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20th International Symposium FORAGE CONSERVATION

CONFERENCE PROCEEDINGS

Ing. Václav Jambor, CSc., Ing. Soňa Malá (eds.)

June 15-18, 2026
Lednice, Czech Republic



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PART 1

PLENARY PAPERS

INTERNATIONAL SILAGE SCIENCE SOCIETY AIMS TO STRENGTHEN THE SCIENTIFIC UTILIZATION OF ENSILED BIOMASSES

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Abstract

The mission of the International Silage Science Society (ISSS) is to promote and advance silage science and technology through research, education and industry for enhanced fermented feeds for animal production as well as for efficient biomass preservation for bioenergy, biorefineries, and environmentally friendly recycling of agricultural and food industry by-products.

Keywords: silage science, biomass preservation, ensiling, fermented feeds, bioenergy, international collaboration, knowledge exchange

OUR STORY

ISSS is a non-profit international organization voluntarily organized by scientists and industrial society related to silage science and technology. It aims to establish an international platform and network to prompt worldwide exchanges and communications on silage research, extension and education. ISSS also wishes to extend silage science and technology to regions of the world where it is undeveloped.

INTRODUCTION

ISSS builds on the long tradition of the International Silage Conferences, which have connected researchers and professionals in silage science and forage preservation since 1970. The need for the Society was acknowledged at the XIX International Silage Conference in Beijing, China in August 2023. The International Silage Science Society had its founding meeting at the XX International Silage Conference in Gainesville, Florida, USA on 23 July 2025. The Executive Board consists of chairpersons of previous International Silage Conferences: Richard Muck (USA 2009), Marketta Rinne (Finland 2012), Luiz Gustavo Nussio (Brazil 2015), Karl-Heinz Südekum (Germany 2018), Fuyu Yang & Xusheng Guo (China 2023) and Adegbola Adesogan (USA 2025). In addition, regional members to cover the different Continents will be invited to the Executive Board to increase the representativeness of the group.

THE ISSS VISION

As a newly founded organization, ISSS aims to provide a platform for communication, collaboration and exchange of knowledge between researchers, educators, students and industry representatives worldwide. One of the main objectives of ISSS include to support the continuity and future development of the International Silage Conferences. The XXI International Silage Conference is scheduled to be held in 2028 in Abuja, Nigeria.

JOIN THE FUTURE OF SILAGE SCIENCE

This contribution introduces the foundation of ISSS, its mission and planned activities, and outlines the vision of the Society as a growing international network supporting the future development of silage and biomass preservation science. ISSS aims to develop into a multidisciplinary global community and it calls for all silage enthusiasts to join the community. All individuals committed to the Society targets can join the Society as members including academics, students, industry representatives, companies and organizations.



Fig. 1: The logo of the International Silage Science Society

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HOW CAN FORAGE MIXTURES HELP WITH FORAGE QUALITY MANAGEMENT?

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Abstract

Effective forage production from perennial fodder crops primarily depends on biomass yield and high forage quality. In this review, we highlighted the potential of multispecies mixtures to influence forage nutritive value, along with forage yield and environmental aspects of production. Multispecies mixtures have clear potential to increase forage yield without decreasing desirable nutrient concentrations. Besides, multispecies mixtures had higher concentrations of macro- and micronutrients. They could better balance protein and energy ratios, although this effect on N use efficiency may be unstable across seasons and years. Integrating bioactive-rich forage species into multispecies grassland systems is a promising strategy to enhance ruminant productivity and mitigate environmental impacts. Still, it is limited by lower content of desirable compounds in bioactive-rich species or their fluctuating proportions in the mixture, a lack of operative analytical methods, limited knowledge and control over appropriate agronomic management, and the functional doses required for consistent biological effects. Optimization of the contribution of mixtures to forage quality requires a deeper understanding of interactions among species, complementarity among functional groups, harvest timing, and environmental conditions, in close relation to transdisciplinary innovation in crop-livestock systems.

Keywords: multispecies mixtures, perennial fodder crops, forage yield, nutrient composition, bioactive forage species, ruminant nutrition, nitrogen use efficiency, sustainable grassland systems, crop-livestock systems

INTRODUCTION

Fodder crops, such as legumes and grasses, traditionally play a dominant role in ruminant nutrition worldwide, and their roughage—mainly from grasslands—accounts for approximately 73% of global ruminant feed intake (Mottet *et al*., 2017). Along with them, other dicotyledonous herbs are increasingly being introduced into these forage mixtures. Farmers have long recognized the benefits of various seeded forage mixtures, which have been used for at least 200 years, probably longer (Foster, 1988). Species or cultivar selection generally represents a simple yet highly effective tool for adapting forage production to environmental conditions. Despite today‘s emphasis on ecology and sustainable farming methods, the various multispecies mixtures are not only created to enhance diversity and ecological benefits but also can provide agronomic advantages and improve the resulting forage quality. In addition to seed-sown grasslands on arable land, where diversity can be managed through seed mixture composition, there are also diverse permanent grasslands where a wide range of wild species can occur naturally, thereby influencing the resulting forage quality. The aim of this review is not to describe all the benefits of sown or natural multispecies mixtures, but to provide an overview of key areas where the mixtures can bring benefits in forage quality in relation to biomass yield.

MIXTURE DIVERSITY INCREASES FORAGE YIELD

Multispecies swards are increasingly recognized for their potential to enhance the agricultural performance and ecological resilience of grasslands (Marley *et al.* 2025). The concept of multispecies swards included introducing a range of species into a grass-based mixture that would constitute a natural mixed species sward, where species from the three broad categories of grass, legume, and forb were each included with a minimum of two representatives of each (Wilkinson *et al.* 2025). Selection of proper species or composition of a mixture of species remains the basic farmer’s tool for successful and effective forage production. Although higher species diversity is highlighted especially for nutrient-poor systems, Lüscher *et al.* (2008) found that a mixture of four agronomic plant species (grasses and legumes) generally yielded more than even the best-performing monoculture (transgressive overyielding), where these diversity effects were consistent across a wide range of environmental conditions and persisted for three harvest years, even under highly fertilized conditions. The productivity benefits of multispecies swards were irrefutably demonstrated by the Agrodiversity experiment, which spanned 33 sites across a wide range of climatic regions in Europe (Kirwan *et al*., 2014). According to O’Malley *et al.* (2026), the multispecies mixtures outyielded two widely used grassland practices: a grass monoculture with higher nitrogen fertilizer and a two-species grass-legume community. In their multi-site experiment net across a large gradient, high yields in multispecies mixtures were driven by strong positive grass-legume and legume-herb interactions. Although the negative effect of low temperature on forage yield was similar on all monocultures and grass-herb mixtures, any mixture containing legumes yielded proportionately more as temperature increased, because the grass-legume and legume-herb interactions were stronger at higher temperatures. In summary, more diverse multispecies mixtures generally deliver higher yields than either a grass monoculture with higher nitrogen input or a two-species grass-legume community. It was realized that combining high-performing plants from different functional groups could enhance forage yields across a wide range of temperate climates and soil conditions while reducing nitrogen fertilizer inputs. Additionally, mixtures can deliver other benefits, such as a wide range of ecosystem services and sustainable weed control, as summarized by Wilkinson *et al.* (2025).

MIXTURE CONTRIBUTION TO FORAGE QUALITY

High-quality forage enhances animal performance, reduces reliance on concentrates, and lowers environmental impacts such as methane emissions (Beauchemin *et al*., 2022). In mixtures, forage quality is shaped by complex interactions among species composition, phenology, harvest timing, and environmental conditions. In multispecies swards, the differing phenological stages of individual species mean that a single harvest date inevitably compromises some species’ quality, as it reflects an average optimal timing rather than the species-specific optimum achievable in monocultures (Malisch *et al.* 2026). In multispecies mixtures, the differences in general nutritional quality between sward types should be related to the achieved proportion of herbs relative to the proportion of grasses and legumes, which can vary over a season and also in harvest years (Cooledge *et al*., 2024). There

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is intensive research on forage quality in legumes and grass mixtures, where grass monocultures clearly tended to have higher neutral detergent fiber (NDF) concentrations than clover monocultures, but also a greater NDF digestibility. As a result, grass monocultures tended to have a slightly lower digestibility of the DM together with much lower CP concentrations than clover monocultures (Sturludóttir *et al.* 2014). Much of the legume benefits for animal performance are related to enhanced intake, since their digestibility is not markedly different from that of grasses. The higher intake of legume forage reflects differences in the cell structure of legume plants, which, combined with high fermentation rates, result in their breakdown into small particles in the rumen and their exit from the rumen more rapidly than with perennial ryegrass (Dewhurst *et al.* 2009). However, the broader effects of functional diversity—particularly the inclusion of herbs such as *Plantago lanceolata* L. and *Cichorium intybus* L.—on forage quality remain underexplored (Baker *et al*., 2023).

MIXTURE EFFECT ON FORAGE NUTRITIVE VALUE

Forage nutritive value measures a plant's ability to supply nutrients (especially energy and protein) to animals, with higher value usually indicating greater digestibility and lower fiber content. Here, the well-known trade-off between forage yield and quality should be considered when the forage quality of perennial forages is evaluated. Because with increasing yield, NDF content increases, but CP content, digestibility, and leaf-stem ratio decrease, it is always effective to evaluate forage quality parameters along with forage yield and/or canopy structure traits (Hakl *et al.* 2021). Previous research showed that legume-grass mixtures were 9, 15, and 7% more productive than the most productive monoculture in the first, second, and third year, respectively. These benefits persisted over the three harvest years of the experiment and were consistent across most sites. In contrast, the positive yield effect was not accompanied by reductions in herbage digestibility and crude protein concentration, as is usually observed with increased DM yield (Sturludóttir *et al.* 2014). A recent study by Malisch *et al.* (2026) demonstrates that multispecies mixtures can outperform or match monocultures in total ME yield per hectare across a wide range of environmental conditions. However, this advantage is primarily driven by biomass overyielding due to niche complementarity, asynchronous growth, and efficient resource use among functional groups (Suter *et al*, 2021). The absence of synergistic effects on ME concentration suggests that combining functional groups primarily results in higher biomass/energy yields while maintaining forage energy content, as shown in Figure 1. In summary, multispecies mixtures with forbs significantly increased total metabolizable energy yields across the multi-site experiment, due to biomass overyielding, without reducing forage energy content. These findings demonstrate their potential to improve nutrient yield in forage-based livestock systems across diverse soil types and environmental conditions, while also delivering other ecosystem services—thus offering a multifunctional alternative to monocultures for forage production (Malisch *et al.* 2026).

Protein degradability in forage legumes is of global importance because N utilization efficiency of forage (NUE) has economic and environmental consequences. Increasing the amount of protein that escapes ruminal digestion could benefit ruminant nutrition and improve the economics of the dairy industry (Chen *et al*., 2009). The most commonly studied factors affecting protein fractions include plant species and harvest maturity (Kirchhof *et al*., 2010; Krawutschke *et al*., 2013), along with plant morphology (Lemaire *et al*., 2005), where lucerne canopy structure contributes by 75% of CP fraction variability (Hakl *et al*., 2016). The findings of this research indicate that plant morphology should be considered, particularly when evaluating the genetic variability of the CP fraction within legume species (Tremblay *et al*., 2003) or measuring protein composition among lucerne cultivars (Chen *et al*., 2009).

Rumen protein degradation and the resulting imbalance between carbohydrate and protein supply lead to lower NUE by ruminants (Broderick, 1995). In forages, it is usually associated with an insufficient readily available energy supply combined with the very fast degradability of a large proportion of forage proteins, which limits the synthesis of microbial proteins in the rumen and causes an excessively large part of the N to be wasted and excreted mostly in urine (Wilson *et al*., 2020). Major dietary strategies to mitigate N emission from cattle operations include reducing dietary N content or increasing energy content, and increasing dietary mineral content to increase urine volume (Dijkstra *et al*., 2013). Considering the differences in water soluble carbohydrates (WSC), pectin or starch fractions between grasses and legumes along with protein fractionation, optimizing the forage energy to protein ratio should be focused on ratio between

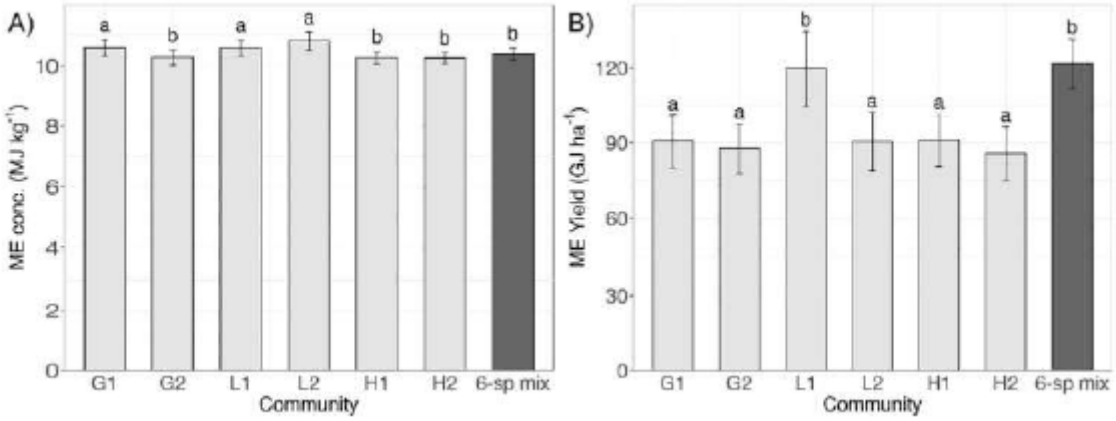


Fig. 1: Metabolizable energy (ME) concentrations in MJ per kg dry matter (A) and ME yields in GJ per hectare (B) of monocultures from *Lolium perenne* (G1), *Phleum pratense* (G2), *Trifolium pratense* (L1), *Trifolium repens* (L2), *Cichorium intybus* (H1) and *Plantago lanceolata* (H2), as well as their respective equi-proportional six-species mixtures (6-sp mix). Error bars represent 95% confidence intervals. (taken from Malisch *et al*. 2026)

nonfibrous carbohydrates and nonprotein nitrogen (NPN) and the rapidly degraded (PB1) protein fractions: NFC/(NPN + PB1) which could better predict the synchrony of readily available carbohydrates and N compounds in the rumen. In lucerne-based mixtures, the energy-to-protein ratio can be optimized through management practices, such as selecting accompanying species, to improve dairy NUE and profitability while minimizing environmental impacts (Tremblay *et al.* 2023). Therefore, in potential mixtures, grasses can contribute with higher WSC and lower CP, whilst legume species can differ in CP content and proportion among protein fractions. According to Tremblay *et al.* (2023), the addition of either red clover or birdsfoot trefoil to a lucerne-based mixture improved the forage energy-to-protein ratio and has the potential to improve NUE in ruminants. In contrast, the addition of timothy or tall fescue had no detrimental effect on annual yield and did not improve the forage readily available carbohydrate concentration or the energy-to-protein ratio. Another issue is the instability of the achieved ratio over the growing season and during the productive years, which is related to the competitiveness and persistence of the companion species. This can be demonstrated by the decline in the NFC/(NPN + PB1) ratio over three harvest years when mixtures of sainfoin and lucerne were harvested under three-cut management, compared to lucerne alone (see Fig 2). This is consistent with the study by Malisch *et al.* (2017), who observed a decline in the proportion of sainfoin in a three-year experiment, with the most pronounced decline in the third productive year, regardless of the accompanying grass species.

MIXTURES AND MINERAL NUTRIENTS

The complex multispecies swards necessarily include variable species that are better able to access macro- and micronutrients from the mineral soil components, slowly released due to combinations of weathering and the impact of soil microbes (Wilkinson *et al.* 2025). Recent research has shown that lambs fed on multispecies swards ingested significantly more key minerals, such as sodium, calcium, magnesium, and potassium, than those fed a legume-grass mixture, leading to more optimal growth (Cooledge *et al.*, 2024). Other studies have shown similar results for trace elements, where multispecies swards containing red clover or chicory provided synergistic multiple-nutrient services by acquiring as much, or more, of cobalt, selenium, zinc, and iodine than pure sown ryegrass swards (Marley *et al.*, 2025). Although there can be an absence of differences in general nutritional quality (e.g., crude protein content, sugar, metabolizable energy, digestibility) between the herbal and grass-clover ley, the multispecies mixtures had greater concentrations of certain macro- and micronutrients (Cooledge *et al.*, 2024).

MIXTURES AS A SOURCE OF BIOACTIVE COMPOUNDS

In the last decades, there has been a surge of scientific interest in bioactive-rich forage species, including in the context of multi-species swards aiming to enhance ruminant productivity while reducing environmental emissions (Niderkorn and Jayanegara, 2025). Especially forbs with legumes represent plants naturally rich in secondary metabolites such as tannins and other phenolic compounds, or saponins, and are increasingly recognized for their potential to modulate ruminal fermentation, reduce enteric methane (CH_4) emissions and nitrogen excretion, and improve animal health and resilience (Mueller-Harvey *et al.* 2019). According to Kleen *et al.* (2011), red clover and birdsfoot trefoil improve protein quality in the mixtures, likely due to their bioactive compound content. It seems that integrating bioactive-rich forage species into multi-species grassland systems is a promising strategy to enhance ruminant productivity and mitigate environmental impacts. The potential of plant secondary metabolites is usually realized through their protein-binding ability and their ability to alter rumen microbial ecology (Zeller *et al.* 2020). While the magnitude of this effect depends on compound type, concentration, and forage context, the mechanism consistently involves protozoa suppression and shifts in fermentation end-products. However, this mitigation

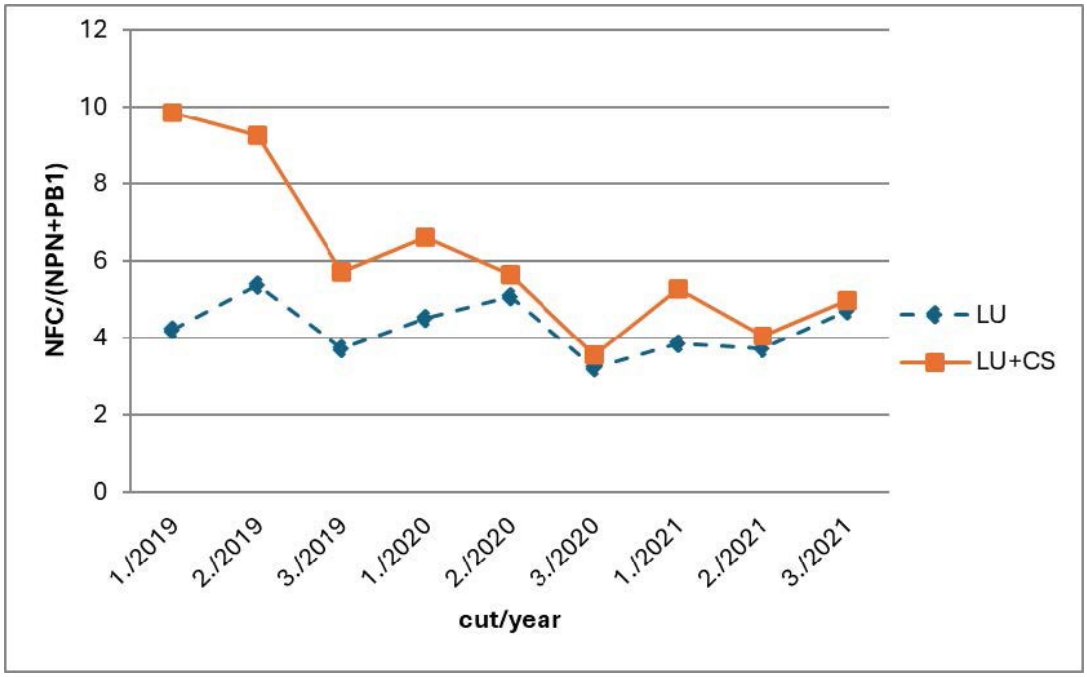


Fig. 2: Ratio of non-fibrous carbohydrate (NFC) to nonprotein N (NPN) and the rapidly degraded (PB1) protein fraction in pure lucerne (LU) in comparison with lucerne + common sainfoin mixture (LU + CS) for a three-harvest year period (Hakl *et al.*, unpublished)

is often subtle *in vivo*, rarely linear beyond certain thresholds. It may result in adverse effects on digestibility or feed intake, underscoring the need for a carefully calibrated integration of such compounds into grazing systems to balance environmental benefits with animal performance. (Niderkorn and Jayanegara, 2025).

There is currently considerable global interest in harnessing the benefits of CTs in forage legumes to support the sustainability of agriculture, particularly in Europe, focusing on birdsfoot trefoil (*Lotus corniculatus*) and sainfoin (*Onobrychis viciifolia*) (Malisch *et al*., 2017). Naturally occurring condensed tannins (CT) in legume forages have been the most intensively studied, especially for their ability to limit rumen degradability of CP and improve protein utilization, arising from protection of dietary protein from excessive rumen fermentation. Other benefits include reducing bloat and parasitism in ruminants, as well as reducing ammonia and methane emissions from livestock operations (Coblentz and Grabber, 2013). The benefits and limitations of feeding CT-containing forage to ruminant livestock have been summarized in several review publications. Waghorn (2008) concluded that the CT in browse, typical of warm and hot climates, is nearly always detrimental to ruminants, except for reducing internal parasite numbers, in contrast to the temperate farming system. Grasses grown in these warm regions contain less protein (and usually more fiber) than temperate forages, and the inclusion of CT from browse further reduces protein availability for absorption by limiting ruminal microbial growth and lowering the fractional absorption of amino acids from the intestine. Intakes of CT from browse, in combination with a medium–to poor-quality diet, are detrimental to performance.

In a study by Coblentz and Grabber (2013), rumen degradable protein (RDP) averaged 77.5% and 88.7% of CP for hay and silage, respectively, and higher estimates of RDP for silage were associated with extensive proteolysis in laboratory silos, resulting in conversions of protein into nonprotein N forms. However, their results suggest that CT suppressed ruminal protein breakdown to the same extent in both hay and silage. When RDP and CT were expressed on a CP basis, regression slopes indicated that each unit of CT protected 0.61 units of CP from ruminal degradation. Applying this relationship to a typical mid-maturity forage legume containing 21% CP suggests that a CT concentration of 3.8% of DM would be required to reduce RDP from 81% to a 70% target considered optimal for improving protein utilization and milk yields by dairy cattle. Inconsistent animal responses to CTs were initially attributed to dietary concentration, but recent research has highlighted the importance of their molecular structures, concentration, and the diet's composition. Therefore, the importance of CT structural traits cannot be underestimated, as reviewed by Mueller-Harvey *et al.* (2019), in which procyanidin (PC)-type CTs are much more widespread than prodelphinidin (PD)-type CTs. Procyanidins are composed of catechin or epicatechin and prodelphinidins of gallocatechin or epigallocatechin subunits. Still, many more plant species contain PC/PD mixtures.

Regarding the animal health dimension of plant secondary metabolites, most studies report antiparasitic, antioxidant, or anti-inflammatory properties associated with specific classes of secondary metabolites. These effects, while context-dependent, may enhance animal resilience to parasites or oxidative stress and reduce reliance on chemical inputs. However, the variability in responses across species and environments, and the potential toxicity of certain alkaloids, highlight the need for cautious, evidence-based application (Niderkorn and Jayanegara, 2025). These animal health effects could be combined with reduced degradability of rumen CP, where the CT in birdsfoot trefoil has been beneficial for ruminant production when forages are fed as a sole diet. Still, the CT in sainfoin, sulla (*Hedysarum coronarium*), and *Lotus major* (syn. *L. pedunculatus*) does not appear to benefit productivity beyond mitigating the impact of parasites. The sainfoin, sulla, and *L. major* have a high feeding value, but the CT *per se* offers no benefits for nutrition (Waghorn 2008). The anthelmintic impact is in line with research undertaken by two research projects' Healthy Hay' and 'Legume Plus' indicated that the reduction in worm numbers seen in ruminant and hind gut livestock was due to the presence of large molecular weight condensed tannins in the foliage of certain key species (sainfoin, birds-foot trefoil and burnet) which reduced parasitic worm reproductive rates. This provides a welcome improvement in livestock welfare, reduces or removes the need for expensive, sometimes ineffective synthetic anthelmintic medicines (Wilkinson *et al.* 2025).

The use of bioactive forages in ruminant systems faces several important constraints. One of the main challenges is the high variability and often marginal concentrations of plant bioactive compounds in real field systems. Further, current analytical methods are often laborious and poorly suited to field monitoring; therefore, the development of portable or predictive approaches, such as near-infrared spectroscopy (NIRS) or metabolomic fingerprinting, would greatly enhance the ability to monitor active compound dynamics in real field conditions (Ortuño *et al*., 2021). Other obstacles include the high cost of traditional analytical methods, fluctuating proportions of bioactive-rich species, and limited knowledge and control over the functional doses required for consistent biological effects (Niderkorn and Jayanegara, 2025). It is also necessary to take into account the agronomic limits of the species of interest. According to Kleen *et al.* (2011), different forage legumes did not perform equally in cutting and grazing systems, and both legume species and defoliation systems interacted to affect the production of forage of high protein quality for ruminant nutrition. Other agronomic practices, such as defoliation regime or fertilization, could be tailored to enhance both the botanical composition and forage quality of grassland (Dindová *et al.* 2019). Especially in multi-species swards, the challenge of achieving a certain level of bioactive metabolites is compounded by dilution effects, as bioactive-rich species are often minor components among dominant plants that lack secondary metabolites (Niderkorn and Jayanegara, 2025). Moreover, even if some forage species can reach these levels, especially under specific management or environmental conditions, they often fall short under practical grazing scenarios (Mueller-Harvey *et al.*, 2019). Consequently, the overall intake of these compounds by ruminants tends to hover around efficacy thresholds, leading to modest or inconsistent responses in terms of emission mitigation or health-related outcomes such as parasite control and antioxidant activity (Niderkorn and Jayanegara, 2025). Tremblay *et al.* (2023) attributed the low effect of BT on the crude protein fraction in a mixture with lucerne to the low BT proportion in the mixture (7%), which was associated with a low CT content (2.5 $g.kg^{-1}$). Malisch *et al.* (2017) reported sainfoin proportions of around 20% in the third harvest year of their experiment, depending on the accompanying grass species. They concluded that the benefits of sainfoin mixtures with *Lolium perenne* and *Festuca pratensis* include high symbiotic N_2 fixation, suppression of unsown species, and increased total yields. Sainfoin has the potential to broaden the options for cropping grass–legume mixtures when management includes low cutting frequency and N fertilizer.

Research on spatial and temporal complementarity between species is essential to stabilize plant bioactive compounds intake across seasons and landscape heterogeneity (Niderkorn and Jayanegara, 2025). For successful utilization of bioactive-rich species, future breeding programs should target not only yield and crop adaptability but also the stability of bioactive compounds production across environments (Mueller-Harvey *et al*., 2019). The choice of which forage species to grow should not be based solely on potential nutritional effects or bioactive compound content, but should always be driven by agronomic performance in the mixture. Effectively integrating forbs and legumes as bioactive-rich species into multispecies swards represents a promising yet complex path that calls for transdisciplinary innovation and context-adapted strategies.

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NIR FORAGE ANALYSIS: GLOBAL NETWORKS AND LOCAL CALIBRATION STRATEGIES

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Abstract

The authors review the methodological background of NIR forage analysis, with particular attention to database structures and size, inconsistencies among chemical reference methods, and the need for harmonization. The advantages and limitations of NIR spectroscopy and classic 'wet' chemical analysis are critically compared. Applications of NIR technology across harvesting, preservation, and feeding processes, including in situ measurements, are presented. The paper emphasizes a comparative evaluation of globalized NIR laboratory networks versus locally developed, customized NIR databases, highlighting their respective strengths, limitations, and interdependencies. The benefits and risks associated with reliance on global NIR networks are discussed in contrast to local calibration development.Furthermore, the study examines the relevance of specific NIR parameters to key agricultural domains, including crop production, machinery operation, livestock management, feed formulation and veterinarian aspects. The role of global NIR laboratory networks in improving efficiency across forage production, harvesting, storage, and feeding is highlighted. Parameter sets and performance outcomes of selected global NIR networks are compared.

Keywords: NIRS, forage, NIR calibration, handheld NIR device

INTRODUCTION

Forage quality is central to ruminant nutrition and determines the energy, protein and structural carbohydrate supply of dairy cattle, beef herds and small ruminants (Van Soest, 1994; Mertens, 1997). Despite being both accurate and well-validated, traditional analytical techniques like Kjeldahl, Dumas, Soxhlet extraction and the van Soest fiber system are time-consuming, laborious and costly (Van Soest and Wine, 1967; Thompson *et al*., 2002). Because they take a long time to complete, they are not widely employed in the daily feeding management. Near infrared spectroscopy (NIRS) is a fast alternative for investigating forages and total mixed rations (TMR). A standard NIR scan can be performed in only seconds with minimal sample preparation and negligible consumable costs (Shenk and Westerhaus, 1991; Roberts *et al*., 2004). Due to the fact that NIRS assesses chemical composition in terms of broad absorbing characteristics of organic molecules, sophisticated chemometric calibration models are indispensable in order to attain an accuracy similar to reference techniques (Martens and Næs, 1989; Cozzolino, 2014). In the last decades, global collaborative efforts, centralized calibration centers and extensive spectral databases have positioned NIRS as a critical technology in contemporary forage laboratories and during on-farm decision making (Williams and Norris, 2001; Givens *et al*., 1997; Decruyenaere *et al*., 2009).

DIFFERENCES IN NEAR-INFRARED (NIR) SPECTROSCOPY

NIR spectroscopy (NIR) spectroscopy is a technique for the detection of near infrared (NIR) light by the absorption of molecular overtones and combinations mainly with O–H, C–H, and N–H bonds. These vibrations are the dominant chemical forms of water, carbohydrates, lipids and proteins in plant matter. Shenk and Westerhaus (1976) state that NIR based methods (with high correlation coefficient) are possible in prediction of dry matter, crude protein and fibre fractions in forages. It does not need much sample preparation, typically only drying and grinding, and returns results in seconds, not hours or days. The near infrared range (780-2500 nm) preserves CH, OH and NH bond overtones and combination bands (Pasquini, 2018; Workman and Weyer, 2012). Spectral regions of significant importance for forage analysis are:

- 1450 nm – water (OH overtone).
- 1730 nm – lipid CH combinations.
- 1940 nm – strong water absorption band.
- 2100–2300 nm – combinations protein and fiber composition (amide, cellulose, hemicellulose) (Osborne *et al*., 1993; Williams and Norris, 2001).

NIR absorption bands are broad and overlapping, difficult for direct interpretation when compared with mid infrared (Pasquini, 2018). Chemometric modeling provides the link between the spectra and the composition (Martens and Næs, 1989; Cozzolino, 2014).

NIR SPECTROSCOPY TECHNICAL BACKGROUND

Strategies for NIR Instrumentation

Near-infrared spectroscopy (NIRS) techniques have become an important analytical technique in forage quality assessment, with advances in instrumentation, as well as with the development of global calibration networks, (Williams and Norris, 2001; Roberts *et al*., 2004; Givens *et al*., 1997). There are four broad classifications of near-infrared spectroscopy (NIRS) instrumentation for forage analysis:

- Fourier-transform NIR (FT-NIR).
- Diode array systems.
- Monochromator instruments.
- Emerging MEMS-based portable NIR devices (Pasquini, 2018; Crocombe, 2018; Beć *et al*., 2020a).

All the methods differ in optical configuration, spectral acquisition and operational suitability, all of which are directly relevant in the application of the technology in today's feed laboratory systems and on farm applications (Workman and Weyer, 2012; Pasquini, 2018). The efficacy of an NIR technology depends not only on the type of spectrometer used (e.g., Fourier transform NIR, diode array, monochromator, etc.), but also on calibration strategy on the spectral interpretation. These two dimensions are increasingly becoming intertwined in current analytical networks. Types of Instruments and their Function; NIR instruments applied for the analysis of forage include three main types that have different functional functions in analytical systems (Table 1).

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FT-NIR instruments employ an interferometer for simultaneous acquisition of all wavelengths, which could also be transformed mathematically into spectrum (Workman and Weyer, 2012; Pasquini, 2018). This method offers good spectral resolution, better wavelength reproducibility, and better signal-to-noise ratios (Burns and Ciurczak, 2007; Workman and Weyer, 2012). As such, these are ideal for developing calibration models using chemically-validated datasets in reference labs (Williams and Norris, 2001; Roberts *et al*., 2004).

Their stability facilitates a relatively reliable transfer of calibration between instruments and physical locations, which is vital for analytical networks of wide scale (Shenk and Westerhaus, 1991; Martens and Næs, 1989). It is stable enough to be especially useful for transfer of calibration in international laboratory networks. In worldwide forage laboratory networks (e.g. Eurofins Agro), FT-NIR is frequently used, for instance, in reference or master laboratories for:

- Calibration model development.
- Compositional analysis for high precision.
- Definition of reference spectra databases (Williams, 2001; Roberts *et al*., 2004).

Diode array instruments use a fixed detector array to measure multiple wavelengths simultaneously (Workman and Weyer, 2012; Pasquini, 2018). The ideal of a diode array is rapid and portable systems that allow near-instantaneous acquisition of spectral information without the need for moving parts. They remain lightweight, rugged, and low-pressure, as there is no moving optical component (Crocombe, 2018; Beć *et al*., 2020a) and the main reason to choose a diode array instrument. These instruments are applicable in on-farm settings (e.g., ASD, Büchi, AgriNIR) where accuracy of critical parameters such as dry matter and crude protein need to be quickly estimated (Roberts *et al*., 2004; Williams and Norris, 2001).

Their performance is significantly reduced by the need to rely on externally developed calibration models derived from centralized databases (Shenk and Westerhaus, 1991; Martens and Næs, 1989). These instruments are generally employed in forage systems:

- On-farm (i.e. silage pits, TMR analysis).
- For rapid evaluation of dry matter and crude protein.
- In real-time decision support systems (Decruyenaere *et al*., 2009; Givens *et al*., 1997).

More and more organizations, including Dairy One and Rock River Laboratory, also implement handheld diode-based NIR equipment in advisory service. However, their reduced spectral resolution and sensitivity to calibration transfer means high levels of reliance on centralized calibration models are needed (Williams and Norris, 2001; Pasquini, 2018).

Monochromator-based instruments use diffraction gratings or filters to filter out wavelengths in an ordered sequence of time (Workman and Weyer, 2012; Pasquini, 2018). These sensors are intermediate and are a widely accepted compromise between performance, cost, and robustness (Williams and Norris, 2001; Roberts *et al*., 2004). They are often used for regular laboratory assays of the subject (e.g. FOSS, Bruker, Unity) with standardized protocols and moderate throughput requirements. It is the consistent use in regional laboratories that is testament to their reliability and compatibility with established calibration procedures (Shenk, 1991; Martens and Næs, 1989). They are widely used in conventional laboratory forage analysis; industrial feed testing laboratories; and in state-of-the-art quality control environments (Givens *et al*., 1997; Decruyenaere *et al*., 2009). Monochromator systems are the basic standard for commercial or industrial use of monochromator (as in case of CVAS) based laboratory networks, where the monochromator is used for the daily high-throughput analysis, because of the consistency provided by the calibration of monochromators, the reproducibility in the output signal, and their intermediate maintenance requirements (Williams and Norris, 2001; Roberts *et al*., 2004).

Integration within NIR Networks in the World: Modern forage analysis systems (e.g. Eurofins Agro, Dairy One, Rock River Laboratory, CVAS) do not depend on one tool type but rather on a hierarchical and complementary tool type (Different tool types are integrated under a common calibration model). In such systems:

- FT-NIR instruments are developed in central or master laboratories to produce high quality reference spectra and calibration models (development and validation for calibration)
- Monochromator instruments in regional laboratories conduct standardized, high-throughput routine analyses (routine, standardized analyses)
- Diode array instruments provide farm level analytical capability for real-time decision-making (real, near real time measurements) This architecture facilitates

Tab. 1: Technical background and comparison of different systems (NIR spectroscopy) Only examples, not complete lists (Williams and Norris, 2001; Burns and Ciurczak, 2007; Workman and Weyer, 2007; Griffiths and de Haseth, 2006; Fearn, 2001; Feudale *et al*., 2002; Roberts *et al*., 2004; FAO, 2011)

Feature	FT-NIR	Diode Array	Monochromator
Role in network	Master/reference lab	Field/on-farm	Routine lab
Spectral resolution	High	Medium–low	Medium
Moving parts	Yes (interferometer)	No	Yes
Field use	Limited	Excellent	Moderate
Lab accuracy	Excellent	Moderate	Good
Measurement speed	Medium	Very fast	Medium
Robustness	Medium	High	Medium
Calibration transfer	Excellent	Moderate	Good
Typical users	Eurofins central labs	Dairy One, Rock River field tools	CVAS, regional labs
Main advantage	Accuracy and stability	Speed and portability	Reliability and balance
Cost	High	Low–medium	Medium
Main limitation	Cost, complexity	Lower precision	Moving parts

distribution of calibration models and guarantees analytical consistency at several sites (Shenk and Westerhaus, 1991; Williams and Norris, 2001).

Standardized protocols for sample preparation and spectral acquisition also help ensure comparability, as local laboratories are guided by procedures established by central labs (Martens and Næs, 1989; Roberts *et al*., 2004).

MEMS based NIR spectrometers (Micro Electro Mechanical Systems) are based on different principles than monochromators or diode array spectrometers but do include other aspects of both in a miniature-sized, integrated optical system (Crocombe, 2018; Beć *et al*., 2020a). The MEMS NIR instruments obtain spectra in some processes with a moving micro mirror, micro grating diffraction system, or micro-interferometer (Pasquini, 2018; Beć *et al*., 2020a). These small mechanical features (mirrors, gratings and lamellae) are actuated electrostatically or thermally depending on the structure of the system (Crocombe, 2018). The direction dictates what wavelength range the detector receives–so rather than a rotating or prism-based monochromator, the measurement is carried out by a vibrating MEMS element that dynamically determines wavelengths (Beć *et al*., 2020a; Crocombe, 2018). Main types:

1. **MEMS micro mirror** (DMD, Digital Micromirror Device concept): use the principle of determining wavelengths by tilting thousands of microscopic mirrors (e.g., those using TI DLP technology in multiple devices) in a differential manner.
2. **MEMS diffraction grating principle:** a micro grating pivoting mechanism tunes the wavelength of the reflected or transmitted light.
3. **MEMS Fabry–Perot interferometric principle:** an oscillating mirror pair alters the optical path length to selectively allow different wavelengths to reach the detector. Examples include Si Ware NeoSpectra System and Texas Instruments DLP NIRscan System.

New MEMS enabled portable NIR devices provide more analyses for on-farm decision making (Crocombe, 2018; Beć *et al*., 2020b). Most conventional inline NIR systems are now MEMS-based or contain MEMS elements, which are critical for industrial or agricultural machinery that need to be compact, vibration-resistant, and dust-proof (Pasquini, 2018; Crocombe, 2018). Feed mixer, unloading facers, and forage harvester mounted sensors enable continuous feedback during processing and feeding (Sørensen *et al*., 2021; Müller *et al*., 2019).

Inline NIR technology is specifically designed for continuous measurement between individual steps for solids, slurries, or feeds on conveyors, augers, or mixers (Workman & Weyer, 2012). Although a majority of modern sensors are MEMS based, their best analytical precision is mainly reserved by monochromator and diode array systems, especially in laboratories or industrial reference systems (Williams & Norris, 2001; Roberts *et al*., 2004).

- Classical inline NIR systems – Many of the classical inline NIR solutions like FOSS ProFoss, Bruker Matrix, Unity SpectraStar, etc., are monochromator or diode array optics. They are high-reliability, laboratory-grade systems (Williams & Norris, 2001; Workman & Weyer, 2012).
- Contemporary "compact inline" systems: MEMS-based inline sensors are developed recently, making these smaller, cheaper, and easier to incorporate into feed mixers, silage unloaders, and forage harvesters (Beć *et al*., 2020b; Crocombe, 2018). NeoSpectra Scanner / NeoSpectra Puck can be applied in silage facers and mixer wagons, as well as AgriNIR Inline systems that utilize MEMS or compact diode-array spectrometers (Crocombe, 2018; Sørensen *et al*., 2021).

The variety of NIR sensors determines spectral range (the range for MEMS instruments is typically from 1350 to 2500 nm), desired analytical accuracy, and environmental conditions (vibration, temperature, dust, moisture or feed residues) (Pasquini, 2018; Beć *et al*., 2020b).

The NIR market is progressing rapidly from laboratory to inline, and then to handheld "smart sensor" devices (Table 2).

- The AgriNIR and X NIR devices based on the diode array model are better suited with rapid acquisition, moderate resolution and robustness in the field; though lower precision than monochromator based devices, they are much smaller and applicable for use on farms. However, diode array systems, such as the AgriNIR are a compromise: they have higher spectral stability and wavelength range than MEMS devices, but are smaller and faster than larger monochromator based laboratory spectrometers.
- The HarvestLab 3000 is closest to a laboratory grade NIR instrument (as for example in FOSS or Bruker systems). It provides both a wider spectral range and very fine resolution: that is why it is frequently used as a reference grade inline analyzer.

Tab. 2: NIR instrumentation strategies in the case of some portable NIR devices. A limited list here, mainly examples (based on manufacturer specifications and scientific publications – Williams and Norris 2001; Burns and Ciurczak 2007; Workman and Weyer 2007; Roberts *et al*. 2004; FAO 2011; Dinamica Generale; ITPhotonics; John Deere)

Device	Manufacturer / Origin	Optical Principle / Technology	Notes / Typical Application
AgriNIR	Dinamica Generale (Italy)	Diode array spectrometer with 19 optical filters (SW NIR, 1150–2500 nm)	Used either as a portable or inline analyzer on farms and feed mixers; not MEMS based.
X NIR	Dinamica Generale (Italy)	Same diode-array principle as AgriNIR (identical optical platform) but in a handheld version	Portable field device for rapid forage analysis.
PoliSPEC NIR	ITPhotonics (Italy) / Caltech Instruments	MEMS-based spectrometer, likely Fabry–Perot interferometer type	Compact, IP67-rated instrument for on site TMR and plant sample analysis.
John Deere HarvestLab 3000	John Deere / Zeiss	Monochromator type spectrograph (optical grating based), covering ~1100–2500 nm	High-accuracy inline system mounted on forage harvesters, tankers, and tractors. Not MEMS but diffraction grating based.

- PoliSPEC NIR, the new MEMS based generation highlighting miniaturization, low energy consumption, and field mobility — albeit usually with a narrower spectral range and also a lower signal to noise ratio than full scale laboratory instruments.

Spectral Pre-Processing

To minimize the influence of light scattering, baseline drift, and particle size effects in near-infrared spectroscopy (NIRS), several standardized spectral transformations are routinely applied prior to chemometric modeling (Burns and Ciurczak, 2007). The most widely used methods include Standard Normal Variate (SNV) correction (Williams and Norris, 2001), which normalizes each spectrum to its own mean and variance to reduce multiplicative scatter effects; and Multiplicative Scatter Correction (MSC), which aligns individual spectra to a reference spectrum to correct additive and multiplicative differences across samples (Williams and Norris, 2001). Savitzky–Golay derivative filtering (Savitzky and Golay, 1964) is commonly used to enhance spectral resolution and remove baseline offsets by calculating first or second derivatives of the spectra. Detrending further eliminates slope variations and low-frequency baseline curvature, improving model robustness. Finally, wavelength selection algorithms—including interval Partial Least Squares (iPLS) (Nørgaard *et al*., 2000) and heuristic optimization techniques such as genetic algorithms—help identify the most informative wavelength regions, thereby reducing noise and computational load while retaining analytical sensitivity (Workman, and Weyer, 2007; Brereton, 2023; Rinnan *et al*, 2009).

Chemometric Modeling

The predominant modeling technique in forage and feed NIR analysis is Partial Least Squares (PLS) regression, which effectively reduces spectral dimensionality while establishing correlations between latent spectral variables and reference chemical data. Owing to its robustness against multicollinearity and high interpretability, PLS has long remained the benchmark method for routine calibration in spectroscopic applications (Martens and Næs, 1989; ge K. Smilde *et al*., 2004; Cozzolino, 2014).

In recent years, however, there has been a marked increase in the application of machine learning approaches capable of modeling nonlinear relationships between spectral features and compositional parameters. These include Artificial Neural Networks (ANN), Support Vector Regression (SVR), Random Forest Regression, and Gaussian Process Regression (GPR), which have demonstrated improved predictive performance, particularly for heterogeneous sample sets and complex feed matrices (Zhang *et al*., 2022; Roger *et al*., 2016). Such methods often outperform traditional PLS models in nonlinear contexts, including regions characterized by strong interactions between constituents (e.g., moisture–protein interactions) (Balabin and Smirnov, 2011; Bevilacqua and Marini, 2014).

Forage and Feed Samples Examined

A wide spectrum of forage and feed types has been evaluated in NIRS calibration studies.

- Typical materials include corn silage, grass silage, and alfalfa silage, whole crop cereal silages cut in different growing stages (boot stage, in heading, milky-dough stage), whole crop cereal-legume blends, as well as alfalfa and meadow hays derived from temperate climates. In addition, fresh forages such as ryegrass, clover, early cut whole crop cereals are frequently analyzed.
- Industrial by-products—such as distillers dried grains with solubles (DDGS), sugar beet pulp, brewery grains, corn gluten feed, soybean hulls—are also included to expand calibration range and enhance model generalizability across diverse feed matrices.
- Cereal grains (corn, wheat, barley, oat, rye), full fat seeds (soya, rapeseed, sunflower), extracted meals (soybean meal, sunflower meal, rapeseed meal) and mixed concentrates are frequently analyzed.
- Complex blends like total mixed rations (TMRs). However, the accuracy and reliability of the mixture results raise some concerns. In this case, the size of the calibration database plays a critical role.

These global NIR-networks generally -besides forage samples- have databases for water, soil, milk and manure samples, also.

Chemical and In Vitro Reference Methods

Reference laboratory analyses underpin all NIR calibration models and are typically conducted using standardized wet chemistry protocols:

- Gravimetric oven drying at 70°C and 105 °C to determine dry matter content.
- Kjeldahl or Dumas methods for crude protein determination; On average the Dumas method provided results that were relatively higher by about 1.4% than the Kjeldahl method, but the difference between the methods depended on the type of feedstuff.
- Soxhlet ether extraction for crude fat quantification;
- Van Soest procedures for neutral detergent fibre (NDF and aNDFom), acid detergent fibre (ADF), and acid detergent lignin (ADL). Original publication for NDF, ADF and ADL: Van Soest and Wine, (1967)
- Assays include enzymatic starch analysis (fotometric method).

However, **reference methods are not standardized**. When NIR results are questioned, it is often the case that differences in the reference methods are responsible for discrepancies between chemical analytical results and NIR outcomes, as well as for variations among results obtained by different NIR laboratories. The **worldwide harmonization of reference methods would significantly enhance comparability**, transparency, and the reliability of results.

For crude protein determination, both the Kjeldahl and the Dumas methods are in use (While *et al*.,1998; Thompson *et al*., 2002). Even within the Kjeldahl method, procedural variations exist.

- Crude protein without NH_3-N: This is the result of the Kjeldahl method where all the NH_3-N has been substracted. This crude protein is used in the Dutch calculation of VEM (Net Energy for Dairy Cows).
- Crude protein with NH_3-N after pre-drying: This is crude protein + the amount of N which remains in the material, after the first drying process (at 60 degrees). This value is used in the German and Danish energy and protein calculation.
- Crude protein total: First the NH_3-N is measured in the sample before it is dried. Afterwards this NH_3-N can be re-calculated and added as protein to the crude protein value. This value is used in the Dutch calculation of DVE (Digestible Protein in the Small Intestine) and OEB (Degraded Protein Balance - rumen nitrogen balance).

Furthermore, there are new protein parameters required by the modern softwares using CNCPS-model: e.g. adjusted protein (Crude Protein – ADICP), ADICP (Acid Detergent Insoluble Crude Protein), NDICP (Neutral Detergent Insoluble Crude Protein), NDRCP (Neutral Detergent Residue Crude Protein).

The crude fiber value is no longer reported in modern analytical reports. Moreover, further harmonization is required for fiber analysis, especially in forage/TMRs/PMRs containing starch. The aNDFom (amylase-treated, organic matter corrected neutral detergent fiber) would be preferable by the AOAC (AOAC Official Method 2002.04; Mertens, 2002) compared to the original NDF method (Van Soest and Wine, 1967). Some laboratories, such as Cumberland Valley Analytical Services (CVAS) report aNDF and NDFom values for corn silage and use a new parameter: the NDR (neutral detergent residue). Dairyland Laboratories Inc. provide both aNDF and aNDFom values for corn silage and TMR/PMR samples. Moreover, there are other technical alternatives (ANKOM or Fibertec methods) for fiber fraction analyses (Hall and Mertens, 2023). Modern softwares using CNCPS-model requires soluble fiber content, also. The physial structure of the NDF (peNDF) can be determind by the Ro-Tap Sieve Shaker and method (Mertens, 1997) applied both classic chemistry or NIR-technology. The concept of physically effective undigested NDF (peuNDF), combining uNDF and physical effectiveness, has been discussed in applied dairy nutrition, particularly in the work of Grant and colleagues, as a new parameter developed by the Miner Institute (Grant *et al.* 2020).

The determination of starch is often still carried out polarimetrically in many European countries, whereas the AOAC method now recommends enzymatic-photometric starch quantification (AOAC Official Method 2014.10 in AOAC INTER NATIONAL, 22nd Edition, 2023). This enzymatic-photometric method is recognized as an AOAC and AACC harmonized approach for starch measurement in feeds and foods. Especially in wet by-products, total mixed rations (TMRs) and partial mixed rations (PMRs) containing viscous, gel-forming substances, part of the sugar fraction is mistakenly measured as starch in polarimetric assays. In contrast, such interference is eliminated in photometric methods.

The determination of total sugars is also critical, as multiple analytical protocols are in use. The WSC (water-soluble carbohydrates) method employs water for extraction, whereas the ESC (ethanol-soluble carbohydrates) method. Both Dairyland Laboratories Inc. and CVAS report results derived from each method. There are other differences, also. The Luff–Schoorl titrimetric method, uses an alcohol-based extraction. It is a cheap, robust but slow method and give total sugar content. The DNS method (3,5-dinitrosalicil acid) quick and has lower cost giving total sugar concentration. The HPLC method advantages: high specificity, simultaneous determination of multiple components and high accuracy, but expensive instrumentation needed, sample preparation required and requires technical expertise. (AOAC Official Method 923.09 titrimetric method; AOAC Official Method 945.66 titrimetric method; AOAC Official Method 982.14 HPLC method; AOAC Official Method 977.20 HPLC method; AOAC Official Method 986.25 enzymatic method)

When measuring fat content, the question arises as to whether hydrolytic pre-treatment is applied universally to all samples or solely to those containing protected fat sources (TMRs/PMRs), as practices differ among laboratories. For example, Eurofins Agro provide total fat content, independently of the sample type. Hydrolytic pretreatment is discussed in AOAC 2003.05, indicating it's required when samples contain protected or bound fats. AOAC Official Metho d 920.39 / 922.06 / 2003.05:

- 920.39: *Crude Fat in Feed – Ether Extraction (Soxhlet).*
- 2003.05: *Total Fat in Foods – Hydrolytic Pre Extraction and Solvent Extraction.*

Wet chemical methods are not capable of determining ruminal degradability, degradation rates, or the proportion of undegradable fractions of protein, starch, or fiber fractions. For this purpose, NIR laboratories require *in vitro* reference databases. As one of the key determinants of competitiveness for NIR laboratories is their ability to provide input data for software based on the CNCPS model, such *in vitro* databases have been extensively developed over the past decade. However, methodological inconsistencies remain. In the United States, 30-hour NDF degradability has been widely used, whereas in Europe the 48-hour value has become standard. The National Academies of Sciences, Engineering, and Medicine recommended in 2021 that the 48-hour *in vitro* degradability should be adopted as the standard, as it shows a closer correlation with *in vivo* data. Unfortunately, this implies that many NIR laboratories in the United States still report 30-hour values, while robust background databases for 48-hour values have not yet been fully established. This creates challenges for European users.

Parameters and parameter packages

As the large number of parameters may be confusing for users, the common international practice is for laboratories to provide pre-defined **data packages tailored to specific forage types**. This user-friendly approach supports non-expert livestock producers in their daily decision-making. However, these data packages are not always sufficiently flexible; therefore, many laboratories also offer optional parameters that can be selected for specific or specialized applications.

The large number of parameters reported in NIR result sheets can be confusing, and even experts often express negative opinions about them. However, it is important to recognize that these data can be categorized into distinct groups (Table 3).

Certain groups of parameters support or evaluate **crop production practices**. Other parameter-groups provide information on the **technical aspects of harvesting**, enabling improved machine settings in subsequent seasons. A third major group is relevant for **livestock managers**, who need to assess the quality, feed value, and potential risks associated with a given forage. A fourth group, which is often less intuitive for professionals with a classical background, consists of **input data for software applications**. Many of these programs are now based on dynamic models, which account not only for quantitative balances but also for temporal synchronization. Finally, there is a group of parameters that can assist **veterinarians** in determining whether observed production depression or herd-level health issues are related to nutritional factors or are caused by other problems, such as management deficiencies, bacterial or viral infections, or parasitic infestations.

We present several examples illustrating which parameters provide relevant information for different divisions within a specialized large-scale farm, and from which perspective this information is important. In smaller farms, such a level of differentiation is not feasible, as the farmer typically performs crop production, harvesting, preservation, and dairy production tasks alone. Nevertheless, even in such cases, it can be informative to distinguish which parameters are primarily relevant for the nutritionist and which are of greater importance to the veterinarian.

Tab. 3: The data presented in the NIR-report are categorized according to the target division and their intended use. Not a complete list, only examples (own compilation)

Crop manager	Crude protein (fertilization, growth stage), nitrate content (fertilization technology: dose, application, timing), NDF, ADF, ADL (growth stage at harvest), Fiber digestibility (NDFd30 or 48: growth stage at harvest), Starch content and digestibility (growth stage at harvest), RFV (relative feed value), RFQ (relative forage quality), Milk kg per ton silage, Milk kg per ha, Beef kg per ton silage
Mechanical specialist	DM (wilting technology), Ash (soil contamination), peNDF (chop size), CSPS (seed cracking efficiency of the harvestor), Fermentation profile: lactic acid, acetic acid, LA/AA ratio, propionic acid, butyric acid, Ammonia and pH (compaction and sealing technology).
Livestock managers	DM (dry matter intake, effluent, face management), Crude protein (milk production), nitrate content (reproductive performance, animal health risk), NDF (fiber supply, milk fat and acidosis risk), peNDF (acidosis risk), Starch (energy content, ketosis and acidosis risk), Total sugar (rumen supply and silage aerobic stability, face management), NFC, NSC (ketosis and acidosis risk), Fermentation profile: lactic acid, acetic acid, LA/AA ratio, propionic acid, butyric acid, ammonia and pH (as silage anaerobic stability and animal health risk)
Diet formulation (input data)	$NDFd_{12,30,48,120,240}$ (*kd*, dry matter intake, CNCPS), $dNDF_{48}$ (milk fat content, CNCPS), $uNDF_{240}$ (dry matter intake, CNCPS), ADICP, NDICP, adjusted protein (available protein for milk production, CNCPS), starch content and digestibility (rumen supply and CNCPS), total sugar (rumen supply and CNCPS), NFC, NSC (energy supply, ketosis and acidosis risk), NDF dig. rate (CNCPS), Starch dig. rate (CNCPS), Crude fat, fatty acids, unsaturated fatty acids (rumen health, milk fat, reproductive performance), Amino acids (nitrogen utilisation efficiency)
Vet. aspects	NDF (acidosis risk), peNDF (acidosis risk), Starch, NFC, NSC (ketosis and acidosis risk), Fermentation profile: lactic acid, acetic acid, LA/AA ratio, propionic acid, butyric acid, ammonia and pH (rumen and animal health risk)

Examples are presented of parameters that are currently reported in NIR reports for forages by some of the major NIR laboratory networks (Table 4).

Tab. 4: Parameters reported in NIR reports for forages by some of the major NIR laboratory networks (parameters listed were compiled from technical documentation of CVAS, Dairyland Laboratories, Eurofins Agro, Rock River Laboratory, Dairy One).

Parameter	CVAS	Dairyland Labs	Eurofins Agro	Rock River Lab	Dairy One
Dry Matter (DM)	✓	✓	✓	✓	✓
Crude Protein (CP)	✓	✓	✓	✓	✓
ADICP, NDICP	✓	✓	✓	✓	✓
NDRCP	✓	✓	–	✓	–
RDP / RUP	✓	✓	✓	✓	✓
Soluble Protein	✓	✓	✓	✓	✓
Amino Acid, total	✓	✓	–	✓	✓
Neutral Detergent Fiber (NDF)	✓	✓	✓	✓	✓
aNDFom (ash-corrected NDF)	✓	✓	✓	✓	✓
NDF Digestibility (NDFd 12,24,30,72,120,240)	✓	✓	✓	✓	✓
NDF Digestibility (NDFd48)	–	–	✓	–	–
uNDF240 (undigestible NDF)	✓	✓	✓	✓	✓
Acid Detergent Fiber (ADF)	✓	✓	✓	✓	✓
Acid Detergent Lignin (ADL)	✓	✓	✓	✓	✓

Parameter	CVAS	Dairyland Labs	Eurofins Agro	Rock River Lab	Dairy One
Starch	✓	✓	✓	✓	✓
Sugar (WSC)	✓	✓	–		✓
Sugar (ESC)	✓	✓	✓	✓	
Crude Fat (EE)	✓	✓	✓	✓	✓
Fatty acids	✓	✓	–	✓	optional
Ash	✓	✓	✓	✓	✓
NEL (Net Energy for Lactation)	✓	✓	✓	✓	✓
ME (Metabolizable Energy)	✓	✓	✓	✓	✓
TDN (Total Digestible Nutrients)	✓	✓	✓	✓	✓
RFV (Relative Feed Value)	✓	✓	✓	✓	✓
RFQ (Relative Forage Quality)	✓	✓	✓	✓	✓
DCAD	✓	✓	✓	✓	✓
NFC (Non-Fiber Carbohydrates)	✓	✓	✓	✓	✓
pH (silage)	✓	✓	✓	✓	✓
Fermentation acids (lactic, acetic, butyric)	✓	✓	✓	✓	✓
Ammonia-N (% of CP)	✓	✓	✓	✓	✓
CNCPS carbohydrate pool	–	–	✓	–	–
CNCPS protein pool	–	–	✓	–	–
French dataset (PDIA, PDIN. PDIE, UFL)	–	–	✓	–	–
German dataset (nXP, RNB)	–	–	✓	–	–
Dutch dataset (DVE, VEM, VEVI, OEB, NELVC)	–	–	✓	–	–

A key limitation of U.S.-based laboratory networks in Europe is that they primarily provide data aligned with American feeding systems, most notably the parameters required for the CNCPS model (e.g., AMTS profiles). As a result, values corresponding to European feed evaluation systems—such as the French, German, Dutch, or Nordic models—are typically not available. Consequently, users relying on French, German, or other region-specific ration formulation software cannot fully utilize the outputs of these laboratories.

From this perspective, the Dutch central laboratory of Eurofins Agro is more competitive, as it incorporates equations from local feed evaluation systems and provides results adapted to regional requirements, including the use of local languages.

Table 5. presents the correlation of parameters measured by different NIR-laboratory networks, compared to reference datasets (obtained from 'wet' chemical analytical methods) and NIR prediction accuracy.

Tab. 5: NIR prediction accuracy for forage analysis of different NIR laboratories. Based on technical reports of Dairy One, Rock River Laboratory, Eurofins Agro, CVAS, Dairyland Laboratories and scientific references include Shenk and Westerhaus (1991), Williams (2001), Deaville and Flinn (2000), Roberts *et al.* (2004), Williams and Norris (2001)

Parameter	CVAS	Dairyland Labs	Eurofins Agro	Rock River Lab	Dairy One	Typical Range
Dry Matter	R^2: 0.90–0.98 RMSE: 1.0–2.5 %	0.92–0.98 / 1.0–2.0	0.90–0.97 / 1.2–2.5	0.92–0.98 / 1.0–2.0	0.90–0.97 / 1.2–2.5	0.90–0.99 / 0.8–3.0
Crude Protein	R^2: 0.92–0.99 RMSE: 0.5–1.5 %	0.94–0.99 / 0.4–1.2	0.92–0.98 / 0.5–1.5	0.94–0.99 / 0.4–1.2	0.92–0.98 / 0.5–1.5	0.93–0.99 / 0.3–1.5
NDF	R^2: 0.88–0.97 RMSE: 1.5–3.5 %	0.90–0.97 / 1.2–3.0	0.88–0.96 / 1.5–3.5	0.90–0.97 / 1.2–3.0	0.88–0.96 / 1.5–3.5	0.88–0.98 / 1.0–4.0
ADF	R^2: 0.85–0.95 RMSE: 1.5–3.0 %	0.88–0.96 / 1.2–2.5	0.85–0.94 / 1.5–3.0	0.88–0.96 / 1.2–2.5	0.85–0.94 / 1.5–3.0	0.85–0.97 / 1.0–3.5
NDFd	R^2: 0.70–0.90 RMSE: 2.5–5.0 %	0.75–0.92 / 2.0–4.5	0.70–0.88 / 3.0–5.0	0.75–0.92 / 2.0–4.5	0.70–0.88 / 3.0–5.0	0.70–0.93 / 2.0–6.0

Calibration and Validation: Model Performance Metrics

Model quality in chemometric applications is typically evaluated using standard statistical indicators, most notably the coefficient of determination (R^2) and several root mean square error metrics, including RMSEC (calibration), RMSECV (cross-validation), and RMSEP (prediction). These parameters are widely used to assess both model fit and predictive performance in spectroscopic calibration studies (Rocha *et al*., 2020; Sadergaski *et al*., 2022).

Lower values of RMSEC, RMSECV, and RMSEP indicate improved model accuracy and robustness, while their comparison enables the detection of overfitting and generalization capability (De Bleye *et al*., 2012).

In addition to these metrics, the Residual Predictive Deviation (RPD), defined as the ratio between the standard deviation of reference data and the prediction error, serves as an integrative indicator of model applicability and predictive usefulness (Kamruzzaman *et al*., 2022). High RPD values are generally associated with models suitable for screening, quality control, or precise quantitative prediction, depending on threshold ranges reported in the literature.

Interpretative thresholds for RPD are widely accepted as follows:

- → RPD > 3.0 – Excellent analytical accuracy suitable for quantitative use.
- → RPD = 2.0 – 3.0 – Good screening ability with acceptable precision.
- → RPD < 2.0 – Limited practical utility.

A highly reliable and industry-grade calibration model typically achieves an RPD ≥ 3.0, indicating strong predictive performance and robustness across varying forage types and measurement conditions.

COMPARISON OF NIR SPECTROSCOPY AND CONVENTIONAL CHEMICAL ANALYSIS FOR FORAGE EVALUATION

Forage quality is a cornerstone of ruminant nutrition, determining the energy, protein and structural carbohydrate supply available to dairy cattle, beef herds and small ruminants. Classical analytical methods such as Kjeldahl, Dumas, Soxhlet extraction and the van Soest fiber system are accurate and widely validated, yet they are labor intensive, time consuming and expensive. Their turnaround time limits their widespread use in daily feeding management.

Near-infrared spectroscopy (NIR) has become an important analytical tool in forage quality assessment due to its speed, non-destructive nature, and ability to support real-time decision-making in livestock nutrition. In contrast, conventional chemical analyses—such as Kjeldahl nitrogen determination, Van Soest fibre fractionation, and solvent-based fat extraction—offer high accuracy but at the cost of time, labor, and chemical use. Recent systematic review highlights that NIR can achieve comparable predictive performance for key nutritive traits, especially when robust calibration models are available.

The quantification of forage quality is fundamental for ruminant nutrition, feed preparation and accurate animal management. Chemical analysis methods have traditionally been the gold standard in nutritional laboratories that are applied to crude protein (Kjeldahl), acid and neutral detergent fibre (ADF/NDF) analysis by the Van Soest system, and Soxhlet extraction for ether extract. But, they are time-consuming and labor-intensive methods that need hazardous reagents regularly. Over the last 30 years, near-infrared spectroscopy (NIR) has been developed as a fast non-destructive approach for determining nutritive value in silages, hays, and TMR. Among the advantages of NIR over classical chemical assays is its non-destructive characteristic and lack of chemical reactants, thus significantly decreasing its operational cost and environmental impact (Fodor *et al*., 2024), which is the primary reason for its implementation. In addition to that, high-throughput analysis of NIR also exist and can be performed in both lab and field applications with low-cost portable spectrometer portable measurements (Alomar *et al*., 2021). Such flexibility is particularly relevant for precision feeding programs that need real-time detection on forage quality to dynamically adapt rations.

Although much slower than pure NIR, chemical methods remain essential as reference procedures. However, their accuracy for the measurement of distinct compounds (e.g. crude protein or lignin) continues to be much greater than that of NIR for poorly absorbing or trace compounds (Chen *et al*., 2013). For instance, mineral elements or secondary metabolites produce weak NIR signals, so measurement is only possible by wet chemistry. In addition for formal feed assessment systems and regulatory laboratories, standard systems adopted by AOAC or ISO for laboratory verification and inter-lab reproducibility are also required.

The data of the NIR spectrum have many limitations including the reliance on calibrated database. Sample diversity, species composition, growing conditions and storage history are all important variables that can affect the robustness of predictive models (Hossain *et al*., 2024). In the absence of representative calibration sets, the outlier samples exhibit drastically lower predictive accuracy. Furthermore, instrument variability—variation in detectors, light sources, and path lengths—requires model transfer procedures or recalibration (Maduro Dias *et al*., 2024). Therefore, NIR, despite its statistical capabilities, is not quantitative-based in principle, its value comes from pre-known relationships to chemical reference data.

Practically, the incorporation of NIR spectroscopy into intelligent farming systems is a quantum leap of progress. Studies in feed laboratories and devices, estimate well dry matter/ crude protein composition of whole crop corn, corn silage and TMR mixtures, which can make immediate management decisions in the field. These on-farm adoption provide economic benefits via immediate forage allocation, decreased waste disposal and enhanced herd nutrition.

Chemical analysis, in contrast, has specialist workers and lab facilities. In addition, sample prep consists of digestion, filtration and reactions in multiple analytical steps, each with individual human errors and cumulative uncertainty. The average batch analysis time for one sample of forage might be 24-72 hours, depending on the parameters. By comparison, with sufficient calibration, NIR can work at times of tens of applications/hour to monitor feed inventories (De Boever *et al*., 2018). Thus, many recently developed food industry research programs are a combination of the techniques. (1) the primary reference information, based on scientific chemical analyses, and, (2) feed (continuous) operational feedback from NIR, such that operational activities are more or less continuous along production chains.

Note that contemporary ration formulation software needs specific quantities of inputs that cannot be easily acquired using common chemical analytical techniques. Some of such systems (among other applications) require the observation of fiber degradability parameters at multiple time points (degradability of NDF at 12, 24, 30, 48, 120, and 240 h *in vitro* incubation, degradation rate (kd in %/h), concentrations of degradable and un-degradable fiber fractions (dNDF48

and uNDF240, g/kg DM), starch degradability (7 h *in vitro* degradability) and soluble/rumen-degradable protein (RDP). Competitiveness of privately owned NIR laboratories generally rely on their customers for obtaining compatible data with their clients' software. Through the proliferation of dynamic rumen systems, this competition has prompted swift advancements in *in vitro* parameters following the development of dynamic rumen models and this pace cannot be achieved by methods based on classical analytical methods. Due to this, the current chemical analytical laboratories in the field are losing competitiveness as a result.

Despite development in technologies, NIR should not be treated as an absolute substitution for wet chemistry. It works rather as a complementary method for standard, nondestructive, statistically derived quality control to act. (Ferré and Garrido, 2022), the reliability of NIR assessment increases significantly where appropriate sampling design, recalibration on a periodic basis, and chemometric verification techniques, including partial least squares regression (PLSR), and cross-validation, are applied. Under these requirements, NIR allows the rapid translation of laboratory data to real-time nutritional insights. It should rely on analytic objective whether they satisfy regulatory targets or are efficient in operations. Continuous improvement of machine learning, spectral and sensor standardization and small-sensor miniaturisation is anticipated to bridge performance disparity further and NIR spectroscopy will be the predominant tool in sustainable forage quality of management and precise feeding to livestock in the next 10 years.

A summary Table 6 illustrates the main differences between the two systems in terms of their respective strengths and weaknesses, summary Table 7 presents the differences between the NIR spectroscopy and 'wet' chemical analysis based on various criteria.

Tab. 6: The key differences between NIR spectroscopy and conventional chemical Analysis in terms of their advantages and disadvantages (based on Shenk and Westerhaus, 1991; Givens *et al.*, 1997; Deaville and Flinn, 2000; Williams and Norris, 2001; Roberts *et al*., 2004; Van Soest *et al.*, 1991; AOAC, 2019; Sniffen *et al*., 1992)

NIR Spectroscopy	
Advantages	**Limitations**
1. Rapid and Non-Destructive Measurement NIR allows for the rapid assessment of dry matter, pro-tein, fiber, and other forage constituents without sample destruction, reducing time from hours to seconds	**1. Dependence on Calibration Quality** NIR accuracy de-pends heavily on representative calibration databases; for-age diversity in plant species, harvest maturity, and ensiling conditions can reduce predictive accuracy
2. No Hazardous Chemicals Unlike traditional methods (e.g., Kjeldahl or Soxhlet), NIR avoids aggressive reagents such as sulfuric acid or organic solvents, reducing cost, environmental impact, and safety risks	**2. Lower Sensitivity to Minor Components** Trace elements, minerals, and compounds with weak NIR absorption often cannot be detected with sufficient precision, necessitating traditional chemical methods
3. Suitability for On-Farm and Real-Time Applications Portable instruments enable in-situ forage eval-uation, supporting precision feeding strategies and enhancing reactivity to variability in silage and TMR quality	**3. Instrument and Model Transfer Issues** Differences be-tween devices, optical setups, or measurement geometries require careful standardization or transfer algorithms for consistent results
4. High Throughput and Low Operating Cost Once calibration models are established, NIR provides near-zero marginal cost per sample, enabling large dataset generation.	
5. Digestibility data for fiber fractions, starch, and protein (based on an *in vitro* reference dataset) can be used as input for software implementing dynamic rumen models. CNCPS data are available.	
Conventional Chemical Analysis	
Advantages	**Limitations**
1. High Analytical Accuracy and Sensitivity Wet chemistry provides reference-level quantification for protein, fibre fractions, minerals, and secondary metabolites. These measurements form the founda-tion for NIR calibration itself.	**1. Time- and Labor-Intensive Processes** Protein, fibre, and lipid assays may require hours to days, limiting throughput.
2. Universality Across Sample Types Unlike NIR, chemical methods require no calibration models and remain robust across highly diverse forage matrices.	**2. Use of Hazardous Chemicals** Procedures rely on strong acids, bases, and solvents, creating waste and environmen-tal burdens
3. Regulatory and Standardised Methods Many wet chemistry methods (e.g., AOAC) are legally required for feed evaluation and food safety assays.	**3. Higher Cost per Sample** Reagents, labor, and waste management lead to significantly higher unit costs relative to NIR.
	4.Digestibility and CNCPS can not be given. It is not capable of supporting modern feed ration formulation software based on dynamic rumen models and is therefore not com-petitive.

Tab. 7: Differences between the NIR spectroscopy and 'wet' chemical analysis based on various criteria. (based on Shenk and Westerhaus, 1991; Givens *et al.*, 1997; Deaville and Flinn, 2000; Williams and Norris, 2001; Roberts *et al*., 2004; Van Soest *et al*., 1991; AOAC, 2019; Sniffen *et al*., 1992)

Criterion	NIR Spectroscopy	Chemical Analysis
Speed	Seconds	Hours–days
Sample preparation	Minimal	Extensive
Chemical use	None	High
Accuracy	High (with calibration)	Very high
Trace components	Limited	Strong
Cost per sample	Very low	High
On-farm usability	Excellent	Poor
Digestibility data (fiber fractions, starch, and protein)	Yes	No
Supporting modern formulation softwares based on CNCPS model	Yes	No
Flexibility	Strengths	Limitations
Market sensitivity and competitiveness	Strengths	Limitations

Corn silage samples comparison

It aims to analyze the variability between conventional chemical analysis and NIR-based predictions for different laboratory networks. The results of the analysis are presented in Table 8. Ten corn silage samples were collected for comparison purposes. The samples were obtained through standard forage extraction and preparation protocols. The samples were dried at 70 °C to constant weight and then ground to pass through a 1 mm sieve. The samples were also homogenized to reflect uniformity following the grinding process. Every such homogenized sample had four representative subsamples. One subsample was subjected to standard wet chemical analysis and the rest of the subsamples were sent to three NIR laboratory networks for laboratory analyses – Cumberland Valley Analytical Services (CVAS), Dairyland Laboratories (Dairyland Laboratories), and Eurofins Agro (Eurofins Agro). All laboratories performed analyses of the samples based on their normal models of calibration with near-infrared spectroscopy (NIR) and as per standard operating procedures. Standard chemical analysis was also performed using reference methods compatible with the forage study.

Data analysis (statistical methods and laboratories) analyzed differences in analysis across laboratories and agreement between laboratories was obtained. We calculated descriptive statistics (mean, standard deviation) for all parameters. Differences among experimental settings were analyzed through one-way analysis of variance (ANOVA). Post-hoc comparisons were conducted to determine the pairwise difference between laboratories when significant ($p < 0.05$) effects were observed. The prediction accuracy of NIR results was assessed in relation to traditional chemical analysis based on coefficient of determination and root mean square error.

(1)

$$R^2 = 1 - \frac{\sum \left(y_i - \hat{y}_i\right)^2}{\sum \left(y_i - \bar{y}\right)^2}$$

(2)

$$RMSE = \sqrt{\frac{1}{n} \sum \left(y_i - \hat{y}_i\right)^2}$$

where y_i represents the reference (wet chemistry) values, $\hat{y}_i$ the NIR predicted values, and $\bar{y}$ the mean of reference values.

All statistical studies were performed at $\alpha = 0.05$. The results of the systematic differences among the laboratories and the predictive performance of NIR spectroscopy over traditional analytical analyses were examined for statistical methods (Table 9).

Starch: One-way ANOVA group differences were significant with each other ($F = 6.05$, $p = 0.0019$). Post hoc Tukey analysis showed that NIR2 was significantly different from all other groups, but no difference was found among wet chemical data, NIR1, and NIR3. R^2 values proved the good consensus between wet chemical and NIR1 ($R^2 = 0.66$), between wet chemical and NIR3 ($R^2 = 0.68$), but poor consensus for NIR2 ($R^2 = -0.73$),

Tab. 8: Comparative analysis of starch- and ADF content in corn silage samples (n = 10)

	Starch			ADF		
	Mean	**Std**	**CV%**	**Mean**	**Std**	**CV%**
Wet chemistry	345,3	46,9	13,6	220,6	24,3	11,0
NIR1	331,7	34,4	10,4	236,2	21,0	8,9
NIR2	401,8	38,9	9,7	218,2	22,3	10,2
NIR3	355,4	35,0	9,9	218,1	25,4	11,6
Mean	**358,6**	**–**	**–**	**223,3**	**–**	**–**
Std	**30,4**	**–**	**–**	**8,7**	**–**	**–**
CV%	**8,5**	**–**	**–**	**3,9**	**–**	**–**

Tab. 9: Statistical analysis of corn silage samples (n = 10) and complete validation table (R^2, RMSE, bias, RPD) compared to wet chemical data

	Starch				ADF			
Modell	**R^2**	**RMSE**	**Bias**	**RPD**	**R^2**	**RMSE**	**Bias**	**RPD**
NIR1	0.662	25.87	−13.6	1.81	0.468	16.80	+15.6	1.45
NIR2	−0.735	58.60	+56.5	0.80	0.913	6.79	-2.3	3.58
NIR3	0.685	24.99	+10.1	1.88	0.927	6.20	-2.5	3.91

which indicated a systematic difference. Root mean square error (RMSE) analysis clearly confirmed the good agreement observed within wet chemistry and NIR1 (RMSE = 25.9) and between wet chemistry and NIR3 (RMSE = 25.0), while NIR2 showed significantly greater deviation (RMSE = 58.6), showing poor agreement and systematic differences. The residual predictive deviation (RPD) values revealed limited predictive performance of the NIR models, (NIR3 (RPD = 1.88) and NIR1 (RPD = 1.81) were relatively moderate in their suitability for screening purposes; NIR2 (RPD = 0.80) exhibited a poor prediction capability). Systematic biases indicated systematic underestimation and overestimation, with NIR1 (–13.6) vs NIR3 (+10.1), but NIR2 displayed strong positive bias (+56.5), which suggested systematic deviation from the models.

ADF: No significant differences were found for ADF values across laboratories (ANOVA: F = 1.39, p = 0.262). While mean scores of NIR1 were slightly higher, the variation within groups was comparable, revealing comparable performance from laboratory to laboratory and with even distribution across laboratories. Validation Results: The validation showed great prediction performance for NIR2 (R^2 = 0.91, RMSE = 6.79, RPD = 3.58) and NIR3 (R^2 = 0.93, RMSE = 6.20, RPD = 3.91) demonstrating the highest accuracy and high stability. The models presented less of a bias which proved appropriate for quantitative applications. In contrast, NIR1 exhibited only limited predictive power (R^2 = 0.47, RMSE = 16.8, RPD = 1.45) and a strong positive bias, limiting its application to the rough screening for all features.

GLOBAL NIR LABORATORY NETWORKS

Modern global NIR systems operate as multi tiered analytical infrastructures integrating central, regional, and local facilities into a unified data ecosystem. At the core of such systems are central “master” laboratories, which maintain reference instruments and serve as custodians of calibration integrity and spectral standardization. These laboratories generate and validate the master calibration models used for routine forage and feed analysis worldwide. Surrounding this center, regional NIR laboratories function as intermediate nodes, applying and adapting the master calibrations to the specific feed materials and environmental conditions of distinct geographic regions.

At the operational level, on farm and portable NIR instruments extend analytical capacity directly to the production environment. These field deployable spectrometers collect real time spectra of silages, hay, or total mixed rations, and their data are synchronized with the central network through cloud based calibration databases. Within these databases, global spectral libraries are continuously updated, incorporating new sample data and verified reference analyses.

The integration of automated calibration updates and bias correction algorithms ensures that instruments remain harmonized despite differences in hardware, optical configuration, or climate induced sample variability. It is also a common approach that the infrastructure—from grinding to spectral acquisition—and the sample preparation methods are fully standardized, meaning that the local (satellite) laboratories follow a uniform protocol defined and prescribed by the central laboratory. Through these mechanisms, networks such as INGOT, EVEREST, or the International Forage Evaluation Consortium provide globally standardized calibrations, enabling reliable cross laboratory comparability. In the field of forage analysis, global networks are being established by the companies below:

Eurofins Agro network (BLGG 2013): reference forage laboratory is in Wageningen (The Netherlands). Eurofins Agro is presented in Belgium, Bulgaria, Chile, Canada, China, Colombia, Denmark, Ecuador, Finland, France, Germany, Hungary, Mexico, Norway, Portugal, Romania, Tudkey, UK, Vietnam.

Dairy One network: reference laboratory is in Ithaca, New York, US. Satellite labs are in 7 different locations in the US. DL presented in Argentina, Brasil, Chile, Canada, Republic of South Africa, Marocco, Australia, New Zealand, Spain, The Netherlands, Belgium, Luxemburg, France, Italy, Hungary, Poland, Ukraine, Belarus, Turkey, China, Russia

Dairyland Laboratories Inc. network: reference laboratory is in Arcadia, Wi, US. Satellite labs are in 7 different locations in the US. DL presented in Argentina, Brasil, Chile, Canada, Republic of South Africa, Marocco, Australia, New Zealand, Spain, The Netherlands, Belgium, Luxemburg, France, Italy, Hungary, Poland, Ukraine, Belarus, Turkey, China, Russia.

Rock River Laboratory Inc. network: reference laboratory is in Watertown, WI, USA. 5 satellite Rock River Laboratories in the US, 4 satellite laboratories supported by he RRL in the US. International laboratories: 3 laboratories in Brasil, 2 laboratories in Mexico, and RRL are presented in Australia, Ukraine, Chile, Colombia, Germany, Spain, Turkey.

CVAS (Cumberland Valley Laboratories) network: reference laboratory is in Waynesboro, PA, US. Satellite labs are in 6 different locations in the US. There are 3 CVAS supported labs in Canada. CVAS presented in Argentina, Uruguay, Mexico, Canada, Republic of South Africa, Australia, Japan, Spain, the UK, Italy, Hungary, Croatia, Poland, Ukraine, Belarus, Thailand, Chine, Russia.

At the same time, they retain the capacity for localized fine tuning, allowing each participating laboratory or farm to adjust models to regional feed characteristics while maintaining traceability and analytical coherence across the global NIRS community. This architecture exemplifies the convergence of centralized reference control and decentralized operational flexibility, a defining feature of next generation agricultural spectroscopy.

Global NIR laboratory networks provide a rapid catch-up opportunity for countries where laboratory systems for forage analysis are not yet modernized or the opportunities for NIR-calibration development is limited. In such countries, the availability of fast, comprehensive laboratory results—covering a wide range of new analytical parameters—may have a significant impact on the efficiency of forage crop production and harvesting, as well as on the productivity of

milk and beef production systems. Beyond improving production performance, these advancements also may contribute to better rumen- and animal health and may accelerate the overall development of the livestock sector.

These global NIR-networks, in addition to analyzing forage samples, *are expanding the range of sample types to provide broader services*. Thus, besides forage samples, they typically also maintain calibration databases for *water, soil, milk and manure samples*. Generally the farm technology support is also involved in the services.

Global NIR Laboratory Networks and Local Calibration Development in Forage Analysis: Benefits and Risks

The effectiveness of NIR systems is fundamentally linked to calibration strategy. Two primary approaches can be distinguished: global (centralized) calibration models and local (site-specific) calibration models.

Global calibration systems rely on large, multi-regional spectral databases, often maintained by international laboratory networks or instrument providers. These databases incorporate extensive variability in feed composition, environmental conditions, and processing methods, resulting in robust and broadly applicable models. Studies such as Hossain *et al.* (2024) have demonstrated that multi-regional datasets significantly enhance predictive accuracy by capturing diverse sources of variation. Furthermore, continuous updates and algorithm refinement—highlighted by Maduro Dias *et al.* (2024)—improve model stability and reduce systematic bias. However, global calibration systems may reduce local calibration autonomy, as laboratories become dependent on externally maintained models. This can limit adaptability to region-specific conditions, such as novel crop varieties or unique silage practices, and may introduce long-term reliance on proprietary systems.

Local calibration models are developed using region-specific reference samples, allowing for greater contextual accuracy and flexibility. Research by Chen *et al.* (2013) indicates that locally calibrated models can outperform global ones when applied to narrowly defined sample sets. Additionally, local systems provide independence from centralized infrastructure, which is particularly advantageous in regions with limited technical resources. Nevertheless, local calibration approaches require substantial investment in sample collection, laboratory analysis, and continuous model validation. Limited dataset diversity may also lead to overfitting, reducing model generalizability (Cozzolino and Morón, 2014).

Towards a Hybrid Calibration System: given the strengths and limitations of both approaches, modern forage analysis increasingly adopts a hybrid calibration strategy, combining global data resources with local model refinement. In this framework: the global databases provide a robust baseline calibration, while local datasets are used for fine-tuning and validation and instrument-specific standardization techniques ensure compatibility across platforms. Emerging developments in cloud-based data sharing and machine learning further support this integration, enabling continuous model improvement while preserving local adaptability.

The performance of NIR spectroscopy in forage analysis is determined by the combined effects of instrument technology and calibration strategy. Global laboratory networks leverage FT-NIR, monochromator, and diode array systems in a complementary manner to achieve both analytical precision and operational efficiency. At the same time, the balance between global standardization and local adaptability remains a critical consideration.

A sustainable and effective NIR system will therefore depend on maintaining transparency, supporting calibration flexibility, and fostering collaboration between instrument manufacturers, laboratories, and end users. Over the past decades, global collaborative initiatives, centralized calibration centers and large spectral databases have elevated NIRS to a key technology in modern forage laboratories and on farm decision making.

The recent expansion of global near-infrared spectroscopy (NIR) laboratory networks has dramatically reshaped how analytical calibration models are developed, shared, and maintained within the feed and forage industry. Large international organizations—such as the global NIRS consortiums established by commercial instrument providers and public–private research alliances—now maintain centralized databases containing tens of thousands of spectra from diverse feed and forage materials. This approach has improved model robustness and promoted cross-instrument standardization, particularly for common nutritional parameters such as dry matter, crude protein, neutral detergent fibre (NDF), and acid detergent fibre (ADF).

By pooling global data, these NIR networks reduce the need for each laboratory to collect extensive local reference samples before achieving acceptable model performance. For instance, Hossain et al. (2024) demonstrated that multi-regional calibration datasets significantly increased prediction accuracy in livestock diet quality assessment by capturing variability in growing conditions, cultivar, and processing. Similarly, the review by Maduro Dias, Nunes, and Borba (2024) emphasized that global collaboration allows continuous updates of algorithms and model parameters across heterogeneous environments, thereby minimizing systematic bias linked to specific optical setups or geographic differences.

Beyond accuracy, the global-network approach promotes inter-laboratory harmonization. Calibration transfer between instruments from the same manufacturer can be readily achieved through standardized reference spectra and mathematical treatments such as piecewise direct standardization (PDS) or external parameter orthogonalization (EPO). Consequently, NIR operators can achieve consistent performance across multiple sites within a corporate or research consortium (Fodor et al., 2024). Such harmonization is essential for international feed trading systems, where analytical uniformity directly impacts nutritional labeling and regulatory compliance.

However, the same integration that offers technical advantages may also introduce structural weaknesses and dependency. The most evident risk is the erosion of calibration sovereignty—that is, the ability of a local laboratory or farm to adapt models to its own regional feed materials and climatic conditions. When calibration databases and prediction equations are proprietary assets held by multinational instrument providers or centralized consortia, local users often lose the capacity to independently refine or recalibrate models. This dependency may hinder adaptability to new crop varieties, novel silage types, or changing preservation technologies, and it can drive long-term vendor lock-in.

Local calibration development, in contrast, provides flexibility and contextual relevance. Locally built models reflect the specific feedstuff composition, soil mineral profile, and processing practices characteristic of a region. Studies such as Chen et al. (2013) showed that site-specific models often outperform global ones when applied to new or regionally limited feed materials, because local reference samples better capture subtle spectral–chemical relationships

Furthermore, in developing regions where infrastructure and internet access may be limited, localized calibration systems offer greater autonomy and resilience. They avoid

dependence on external updates or cloud-based databases, which can be costly or unavailable.

Nevertheless, local calibration strategies also entail substantial drawbacks. They demand large, well-curated reference datasets, continuous validation, and frequent model re-training. Maintaining such datasets can be financially and logistically challenging for small laboratories or farm-scale facilities. Limited sample diversity also risks overfitting, where the calibration performs well only for local materials but fails for imported or cross-seasonal samples. As highlighted by the comprehensive review of Cozzolino and Morón (2014), model transfer and robustness remain primary barriers for the widespread decentralization of NIR forage analysis.

From an operational perspective, NIR networks integrate automated quality control, version control of calibration files, and instrument diagnostics, enhancing analytical traceability and reproducibility.

Yet this centralized model may compromise transparency: users often cannot view the internal structure of multivariate models, making the analytical process a "black box." Such opacity can reduce scientific confidence when NIR results are used in regulatory or litigation contexts. Data-sharing agreements also raise questions about intellectual property and sample privacy, especially when agricultural data include sensitive production information.

A sustainable calibration ecosystem likely requires a hybrid approach combining global knowledge bases with local adaptability. Open-access initiatives and federated calibration frameworks are emerging to allow laboratories to contribute anonymized spectral data while preserving local customization capacity. Decentralized network architectures, leveraging cloud and AI-assisted learning, could help maintain calibration quality without surrendering ownership. As Maduro Dias et al. (2024) suggested, continuous collaboration between instrument manufacturers, research institutions, and end users is vital to prevent dependency asymmetry and ensure that both global and local knowledge are exploited for scientific and economic benefit.

Ultimately, the choice between global NIR network participation and local calibration development is not binary. Instead, the optimal strategy balances global standardization, which ensures comparability and efficiency, with local calibration autonomy, which ensures contextual accuracy and resilience. For the near future, maintaining transparency in model governance and fostering open calibration platforms appear essential to prevent concentration of analytical control in a few global entities while still harnessing the collaborative strength of large-scale spectral data infrastructures.

A summary Table 10 highlights the key differences between the two systems in terms of their advantages and disadvantages, summary Table 11 presents the differences between the two systems based on various criteria.

Near infrared spectroscopy has established itself as a leading technique in forage analysis. When supported by high quality calibration databases, modern preprocessing and robust chemometric models, NIRS achieves accuracy close to classical reference methods for most nutrients. Its rapid, non destructive measurement capability makes it indispensable for real time feeding management and quality control.

Tab. 10: The key differences between the global NIR databases and local NIR databases in terms of their advantages and disadvantages (compiled from literature on NIR calibration systems and database structures – Shenk and Westerhaus, 1991; Williams and Norris, 2001; Roberts *et al*., 2004; Fearn, 2001; Feudale *et al.*, 2002; FAO, 2011; ICAR, 2012; ISO, 2016)

Aspect	Global NIR Databases	Local NIR Databases
Advantages	Large, diverse datasets improve model robustness and predictive accuracy	Models are tailored to local feed composition and environmental conditions
	Enable cross-instrument standardization and calibration transfer	Greater flexibility and adaptability to new crops, silage types, and practices
	Reduce need for extensive local reference sample collection	Better representation of region-specific spectral–chemical relationships
	Continuous updates through global col-laboration improve model performance	Increased autonomy, no dependence on external providers or cloud systems
	Promote inter-laboratory harmonization and comparability	More resilient in regions with limited infrastruc-ture or internet access
	Support large-scale monitoring and standardized feed evaluation	Greater transparency and control over calibration models
	They are more competitive and can re-spond more quickly to market dynamics.	
Disadvantages	Reduced calibration sovereignty for local users	Require large, well-curated local reference da-tasets
	Dependency on centralized systems and instrument providers (vendor lock-in)	High cost and effort for maintenance, validation, and recalibration
	Limited flexibility to adapt to local varia-bility	Limited sample diversity may lead to overfitting
	Models may not fully capture region-specific characteristics	Poor transferability to non-local or imported feed materials
	Lack of transparency ("black box" mod-els)	Slower model development compared to global systems
	Data privacy and intellectual property concerns	Lack of standardization across laboratories
	Potential bias from centralized optical setups or geographic differences	No automatic updates or shared improvements
		Less compatitive, can not respond quickly to market dynamics.

Tab. 11: Differences between the global NIR databases and local NIR databases based on various criteria (compiled from recent literature and international guidelines on NIR calibration systems Shenk and Westerhaus, 1991; Hossain *et al*., 2024; Williams and Norris, 2001; Chen *et al*., 2013; Roberts *et al*., 2004; Fearn, 2001; Feudale *et al*., 2002; Fodor *et al*., 2024; Cozzolino and Morón, 2014; FAO, 2011; ICAR, 2012; ISO, 2016).

Criterion	Global NIR Databases	Local NIR Databases
Data scope	Large, multi-regional spectral datasets improve robustness and variability coverage (Hossain *et al*., 2024)	Limited to region-specific samples reflecting local feed composition and conditions (Chen *et al*., 2013)
Predictive performance	High accuracy for common parameters due to diverse calibration sets (Maduro Dias *et al*., 2024)	Often superior for niche or region-specific materials due to contextual relevance (Chen *et al*., 2013)
Calibration effort	Reduced need for local reference sample collection	Requires extensive local sampling, wet chem analyses, validation, and model development. Limited opportunities
Standardization	Enables cross-instrument calibration transfer (PDS, EPO) and inter-laboratory harmonization (Fodor *et al*., 2024)	Limited standardization; models often instrument- and site-specific
Adaptability	Less flexible to local variability, new cultivars, or novel processing conditions	Highly adaptable to local agricultural practices and environmental conditions
Model maintenance	Continuously updated via centralized networks and collaborations (Maduro Dias *et al*., 2024)	Requires ongoing local recalibration and maintenance
Infrastructure dependency	Strong reliance on centralized databases, cloud systems, and providers	Independent from external systems; suitable for low-infrastructure environments
Transparency	Often limited ("black box" models), restricted access to model structure	Full transparency and control over model development and parameters
Economic aspects	Efficient for large-scale operations; involve subscription or licensing costs	Higher initial and maintenance costs for small laboratories
Risk factors	Vendor lock-in, reduced calibration sovereignty, data privacy concerns	Overfitting risk, limited transferability, smaller datasets (Cozzolino and Morón, 2014)
Operational impact	Facilitates large-scale monitoring and standardized feed evaluation	Best suited for targeted, site-specific decision support
Service development, competitiveness	They possess stronger infrastructural, human, and financial resource capacity to support the development of new sample types and the expansion of measurable parameters. As a result, they are more competitive and can respond more quickly to market dynamics.	They have more limited opportunities for development and are less competitive.

PORTABLE NIR SPECTROMETERS

Recent technological developments have introduced handheld near-infrared (NIR) spectroscopy devices. Interest in compact, portable spectrometers is steadily increasing (Crocombe, 2018; Pasquini, 2018; Beć *et al*., 2020a,b; Digman *et al*, 2022; Feng *et al*, 2022¸ Rego *et al*., 2020; Rukundo *et al*, 2021; Yan *et al*, 2023). These devices have demonstrated good performance when applied to pre-treated samples, such as dried and ground forage (Cozzolino, 2014). Moreover, portable NIR spectrometers are increasingly integrated into forage harvesting machinery and have been adapted for use in other agricultural equipment, such as liquid manure applicators (Griffiths *et al*., 2007; Sørensen *et al*., 2021).

Understanding and effectively managing the variability in forage nutritional composition is essential for improving dairy farm performance and supporting animal health. The nutritive value of forages—such as alfalfa–grass haylage and corn silage—can vary substantially, with direct implications for milk production efficiency and environmental sustainability (Van Soest, 1994). By enabling frequent, data-driven adjustments of animal diets based on real-time forage analysis, handheld NIR devices can improve feed efficiency, reduce environmental burdens, and enhance the economic and ecological sustainability of dairy operations (Batten, 1998; Roberts *et al*., 2004; Decruyenaere *et al*., 2009).

Existing studies highlight both the capabilities and limitations of handheld NIRS systems in real-world applications. For example, Beć *et al*. (2020a) report on predictive model development for different forage types and emphasize the critical role of scanning methodology in determining prediction accuracy. Similarly, Crocombe (2018) evaluates multiple handheld NIR devices, focusing on their precision and accuracy in on-farm applications, particularly in comparison with conventional moisture measurement methods and in relation to the robustness of calibration models for nutritive parameters.

Type of portable NIR spectrometers

NIR devices used in forage analysis can be broadly categorized into three groups based on their functionality, calibration approach, and application context.

"Research-grade" instruments, such as those developed by NeoSpectra, TrinamiX, and AgroCares, are characterized by their strong validation in scientific studies and their ability to predict a wide range of forage quality parameters. These include not only primary chemical constituents—such as crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) (Norris *et al*., 1976; Coleman *et al*., 1995; Andrés *et al*., 2005, Cozzolino and Morón, 2004; Decruyenaere *et al*., 2009; Roberts *et al*., 2004 Givens *et al*., 1997)—but also more complex biological

traits, including neutral detergent fiber digestibility (NDFD) (Decruyenaere *et al*., 2009; Coleman *et al*., 1995; Andrés *et al*., 2005) and *in vitro* total digestibility (IVTD) (Decruyenaere *et al*., 2009; Cozzolino and Morón, 2004; Andrés *et al*., 2005; Roberts *et al*., 2004).

A key advantage of these systems lies in their flexibility, as they allow the development and implementation of custom calibration models. Consequently, they typically provide superior predictive performance. However, their accuracy is highly dependent on the quality and robustness of the applied calibrations (Martens and Næs, 1989; Shenk and Westerhaus, 1991; Williams and Norris, 2001; Decruyenaere *et al*., 2009).

"Plug-and-play" agricultural devices, such as X-NIR and AgriNIR, are designed for immediate use under field conditions. These systems come with pre-installed calibrations for commonly required parameters, including dry matter (DM), crude protein (CP), starch, NDF, ADF, fat, and ash. Their primary advantage is ease of use and rapid deployment without the need for advanced calibration development. However, this convenience is often associated with limitations in the range of measurable parameters, particularly with respect to more complex digestibility-related traits such as NDFD and IVTD, which are less frequently available or less reliably predicted.

Industrial or on-line NIR systems are the third category, exemplified by Polispec NIR and HarvestLab 3000. Polispec NIR instruments are designed for continuous, real-time monitoring of forage materials during processing or handling (e.g., in mixer wagons or along conveyor systems) and represent typical industrial process-NIR applications (Burns and Ciurczak, 2007; Pasquini, 2018). They can cover a broad spectrum of parameters depending on the applied calibration models and are particularly valuable for dynamic process control. Their main strength lies in enabling real-time decision-making and continuous quality monitoring in operational environments (Workman and Weyer, 2012).

HarvestLab™ represents an on-machine near-infrared (NIR) sensing system designed for integration into agricultural equipment, such as forage harvesters and slurry tankers. Previous research has shown that HarvestLab™ systems are capable of estimating the quality of mixed, undried forage, although systematic errors may occur and require calibration adjustments (Griffiths *et al*., 2006; Sørensen *et al*., 2021). Its primary advantage lies in its ability to perform continuous, real-time measurements during field operations, thereby providing immediate feedback that supports precision agriculture practices, including variable rate application (Müller *et al*., 2019; Sørensen *et al*., 2021).

The system is well established for the estimation of key compositional parameters, such as dry matter (DM), crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), and starch (Decruyenaere *et al*., 2009; Roberts *et al*., 2004). In addition, it can be used to predict more complex variables, such as neutral detergent fiber digestibility (NDFD) and *in vitro* digestibility (IVTD), although the reliability of these predictions is highly dependent on the applied calibration models (Coleman *et al*., 1995; Andrés *et al*., 2005). The success of such applications relies heavily on the development of robust and reliable calibration models (Martens and Næs, 1989; Shenk and Westerhaus, 1991).

Despite its strengths, HarvestLab™ has certain limitations. Its performance is strongly influenced by crop type and calibration quality, and it offers less flexibility compared to research-grade handheld NIR instruments, which allow for more extensive customization of predictive models (Crocombe, 2018; Beć *et al*., 2020b). With the inclusion of HarvestLab™, the category of on-line NIR systems can be more clearly defined. Systems such as Polispec NIR function as flexible, industrial-grade solutions suitable for a wide range of environments and applications, whereas HarvestLab™ is specifically designed for field integration and optimized for use during forage harvesting operations. This distinction highlights the different application domains: Polispec systems are more adaptable for multi-environment, process-oriented monitoring, while HarvestLab™ is tailored to real-time data acquisition directly during harvesting (Pasquini, 2018; Workman and Weyer, 2012).

Comparison of handheld and on-line NIR devices: parameters

Across all device categories, most handheld NIR instruments are capable of reliably measuring core compositional parameters, including DM, CP, NDF, and ADF (Table 12). The primary differences between systems arise in their ability to accurately predict digestibility-related variables, the quality and adaptability of their calibration models, and their intended operational context (handheld versus on-line applications). In general, chemical constituents are more robustly and consistently predicted by NIR spectroscopy, whereas biological parameters—such as digestibility—are more sensitive to variability and typically exhibit lower predictive accuracy.

Conclusion: NeoSpectra, TrinamiX, and AgroCares are most appropriate for research-based and high-level modeling projects; X-NIR and AgriNIR offer convenient solutions for regular on-farm decision-making; while Polispec and HarvestLab™ are most suitable for continuous and real-time observation. Within this latter range, HarvestLab™ serves as particularly advantageous for harvesting and Polispec is more suitable for monitoring during the processing of feeds and feeding operations.

The continuous progress in NIRS technology allows for miniaturized, portable instruments to reach the predictive efficiencies of classic benchtop systems. As adoption expands, attention will need to be paid to the effect of device type and scanning approach on reliability and accuracy of forage quality prediction.

Forage evaluation with handheld NIRS technology is aimed at a relatively straightforward approach, where samples can be analyzed directly without prior preparation. This is done through calibration models customized for wet, unprocessed forage (Cozzolino, 2014; Crocombe, 2018). Nevertheless, some issues still exist, namely moisture absorption bands and sample heterogeneity (Pasquini, 2018; Beć *et al*., 2020). Other pragmatic limitations are maintaining appropriate signal-to-noise ratio and a dependable electrical supply on portable systems, and ensuring operational dependability in different and sometimes hostile environmental scenarios (Crocombe, 2018; Beć *et al*., 2020).

Comparison between handheld and on-line NIR systems: the type of forage

Performance characteristics of handheld and on-line near-infrared (NIR) spectroscopy systems are determined by the characteristics of the physical structure and compositional variability of the forage matrix.

Performance variation is observed among forages (Table 13). Corn silage is generally predicted with high accuracy in all categories of devices due to its relatively homogeneous composition. Whereas grass silage and haylage display a more diverse behaviour, requiring more rigorous calibration models. Since TMR is highly heterogeneous and best suited to on-line measurement systems, it should be used accordingly, whereas the more accurate predictions made on dry hay are more reliable, regardless of the device type.

Tab. 12: Comparison of handheld and on-line NIR devices for parameters (own compilation based on published studies and manufacturer specifications – Alomar *et al*, 2021; Cozzolino, 2014; Rinnan e al, 2009; De Boever, Goyvaerts and Van Waes, 2018; Pasquini, 2018; Crocombe, 2018; Rego *et al*., 2020; Rukundo *et al*., 2021; Digman *et al*., 2022; Feng *et al*., 2023; Fodor *et al*., 2024; John Deere, 2024; TrinamiX, 2024; AgroCares, 2024; Dinamica Generale, 2024; PoliSPEC, 2024)

Parameter / Device	NeoSpectra	TrinamiX	AgroCares Scanner	X-NIR	AgriNIR	Polispec NIR	HarvestLab™
Dry Matter	✓	✓	✓	✓	✓	✓	✓
Crude Protein	✓	✓	✓	✓	✓	✓	✓
NDF / aNDF	✓	✓	✓	✓	✓	✓	✓
ADF	✓	✓	✓	✓	✓	✓	✓
ADL	✓	✓	✓	✓ (limited)	✓	✓	(limited)
NDF Digestibility	✓	✓	✓	(limited)	(limited)	✓	✓ (model-dependent)
DM or OM Digestibility	✓	✓	✓	(limited)	(limited)	✓	✓ (model-dependent)
Starch	✓	✓	✓	✓	✓	✓	✓
Crude Fat	(possible)	(possible)	✓	✓	✓	✓	✓
Ash	✓	✓	✓	✓	✓	✓	✓
Energy values (NEL, ME)	(derived)	(derived)	(derived)	✓	✓	✓	✓
Real-time / on-line meas.	×	×	×	×	×	✓	✓

Tab. 13: Comparison of handheld and on-line NIR devices by forage type (own compilation based on published validation studies and manufacturer documentation of Alomar *et al*., 2021; Rego *et al*., 2020; Rukundo *et al*., 2021; Digman *et al*., 2022; Feng *et al*., 2023; John Deere, 2026; TrinamiX, 2025; AgroCares, 2026; Dinamica Generale, 2026; Polispec, 2026).

Parameter / Device	NeoSpectra	TrinamiX	AgroCares	X-NIR	AgriNIR	Polispec NIR	HarvestLab™
Grass silage / haylage	✓✓ (high validation)	✓✓	✓✓	✓	✓	✓✓	✓✓
Corn silage	✓✓ (well validated)	✓✓	✓✓	✓	✓	✓✓	✓✓✓
Fresh forage (green chop)	✓ (moisture sensitive)	✓	✓	✓	✓	✓✓	✓✓✓
Dry hay	✓✓✓	✓✓✓	✓✓✓	✓✓	✓✓	✓✓	✓
Total Mixed Ration (TMR)	✓ (heterogeneity challenge)	✓	✓	✓✓	✓✓	✓✓✓	✓✓
Forage mixtures (multi-species)	✓✓ (with calibration)	✓✓	✓✓	✓	✓	✓✓✓	✓ (model-dependent)
Processed / flowing forage (on-line)	×	×	×	×	×	✓✓✓	✓✓✓

Notes: ✓ acceptable , ✓✓ good performance/reliable applicability, ✓✓✓ excellent performance / highly suitable for the given task.

The ratings (Table 11) are derived from the joint analysis of data which involves: predictive accuracy (R^2, RMSE derived from literature data), sample inclusiveness, moisture effect robustness, measurement conditions (static and dynamic environment), practical application (on-line as an example). Availability of the parameters or extent their performance are determined by literature-derived calibration capabilities and specifications of the manufacturer and are influenced by the quality of calibration, the kind of sample and the conditions concerning measurements.

Sample heterogeneity is one of the leading determinants of predictive performance. NeoSpectra, TrinamiX, AgroCares and other well-known research-grade handheld devices exhibit robust performance over a wide range of forage types, if well-developed calibration models are employed to calibrate it. They are especially robust for relatively homogeneous materials such as dried hay, but they also work well for heterogeneous matrices such as silages and mixtures, provided that calibration strategies are well developed. Farm-oriented "plug-and-play" systems (e.g., X-NIR and AgriNIR) are more efficient at generalizing to standard applications across the forage range to a low scale, but provide limited flexibility to more variable or mixed samples.

Moisture content is another critical parameter governing NIR performance. Fresh and wet forages, especially those used in green chop and silage, can introduce highly damaging spectral noise with the formation of strong water absorption bands, and their high frequency and size can also affect the accuracy of predictions especially as it pertains to more involved or biologically inferred parameters (Pasquini, 2018; Cozzolino, 2014). Research-grade instruments tackle this shortcoming through advanced, moisture-aware calibration models (Martens and Næs, 1989; Shenk and Westerhaus, 1991), while platforms such as Polispec NIR and HarvestLab 3000 adapt to this challenge by making use of continuous data acquisition and signal averaging, increasing robustness under high-moisture conditions (Sørensen *et al*., 2021).

Environmental aspect of measurement also has a major impact. Compared with handheld devices for real-time applications in forage collection, total mixed ration (TMR) preparation and conveyor based processing, on-line NIR systems surpass the capabilities of handheld systems (Pasquini, 2018). Their capacity to perform continuous monitoring increases the representativeness of measurement compared to handheld devices limited by point-based sampling particularly in heterogeneous materials (Crocombe, 2018; Beć *et al*., 2020b; Sørensen *et al*., 2021).

In general, the overall performance of NIR systems is dominated by their inability to estimate the core composition parameters for key forage types when subjected to sample heterogeneity, moisture, and measurement conditions. Research-grade handheld devices are the ideal solution for a wide range of forage types, plug-and-play systems for routine on-farm use, and on-line systems like Polispec, HarvestLab™ offer a clear advantage for the handling of heterogeneous materials, for continuous process processing and for real-time decision support. The choice of a particular NIR apparatus for forage analysis does not depend purely on the measurable variables, but to a great extent on the type and physical state of the forage. Conventional handheld equipment is more flexible and convenient, while on-line tools offer much better quality in the treatment of variability and for measurement of true representatives against reality.

CONCLUSIONS

The use of near infrared (NIR) spectroscopy has been essential to modern forage analysis and it does so in a non-destructive and rapid manner and can predict a multiplicity of nutrition parameters. The use of the device to support decision-making within and outside of the lab allows enhanced feed management efficacy, as well as the development of precision livestock farming. In particular, the NIR approach is well established for the determination of major chemical constituents such as dry matter, crude protein and fiber fractions, with high spectral correlations.

Nonetheless, numerous limitations should be highlighted. Precision in NIR predictions hinges on the reliability, generalizability and representativeness of calibration models used in inference which need constant updating and validation. Additionally, biological-process parameters, including digestibility, are still more complex to evaluate due to their indirect connection to spectral data. Variability in sample types, moisture content and measurement conditions may also compromise prediction accuracy, particularly in fresh or unprocessed forages.

Furthermore, regional differences in feed assessment systems and reporting requirements may restrict the generalization of NIR-based information to the various nutritional models. This points to the potential need for calibration consistency or for flexible calibration schemes.

The investigation of improved calibration robustness in the presence of larger and more diverse datasets, advanced chemometric and machine learning techniques and the performance of portable and on-line devices under volatile field conditions should be the focus of future work. We need to also better characterize complex biological parameters, as well as harmonize NIR outputs with systems across multiple international feed evaluation systems. *A potentially broader approach could be to use an integrated set of parameters supported by the harmonized analytical methodology, a corresponding software framework, for instance, the CNCPS dynamic rumen model (and similar software in the form of AMTS or NDS) adopted across Europe. Such measures would help to overcome fragmentation on the continent, streamline investment-intensive developments through more focused, coordinated action, and strengthen global competitiveness for future developments*. Further technological and methodological development will reinforce the significance of NIR spectroscopy to sustainable harvest-, preservation and feeding technology of diverse forages.

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ESSENTIAL OILS AND FATTY ACIDS AS SILAGE ADDITIVES: A REVIEW

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Abstract

The search for natural alternatives to conventional silage additives has stimulated interest in plant-derived compounds capable of modulating microbial activity during ensiling. This review aimed to compile and critically analyse the available literature on the use of essential oils and fatty acids as additives in silage production. A systematic search of major scientific databases identified 28 studies evaluating essential oils applied to a variety of forage silages, whereas information on fatty acids was extremely limited. Most experiments tested crude plant essential oils, frequently with incomplete descriptions of their chemical composition, and only a few studies evaluated individual bioactive compounds. Overall, the responses to essential oil application varied considerably depending on the forage species, oil composition, application rate and storage conditions. Alfalfa silage appeared to respond more consistently to several essential oils, with reports of enhanced lactic acid production, reduced proteolysis and improved aerobic stability. In whole-plant corn silage, the most consistent effect across studies was the improvement of aerobic stability, whereas impacts on fermentation quality were variable and occasionally suggested compromised acidification. For other forage types, results were inconsistent and often limited by experimental design or lack of microbiological data. The literature on fatty acids as silage additives remains scarce, although available evidence suggests that these compounds may exert selective antimicrobial pressure capable of influencing fermentation processes. Overall, current knowledge remains insufficient to establish clear recommendations for the use of these compounds in silage systems. Future research should prioritise the evaluation of individual bioactive molecules rather than crude oils, incorporate molecular approaches to characterise microbial modulation, and include detailed assessments of the early fermentative phase to better understand the mechanisms through which these compounds influence silage fermentation and preservation.

Keywords: aerobic stability, essential oils, fatty acids, silage, additives

INTRODUCTION

Increasing emphasis has been placed on the adoption of natural compounds as replacements for synthetic chemical additives in various agribusiness systems. In the field of silage processing, the research on vegetable essential oils for use as additives is still quite modest and incipient. The available information is limited to some forage crops, and the reported effects still derive from crude oils extracted from plants and seeds. Potential antimicrobial activities on silage microbiota were summarized by Chen *et al.* (2023), suggesting that plant essential oils and their active components are more inhibitory against yeasts, fungi and pathogenic bacteria, including Gram-negative ones such as *Escherichia coli*, whereas far less inhibitory against lactic acid bacteria (LAB). These effects, found in in vitro studies, suggest that plant essential oils could be very promising as additives for silages, favouring conservation quality while inhibiting undesirable microorganisms. A meta-analysis by Susanto *et al.* (2023), however, did not corroborate these expectations in in silo studies. Unfortunately, the authors recognized that their conclusions were highly biased as all oils were considered as a single entity, regardless of their composition. The same approach was used for the evaluated silages across studies, whose diversity was not considered. Thus, the present review aimed to analyse the effects of each plant essential oil for each type of silage evaluated in the available literature so far.

Fatty acids, which are components of vegetable oils extracted from plant seeds, represent another group of natural compounds that have been even less investigated as silage additives. Although their ability to modulate microbial activity and fermentation is well established within the ruminal microbiota (Ibrahim *et al.*, 2021), their application for similar modulatory purposes in silages remains virtually unknown. Thus, the present review also summarized the available information regarding fatty acids as silage additives.

MATERIAL AND METHODS

A comprehensive search was performed using Scopus, Science Direct, Pubmed and Web of Science platforms through the keywords "essential oil" and "silage" (Figure 1). From the retrieved papers, 28 were related to essential oils being applied in silage processing (Table 1), published in 25 different journals of several impact factors. Most of the published papers were from Brazil (n = 8, 28.6%), Iran (n = 7, 25.0%), and Turkey (n = 6, 21.4%), and four papers were not published in English, being two written in Persian (Maghsoudloo *et al.*, 2017, Besharati *et al.*, 2020a), one in Turkish (Önenç *et al.*, 2017) and one in Chinese (Zhou *et al.*, 2024). Twenty-one different plant essential oils were evaluated, being cinnamon (n = 6, 21.4%) and oregano (n = 6, 21.4%) the most explored, followed by cumin (n = 4, 14.3%), lemongrass (n = 3, 10.7%), lemon seed (n = 3, 10.7%) and others. One paper focused on a commercial blend (Kung Jr. *et al.*, 2008) and only two papers evaluated individual essential oil compounds (Foskolos *et al.*, 2016, Sousa *et al.*, 2022). When not possible to work with essential oil compounds individually, recommendations have already been made in previous meta-analyses (Calsamiglia *et al.*, 2007, Cobellis *et al.*, 2016) to report a description as complete as possible of the composition of essential oils in future research. However, only seven studies presented a full description of components and their proportions in their essential oils, and other six at least described the main components. Eleven studies, however, did not report the composition of their essential oils.

Seventeen different silages were used in the trials, being alfalfa silage the most explored (n = 8, 28.6%), followed by whole-plant corn silage (n = 5, 17.9%), ryegrass silage (n = 2, 7.1%), field pea silage (n = 2, 7.1%) and other forages that were evaluated only once. It is noteworthy that to the moment only forage silages have been used in published studies, with the exception of Andrade *et al.* (2024) who ensiled a total mixed ration (TMR) containing whole plant sorghum and ground corn grains. Despite cereal plants such as whole plant corn, sorghum and barley also include grains when harvested for silage processing, no data for essential oils as additives on exclusive grain silages is available in peer reviewed papers.

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Tab. 1: Compiled studies with essential oils as silage additives

Reference	Journal	Origin[1]	Silage	Essential oil/Compound	Composition data
Kung Jr. *et al*. (2008)	J. Dairy Sci.	USA	Whole plant corn	Commercial Blend	Main components listed
Chaves *et al*. (2012)	J. Sci. Food Agric.	Australia	Whole plant barley	Cinnamon, Oregano, Sweet orange	Main components described
Önenç *et al*. (2015)	Asian Australas. J. Anim. Sci.	Turkey	Field pea	Oregano, Cinnamon	Fully described
Foskolos *et al*. (2016)	Canadian J. Anim. Sci.	Spain	Ryegrass	Thymol, Eugenol, Cinnamaldehyde, Capsaicin, Carvacrol	Essential oil compounds
Hodjatpanah-Montazeri *et al*. (2016)	Iranian J. Anim. Sci.	Iran	Whole plant corn	Cinnamon, Thyme, Oregano, Mint, Cumin	Not reported
Maghsoudloo *et al*. (2017)	Iranian J. Anim. Sci. Res.	Iran	Whole plant corn	Rosemary, Fennel, *Carum copticum*	Not reported
Önenç *et al*. (2017)	J. Anim. Prod.	Turkey	Field pea	Oregano, Cinnamon	Fully described
Turan and Önenç (2018)	Asian Australas. J. Anim. Sci.	Turkey	Alfalfa	Cumin	Fully described
Önenç and Turgud (2019)	J. Agric. Faculty of Ege University	Turkey	Alfalfa	Oregano, Cumin, Cinnamon	Fully described
Cantoia Jr. *et al*. (2020)	Anim. Feed Sci. Technol.	Brazil	Sugarcane	Lemongrass	Not reported
Besharati *et al*. (2020a)	Research on Anim. Prod.	Iran	Alfalfa	Flaxseed	Not reported
Besharati *et al*. (2020b)	Acta agriculturae Slovenica	Iran	Alfalfa	Flaxseed, Cinnamon, Lemon seed	Not reported
Besharati and Niazifar (2020)	J. Anim. Sci.	Iran	Alfalfa	Lemon seed	Not reported
Vaičiulienė *et al*. (2020)	J. Microbiol. Biotechnol.	Lithuania	Ryegrass, red clover, alfalfa	Oregano, Thyme	Main components described
Besharati *et al*. (2021a)	Semina Ciências Agrárias	Iran	Alfalfa	Lemon seed	Fully described
Besharati *et al*. (2021b)	South African J. Anim. Sci.	Iran	Alfalfa	Ricinoleic acid + cardol + cardanol	Main components described
Akinci and Önenç (2021)	J. Agric. Faculty of Ege University	Turkey	Vetch + oat	Cumin	Fully described
Li *et al*. (2022)	Frontiers	China	Paper mulberry	*Amomum villosum*	Not reported
Del Valle *et al*. (2022)	The Journal of Agricultural Science	Brazil	Whole plant shorgum	Lemongrass	-
Sousa *et al*. (2022)	Brazilian Anim. Sci.	Brazil	Sorghum + *Urochloa brizantha*	Rosemary, Thymol	Fully described
Garcia *et al*. (2022)	Anim. Prod. Sci.	Brazil	Mombasa grass	Lemongrass	-
Silva *et al*. (2024a)	Scientia Agricola	Brazil	Whole plant corn	Sweet orange	Main components described
Duru (2024)	J. Hell. Vet. Med. Soc.	Turkey	Vetch + wheat	*Hypericum perforatum*	Not reported
Andrade *et al*. (2024)	Agriculture	Brazil	Total Mixed Ration	Limonene-rich essential oil	Not reported
Silva *et al*. (2024b)	Scientia Agricola	Brazil	Whole plant corn	Blend from *Copaifera langsdorffii* and *Eucalyptus staigeriana*	Main components described
Aniceto *et al*. (2024)	Fermentation	Brazil	Whole plant sorghum	Rosemary, Tea tree, Citronella	Main components described
Zhou *et al*. (2024)	Acta Agrestia Sinica	China	*Stylosanthes guianensis*, Rice straw	*Neolamarckia cadamba* leaf	Not reported
Xu *et al*. (2023)	Letters in Applied Microbiology	China	*Broussonetia papyfera*, *Pennisetum sinese*	*Amomum villosum*	Not reported

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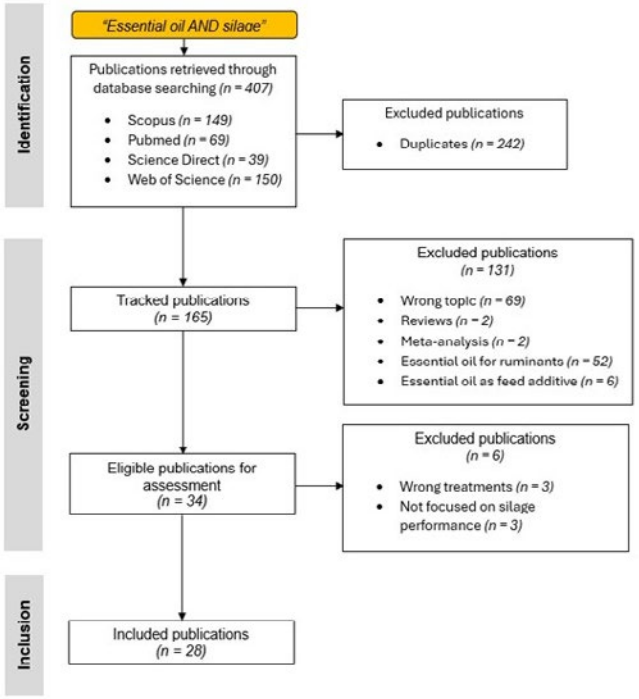


Fig. 1: Flowchart of selection of the studies included in the review of essential oils as additives for silages

RESULTS AND DISCUSSION

Alfalfa silage

Alfalfa has been the relatively most explored forage silage to evaluate essential oils as additives. As a legume forage, it presents some particularities that usually challenge the conservation process, such as the high protein content, which confers buffering capacity and makes the forage more susceptible to extensive proteolysis in the silo. Eight studies were published with seven different essential oils, ranging from 60 to 120 days of storage and application rates from 60 to 600 mg/kg fresh matter (FM). Chemical composition, fermentative profile, microbial counts and aerobic stability results are summarized in Supplementary Tables S1, S2, S3 and S4, respectively. Oregano essential oil was evaluated by Önenç and Turgud (2019) with very promising results, including increased LAB population and greater yield of lactic acid, which favored silage acidification, greater maintenance of crude protein (CP) content and lower dry matter (DM) losses. There was considerable inhibition of yeasts and fungi, although no information on aerobic stability was made available. Vaičiulienė *et al.* (2020) also reported lower silage pH with oregano and thyme essential oils, although only oregano essential oil was able to reduce yeast and fungi counts. With cumin essential oil, two studies reported similar findings, in addition to decreased enterobacteria counts and improved aerobic stability (Turan and Önenç, 2018, Önenç and Turgud, 2019). Lemonseed essential oil at application rates of 60 and 120 mg/kg FM (Besharati and Niazifar, 2020, Besharati *et al.*, 2020b, Besharati *et al.*, 2021a) also enhanced lactic acid production, promoted lower silage pH values, preserved CP content and improved aerobic stability. Cinnamon essential oil was tested at application rates of 60 (Besharati *et al.*, 2020b) and 600 mg/kg FM (Önenç and Turgud, 2019) on alfalfa silage stored for 60 days. Both application rates enhanced lactic acid production, increased LAB population, reduced pH and DM losses, preserved the CP content and reduced ammonia nitrogen (NH_3-N) concentration. Inhibition of yeasts and fungi counts and improved aerobic stability were also reported.

An unidentified oil containing ricinoleic acid, cardol and cardanol at 500 mg/kg FM preserved CP content in alfalfa silage stored for 90 days and enhanced aerobic stability, although without changes in silage pH (Besharati *et al.*, 2021b). No information on microbial counts and organic acids production was made available in that study. Flaxseed essential oil increased lactic acid production and reduced silage pH, managing to preserve the CP content and to enhance aerobic stability (Besharati *et al.*, 2020a, 2020b). No information on microbial modulation was made available. As flaxseed essential oil also contains fatty acids in its composition, mainly unsaturated ones such as oleic and α-linolenic acids, the reported effects may be confounded by multiple classes of bioactive molecules. Unsaturated fatty acids are known to promote microbial modulation on several strains of LAB (Kodicek and Worden, 1945, Talon *et al.*, 2000, Kankaanpää *et al.*, 2001, Endo *et al.*, 2006), either by favoring or inhibiting their growth and activity.

Therefore, essential oils of different compositions have been consistently yielded desirable outcomes when tested as additives for alfalfa silage, including favored LAB development, silage acidification, protein preservation and improved aerobic stability. Only cinnamon essential oil was also associated with decreased deamination. Future research would benefit from molecular techniques to better understand the microbial succession during the early fermentative stage of alfalfa silage treated with essential oils.

Whole-plant corn silage

Five studies were available with whole-plant corn silage, evaluating 11 different plant essential oils and one commercial blend, with application rates varying from 40 to 600 mg/kg FM, and storage lengths from 3 to 256 days. Each essential oil was evaluated in only one study, so it was not possible to compare findings among trials. Chemical composition and fermentative profile are presented in Supplementary Tables S5 and S6, respectively. Although the commercial blend at 40 and 80 mg/kg FM had no effect on whole-plant corn silage after 256 days of storage (Kung Jr. *et al.*, 2008), individual essential oils showed results within 60 days (Hodjatpanah-Montazeri *et al.*, 2016, Maghsoudloo *et al.*, 2017, Silva *et al.*, 2024a, 2024b). The CP content was kept higher than control silage when applying mint and oregano essential oils, which might suggest decreased proteolysis. However, mint, oregano and cumin essential oils favored deamination and hampered the silage acidification. Only thyme essential oil reduced NH_3-N content. Rosemary and fennel essential oils managed to consistently preserve the content of soluble carbohydrates relative to the control silage at application rates of 125 and 250 mg/kg FM up to 45 days of storage. Fluctuations in CP and NH_3-N contents were reported in the first days after ensiling, although without a consistent pattern (Maghsoudloo *et al.*, 2017). The reduced proteolysis and lower carbohydrate consumption reported may suggest a compromised fermentation activity by some oils. The increase in NH_3-N concentration may suggest little or no antimicrobial effect by essential oils on undesirable microorganisms responsible for deamination, which are more active in an insufficiently acidified silage environment. Sweet orange essential oil was the only one able to reduce DM losses in whole-plant corn silage, as reported by Silva *et al.* (2024a) with application rates at 400 and 600 mg/kg FM. Cinnamon, thyme, oregano, cumin, sweet orange, eucalyptus and copaiba essential oils markedly enhanced the aerobic stability of whole-plant corn silage (Hodjatpanah-Montazeri *et al.*, 2016, Silva *et al.*, 2024a, 2024b). This enhancement has been the most consistent effect

reported among trials (Supplementary Table S8). The antimicrobial effects of essential oils are usually more pronounced against yeasts and fungi than against bacteria (Chen *et al.*, 2023), although the available data on microbial counts do not consistently support this hypothesis (Supplementary Table S7). Silva *et al.* (2024a, 2024b) reported increased acetic acid production by sweet orange essential oil applied at 600 mg/kg FM and by blends of *Eucalyptus staigeriana* and *Copaifera langsdorffii* applied at 500 mg/kg FM, which may contribute to enhanced silage stability during aerobic exposure.

Overall, essential oils appear to consistently enhance the aerobic stability of whole-plant corn silage, although the conservation quality may not be guaranteed. A decrease in proteolysis would be of interest in forage silages, although a slower acidification rate would not, as it could result in greater DM losses. There is clearly a need for more information on essential oils as additives for whole-plant corn silage. Maghsoudloo *et al.* (2017) were the only ones to report findings across multiple storage lengths including the early fermentative stage, and this approach would allow better understanding of how essential oils affect fermentation dynamics in future research. The microbial modulation associated to essential oils may also be more relevant at the early fermentative stage of silages, and molecular tools such as metagenomics and metabolomics might help to better describe and understand the microbial succession.

Other silages

A variety of other forages have been explored to test the effect of essential oils on silage quality and aerobic stability. Fifteen studies were compiled including twelve different forages and four combined silages and testing twelve different essential oils and five essential oil compounds. The available results for chemical composition, fermentative profile, microbial counts and aerobic stability are presented in Supplementary Tables S9, S10, S11 and S12, respectively. Besides alfalfa, other legume forages such as field pea and red clover were evaluated. Field pea was used by Önenç *et al.* (2015, 2017) to test oregano and cinnamon essential oils. Oregano essential oil, contrary to observations in alfalfa silage, apparently compromised the fermentation of field pea silage at an application rate of 400 mg/kg FM. Higher NH_3-N contents and decreased lactic and acetic acids production were reported, resulting in higher silage pH and greater DM losses after 120 days of storage. Cinnamon essential oil inhibited deamination in field pea silage stored for 60 days and reduced DM losses, despite the decreased lactic and acetic acids production and higher silage pH. At 120 days, however, greater NH_3-N concentration was observed, and DM recovery was no longer improved. Both oregano and cinnamon essential oils increased yeast counts, an effect completely opposite to the commonly reported on alfalfa silage. Overall, the positive effects consistently reported by applying essential oils in alfalfa silage do not appear to occur to the same extent in field pea silage. Red clover silage was used by Vaičiulienė *et al.* (2020), in which thyme essential oil increased silage pH and both oregano and thyme essential oils reduced LAB counts after 96 days of storage, whereas no effect was observed on yeast and fungi counts. It remains to be clarified whether legume forages other than alfalfa may benefit from essential oil-based treatments.

In addition to whole-plant corn silage, other cereal forages were evaluated. Chaves *et al.* (2012) tested cinnamon leaf, oregano and sweet orange essential oils at 125, 250 and 400 mg/kg FM on whole plant barley. However, the reported findings were obtained after only one year of storage, which may have limited the detection of significant effects. Only a preserved CP content was reported with cinnamon and sweet orange essential oils, whereas no effects on microbiota or fermentation were observed for any of the tested oils. Del Valle *et al.* (2022) tested lemongrass essential oil at application rates between 1 and 4 mL/kg DM on a whole plant sorghum silage stored for 167 days. Increasing application rates linearly reduced butyric acid content and lactic-to-acetic acid ratio. However, NH_3-N concentration increased along with greater CP disappearance in treated silages. Despite reducing gas losses, lemongrass essential oil had no effetc on DM losses and aerobic stability of whole plant sorghum silage.

Grass silages were evaluated in five studies. Garcia *et al.* (2022) tested lemongrass essential oil at application rates up to 2 mL/kg FM on Mombasa grass silage stored for 60 days. Despite not improving DM recovery, desirable outcomes were obtained including reduced gas losses and NH_3-N concentration, increased degradation of acid detergent fibre, and a lower silage pH after aerobic exposure that might suggest an enhanced aerobic stability. Foskolos *et al.* (2016) worked with individual essential oil compounds on ryegrass silage stored for 35 days. Capsaicin had no effect, whereas eugenol, cinnamaldehyde, carvacrol and thymol reduced NH_3-N concentration, although none of them had any effect on CP content. Carvacrol and thymol, main components of oregano essential oil, reduced lactic acid production and increased silage pH, although carvacrol achieved these effects at a lower application rate. Only thymol reduced LAB counts. Vaičiulienė *et al.* (2020) reported contrasting results in which oregano and thyme essential oils reduced pH on ryegrass silage without significant effect on LAB counts. Unfortunately, no other fermentative parameter, such as acids production or NH_3-N concentration, was assessed in that study. These essential oils also reduced yeast and fungi counts on ryegrass silage, although aerobic stability was not assessed. Zhou *et al.* (2024), testing an essential oil from Neolamarkia cadamba leaf, reported increased lactic acid production, decreased pH and reduced NH_3-N content on Stylosanthes guianensis silage stored for 30 days, although LAB counts were reduced relative to the control silage. No information on DM losses and aerobic stability was made available. It is noteworthy in that trial that the essential oil was applied at 5 and 10 g/kg FM, considerably higher application rates than those usually reported in the literature. Xu *et al.* (2023) tested *Amomum villosum* essential oil on *Pennisetum sinese* silage and reported decreased NH_3-N content and increased production of lactic and acetic acids, although without changes in silage pH. Again, no information on DM losses and aerobic stability was made available.

Only one study evaluated sugarcane silage by applying lemongrass essential oil at 1, 2 and 3 mL/kg FM. Cantoia Jr. *et al.* (2020) reported inhibition of yeast and fungi counts, decreased ethanol production and enhanced aerobic stability. There was evidence, however, of compromised fermentation, including decreased lactic and acetic acids yield and lower consumption of soluble carbohydrates, greater NH_3-N concentration and increasing pH with increasing application rates of the essential oil. However, lower DM losses and CP degradation were also reported. Li *et al.* (2022) ensiled paper mulberry treated with Amomum villosum essential oil at 1 mL/kg FM. Despite consuming less soluble carbohydrates, the silage presented increased production of lactic and acetic acids, decreased NH_3-N concentration and lower pH, suggesting enhanced fermentation by desirable bacteria, although no microbial assessment was made available.

Four studies tested essential oils on combined silages. Sousa *et al.* (2022) used sorghum intercropped with Urochloa brizantha ensiled for 15, 30 and 45 days to evaluate Lippia

sidoides essential oil and nanoformulated thymol (62% and 100%) at 400 mg/kg FM. Treatment with pure thymol was able to enhance aerobic stability after 15 days of storage, whereas at 30 and 45 days only the 62% nanoformulated thymol managed to promote this effect. Thymol reduced LAB counts and increased silage pH. Despite being more effective at inhibiting clostridia, pure thymol increased DM, gas and effluent losses relative to the control silage. However, the 62% nanoformulated thymol reduced DM and effluent losses. L. sidoides essential oil was able to reduce DM, gas and effluent losses, but did not inhibit clostridial growth. Aerobic stability was enhanced by thymol at both nanoformulations, whereas L. sidoides was not able to promote this effect. Akinci and Önenç (2021) reported several findings for a vetch + oat combined silage treated with 200, 300 and 500 mg/kg FM of cumin essential oil. Despite the increased lactic acid production and LAB counts, maintenance of CP content and decreased deamination, no effect was observed on silage pH and DM losses. Yeast, fungi and enterobacteria counts were reduced, and the treated silage presented a lower pH after a period of aerobic exposure, which might suggest enhanced aerobic stability. Duru (2024) reported improved fermentation quality when applying Hypericum perforatum essential oil between 20 and 160 mg/kg FM on a vetch + wheat combined silage. Xu *et al.* (2023), testing A. villosum essential oil on Broussonetia papyfera intercropped with Pennisetum sinese ensiled for 3 and 30 days, reported increased CP content relative to the control silage, decreased NH_3-N content and increased production of lactic and acetic acids. No information on DM losses and aerobic stability was made available.

Essential oils as silage additives: general considerations

So far, alfalfa has been the only forage silage that appears to consistently benefit from several different essential oils as additives. Reported outcomes included enhanced lactic acid production and silage acidification, decreased storage losses and proteolysis, and consistent improvement of aerobic stability by inhibiting yeast and fungi presence. For whole-plant corn silage, the only consistent and desirable outcome among multiple essential oils was the enhancement of aerobic stability, whereas there was evidence of compromised fermentation along with extended activity of deaminating microorganisms. Unlike alfalfa silage, other legume silages did not respond well to essential oil application, resulting in compromised fermentation, lower acidification, and greater NH_3-N concentration. For whole plant cereal silages other than corn, the available findings were still inconclusive and probably limited by the extended storage lengths imposed by the trials. For grass silages, the decreased NH_3-N concentration was the most consistent outcome, although evidence of compromised fermentation has also been reported, and no data was available on aerobic stability.

Oregano, cinnamon, and lemonseed essential oils appear to favor LAB activity in alfalfa silage, whereas in other silages they either suppressed lactic acid production or had no effect. Both oregano and cinnamon essential oils either favored and inhibited deamination in silages, depending on type of forage and storage length. Curiously, greater CP contents in treated silages have been consistently reported even in scenarios of increased deamination. As greater CP contents have also been reported in silages with reduced DM losses, protein concentration may be ruled out as an explanation. The most consistent and predictable effect of different essential oils on silages has been the enhancement of aerobic stability, either through inhibition of yeast and fungi growth or by increasing acetic acid production. There was only one contrasting report of favored yeast growth in field pea silage treated with cinnamon and oregano essential oils (Önenç *et al.*, 2015). Testing pure essential oil compounds using appropriate methodologies is required to unveil molecule-specific effects on silage fermentation kinetics. This would contribute to making the development of essential oil–based additives more rational, strategic, and performance-oriented.

Fatty acids and seed oils as silage additives

Fatty acids represent another group of natural compounds that have been even less investigated as silage additives. Although their ability to modulate microbial activity and fermentation is well established within the ruminal microbiota (Ibrahim *et al.*, 2021), their application for similar modulatory purposes in silage remains virtually unknown. The available literature is even more limited than that on essential oils (Table 2). Unlike essential oils, vegetable oils extracted from plant seeds have a high proportion of unsaturated fatty acids, mainly oleic, linoleic and α/γ-linolenic, whose fermentative modulation capacity through microbial inhibition has been reported in ruminal bacteria and protozoa (Ibrahim *et al.*, 2021).

Tab. 2: Compilation of studies on vegetable oils applied as silage additives and their main results

Reference	Silage[1]	Oil	Fatty acids	Application rate[2]	Results[3]
Jackson and Brown (1967)	Fescue (180 d)	Peanut	Oleic, Linoleic	49 g/kg DM	↓ pH, = CP, = Lac, = Ace, ↑ ME
Jackson (1971)	Ryegrass (120 d)	Peanut	Oleic, Linoleic	40 g/kg DM	= pH, = CP, ↓ NH_3, = Lac, ↑ Ace, ↓ But, ↑ ME
Jackson and Anderson (1971)	Ryegrass (120 d)	Peanut	Oleic, Linoleic	40 g/kg DM	↓ SCFA, ↑ Ace, ↓ But
Besharati *et al.* (2020a)	Alfalfa (60 d)	Flaxseed	Linolenic, Linoleic	60 mg/kg DM	↓ pH, = NH_3, ↑ CP, = SC, ↓ NDF, ↑ Lac
		Flaxseed	Linolenic, Linoleic	120 mg/kg DM	↓ pH, ↓ NH_3, ↑ CP, ↑ SC, = NDF, ↑ Lac
Besharati *et al.* (2020b)	Alfalfa (60 d)	Flaxseed	Linolenic, Linoleic	*60 mg/kg FM*	↓ pH, = NH_3, ↑ CP, = SC, ↓ NDF, ↑ Lac, ↑ AS

[1]Forage + storage length in days.

[2]Application rates are presented in the units informed on each study.

[3]↑ = increase, ↓ = decrease, = – no effect, CP = crude protein, SC = soluble carbohydrates, SCFA = short chain fatty acids, NDF = neutral detergent insoluble fibre, Lac = lactic acid, Ace = acetic acid, But = butyric acid, AS = aerobic stability, ME = metabolizable energy.

However, there is a considerable lack of information about microbial modulation by fatty acids in silage. Jackson and Brown (1967) reported no effect of treatment with peanut oil containing oleic (67.9%) and linoleic (22.5%) acids on the fermentation of fescue silage. On the other hand, Jackson (1971) and Jackson and Anderson (1971) reported significant changes in the fermentation of ryegrass silage treated with peanut oil. Although the production of organic acids was reduced, the treated silages presented reduced DM losses, lower extent of proteolysis and reduced presence of mold compared to the control silage. The application rates applied in these studies were about 40 g/kg of forage DM. Besharati *et al.* (2020a, 2020b) treated alfalfa silage with flaxseed oil, which is rich in linolenic and linoleic acids. Despite the considerably lower application rates of 60 and 120 mg/kg DM, a significant improvement in fermentation was observed, such as increased lactic acid production, enhanced silage acidification, reduced extent of proteolysis, and a marked improvement in aerobic stability compared to the control silage. To our knowledge, these are the only studies currently available in the literature that have applied fatty acids via vegetable oils in ensiled forages.

Unsaturated fatty acids can inhibit both Gram-positive and Gram-negative bacteria (Ivan *et al*., 2013) through mechanisms related to changes in cell membrane permeability or the formation of a lipid film on the surface of the microorganism cell (Kodicek and Worden, 1945, Maia *et al*., 2007). Since microbial species have variable susceptibility to these molecules, a selective pressure may occur within the microbiota (Maia *et al*., 2007, 2010, Zhang *et al*., 2008, Ivan *et al*., 2013). As with essential oils, this selective pressure exerted by fatty acids may influence the classical acidification-based microbial control mechanism of silages, interfering with populations of LAB and undesirable microorganisms. Organic acid-based additives, for example, can improve nutrient conservation and aerobic stability of silages by exerting a non-selective antimicrobial effect on enterobacteria, yeasts, and LAB (Morais *et al*., 2017).

Kodicek and Worden (1945) reported that *Lactobacillus helveticus* was inhibited when cultivated in a medium containing linoleic and linolenic acids at 16 µg/mL. Kankaanpää *et al.* (2001) reported inhibition of strains of *Lacticaseibacillus rhamnosus*, *Lactobacillus delbrueckii* and *Lacticaseibacillus casei* when cultivated in a medium containing unsaturated fatty acids ranging from 18- to 22-carbon chain. Talon *et al.* (2000) also reported inhibition of five LAB species, including Lactiplantibacillus plantarum, with linoleic acid. Endo *et al.* (2006) observed inhibition of five LAB strains, including two from L. plantarum (JCM1149 and IFO15891) by α-linolenic, eicosapentaenoic (C20:5) and docosahexaenoic (C22:6) acids. However, L. helveticus was favored by saturated fatty acids, such as palmitic and stearic acids, and less inhibited by oleic acid in the work of Kodicek and Worden (1945). Endo *et al.* (2006) reported that all five LAB strains tested not only survived but also grew in medium containing oleic acid. Partanen *et al.* (2001) reported that L. delbrueckii incorporated oleic acid into its membranes, converting it to cyclopropane (C19) fatty acids. This process was also observed in *Lentilactobacillus buchneri* by Louesdon *et al.* (2014) and in *L. plantarum* strains by Wang *et al.* (2021). The addition of oleic acid to the culture medium may favor the growth of several LAB species (Johnsson *et al*., 1995), and the conversion to cyclopropane fatty acids in the membrane appears to grant the bacteria the ability to survive under adverse conditions, such as freezing (Wang *et al*., 2021) and low pH (Partanen *et al*., 2001). Regarding clostridia, however, oleic acid appears to inhibit spore germination, an effect also observed with linoleic acid (Foster and Wynne, 1948). Skřivanová *et al.* (2005) reported inhibition of clostridia by linoleic acid, although the effect was more pronounced with lauric acid.

Fatty acids are not very susceptible to degradation in the anaerobic environment of silages due to the highly reduced nature of their carbon chains. Jackson and Anderson (1971) observed no evidence of chain breakage or biohydrogenation of unsaturated fatty acids after 120 days of storage. In addition to suppressing undesirable microorganisms after the early fermentative stage, the intact presence of exogenous fatty acids in the silage may enhance microbial control after silo opening, delaying the development of yeasts and spoilage bacteria and enhancing aerobic stability.

CONCLUSION

The available literature indicates that essential oils and fatty acids hold potential as natural silage additives due to their capacity to modulate microbial activity, suppress undesirable microorganisms and, in many cases, improve aerobic stability. However, the current evidence remains inconsistent across forage types, compounds and experimental conditions, reflecting the early stage of development of this approach. A major limitation of the existing research is the predominant use of crude essential oils with highly variable and often poorly characterised composition, which hampers comparisons among studies and obscures the mechanisms responsible for the observed effects. Future research should therefore prioritise the evaluation of individual bioactive molecules, or well-defined combinations of compounds, in order to identify molecule-specific effects on fermentation kinetics and microbial populations. In addition, the adoption of modern molecular approaches, including metagenomic and metabolomic techniques, would allow a more detailed understanding of microbial succession and metabolic shifts occurring in treated silages. Particular attention should also be given to the early fermentative stage, when microbial dynamics and acidification processes are most intense, through experimental designs that include temporal sampling throughout fermentation. For fatty acids, the scarcity of studies and the frequent reliance on whole seed oils highlight the need for targeted investigations into specific fatty acids and their mechanisms of action in silage ecosystems. Collectively, advancing this field will depend on moving from empirical testing of crude plant extracts towards mechanistically oriented studies capable of linking specific molecules to microbial modulation and fermentation outcomes in silage systems.

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APPENDIX
SUPLEMENTARY TABLES

Tab. S1: Chemical composition of alfalfa silage treated with essential oils

Reference	Storage length	Essential oil	Application rate	Δ% DM		Δ% CP		Δ% NDF		Δ% WSC	
Önenç and Turgud (2019)	60 d		Fresh forage	–		222.5 g/kg DM		446.6 g/kg DM			
			Control	254.2 g/kg FM		182.1 g/kg DM		416.8 g/kg DM		95.3 g/kg DM	
		Oregano	600 mg/kg FM	−0.1	ns	+1.5	*	+1.7	*	−5.6	*
		Cumin	600 mg/kg FM	+2.0	*	+3.5	*	+6.0	*	−23.1	*
		Cinnamon	600 mg/kg FM	+6.3	*	+3.1	*	+3.9	*	−58.0	*
Besharati and Niazifar (2020)	60 d		Fresh forage	222.0 g/kg FM		196.0 g/kg DM		248.0 g/kg DM		37.4 g/kg DM	
			Control	244.4 g/kg FM		116.2 g/kg DM		490.7 g/kg DM		40.8 g/kg DM	
		Lemonseed	60 mg/kg FM	−0.8	ns	+6.5	*	−2.1	ns	−35.5	*
			120 mg/kg FM	+5.6	*	+11.4	*	−4.5	ns	+20.3	*
Besharati *et al.* (2020a)	6 d		Fresh forage	222.0 g/kg FM		196.0 g/kg DM		248.0 g/kg DM		37.4 g/kg DM	
			Control	244.3 g/kg FM		116.2 g/kg DM		490.6 g/kg DM		40.8 g/kg DM	
		Flaxseed	60 mg/kg DM	+9.8	*	+5.2	*	−12.0	*	−0.7	ns
			120 mg/kg DM	+22.9	*	+6.4	*	−8.1	ns	+13.0	*
Besharati *et al.* (2020b)	60 d		Fresh forage	222.0 g/kg FM		196.0 g/kg DM		248.0 g/kg DM		37.4 g/kg DM	
			Control	244.4 g/kg FM		116.2 g/kg DM		490.7 g/kg DM		40.9 g/kg DM	
		Cinnamon	60 mg/kg FM	+5.1	*	+5.8	*	−13.5	*	+16.9	*
		Flaxseed	60 mg/kg FM	+9.7	*	+5.2	*	−12.0	*	−1.0	ns
		Lemonseed	60 mg/kg FM	+0.2	ns	+6.5	*	−2.1	ns	+13.2	*
		Mixture 1:1:1	180 mg/kg FM	−0.9	ns	+7.9	*	−19.3	*	+3.7	ns
Turan and Önenç (2018)	120 d		Fresh forage	–		239.0 g/kg DM		384.1 g/kg DM		–	
			Control	325.0 g/kg FM		230.6 g/kg DM		331.5 g/kg DM		–	
		Cumin	300 mg/kg FM	+2.4	ns	+6.3	*	−14.9	*	–	
			500 mg/kg FM	−0.2	ns	+2.2	*	−5.8	*	–	
Besharati *et al.* (2021b)	Alfalfa 90 d		Fresh forage	350.0 g/kg FM		180.2 g/kg DM		418.0 g/kg DM		–	
		Ricinoleic acid + cardol + cardanol	Control	292.9 g/kg FM		149.7 g/kg DM		380.9 g/kg DM		–	
			500 mg/kg FM	+4.8	ns	+12.1	*	−0.4	ns	–	
	3:1 Alfalfa/ apple pomace 90 d		Fresh forage	352.5 g/kg FM		150.5 g/kg DM		414.6 g/kg DM		–	
		Ricinoleic acid + cardol + cardanol	Control	297.0 g/kg FM		133.6 g/kg DM		407.1 g/kg DM		–	
			500 mg/kg FM	+3.4	ns	+14.3	*	−1.1	ns	–	
	1:1 Alfalfa/ apple pomace 90 d		Fresh forage	355.0 g/kg FM		120.7 g/kg DM		411.1 g/kg DM		–	
		Ricinoleic acid + cardol + cardanol	Control	294.0 g/kg FM		108.8 g/kg DM		410.5 g/kg DM		–	
			500 mg/kg FM	+9.2	ns	+5.4	*	+1.6	ns	–	
	1:3 Alfalfa/ apple pomace 90 d		Fresh forage	357.5 g/kg FM		91.0 g/kg DM		407.7 g/kg DM		–	
		Ricinoleic acid + cardol + cardanol	Control	271.0 g/kg FM		93.6 g/kg DM		408.7 g/kg DM		–	
			500 mg/kg FM	+17.7	*	−5.9	*	−0.7	ns	–	

Δ% – Percentage variation relative to the control mean; CP – crude protein; DM – dry matter; FM – fresh matter; NDF – neutral detergent insoluble fiber; WSC – water soluble carbohydrates; * – significant at $P < 0.05$; ns – not significant at $P < 0.05$.

Tab. S2: Fermentative profile of alfalfa silage treated with essential oils

Reference	Storage length	Essential oil	Application rate	Δ% DM losses		Δ% pH		Δ% NH_3–N		Δ% Lactic acid	
Önenç and Turgud (2019)	60 d		Fresh forage	–		–		–		–	
			Control	19.1 g/kg		5.5		105.6 g/kg N		26.5 g/kg DM	
		Oregano	600 mg/kg FM	−16.8	*	−5.5	*	−6.0	ns	+111.7	*
		Cumin	600 mg/kg FM	−24.1	*	−4.2	*	−9.7	ns	+165.0	*
		Cinnamon	600 mg/kg FM	−27.2	*	−8.5	*	−27.3	*	+316.2	*
Besharati and Niazifar (2020)	60 d		Fresh forage	–		6.14		–		–	
			Control	–		4.66		84.93 g/kg N		69.4 g/kg DM	
		Lemonseed	60 mg/kg FM	–	–	−22.5	*	+0.3	ns	+9.9	*
			120 mg/kg FM	–	–	−18.7	*	0.0	ns	+14.5	*
Besharati *et al.* (2020a)	60 d		Fresh forage	–		6.14		–		–	
			Control	–		4.65		84.93 g/kg N		69.4 g/kg DM	
		Flaxseed	60 mg/kg DM	–	–	−19.4	*	−1.6	ns	+2.7	*
			120 mg/kg DM	–	–	−17.0	*	−14.3	*	+6.9	*
Besharati *et al.* (2020b)	60 d		Fresh forage	–		6.14		–		–	
			Control	–		4.65		84.93 g/kg N		69.4 g/kg DM	
		Cinnamon	60 mg/kg FM	–	–	−21.3	*	−6.0	*	+15.7	*
		Flaxseed	60 mg/kg FM	–	–	−19.8	*	−1.6	ns	+2.7	*
		Lemonseed	60 mg/kg FM	–	–	−22.4	*	+0.3	ns	+9.9	*
		Mixture 1:1:1	180 mg/kg FM	–	–	−18.5	*	−1.6	ns	+2.1	*
Turan and Önenç (2018)	120 d		Fresh forage	–		–		–		–	
			Control	–		4.88		–		–	
		Cumin	300 mg/kg FM	–	–	−2.3	*	–	–	–	–
			500 mg/kg FM	–	–	−10.5	*	–	–	–	–
Besharati *et al.* (2021b)	Alfalfa 90 d		Fresh forage	–		8.4		–		–	
		Ricinoleic acid + cardol + cardanol	Control	–		5.1		700.0 g/kg N		–	
			500 mg/kg FM	–	–	0.0	ns	+6.7	ns	–	–
	3:1 Alfalfa/ apple pomace 90 d		Fresh forage	–		7.2		–		–	
		Ricinoleic acid + cardol + cardanol	Control	–		4.2		490.0 g/kg N		–	
			500 mg/kg FM	–	–	0.0	ns	−12.2	ns	–	–
	1:1 Alfalfa/ apple pomace 90 d		Fresh forage	–		6.0		–		–	
		Ricinoleic acid + cardol + cardanol	Control	–		4.1		396.0 g/kg N		–	
			500 mg/kg FM	–	–	0.0	ns	−35.2	*	–	–
	1:3 Alfalfa/ apple pomace 90 d		Fresh forage	–		4.7		407.7 g/kg DM		–	
		Ricinoleic acid + cardol + cardanol	Control	–		3.8		408.7 g/kg DM		–	
			500 mg/kg FM	–	–	−2.6	ns	−0.7	ns	–	–

Δ% – Percentage variation relative to the control mean; CP – crude protein; DM – dry matter; FM – fresh matter; NDF – neutral detergent insoluble fiber; WSC – water soluble carbohydrates; * – significant at $P < 0.05$; ns – not significant at $P < 0.05$.

Tab. S3: Microbial counts of alfalfa silage treated with essential oils

Reference	Storage length	Essential oil	Application rate	Δ% Yeasts (log cfu/g)		Δ% Molds (log cfu/g)		Δ% Enterobacteria (log cfu/g)		Δ% LAB (log cfu/g)	
Önenç and Turgud (2019)	60 d		Fresh forage	–		–		–		–	
			Control	5.52		5.31		–		4.87	
		Oregano	600 mg/kg FM	-40.9	*	-15.4	*	–	–	+7.8	*
		Cumin	600 mg/kg FM	-92.0	*	-15.1	*	–	–	+10.1	*
		Cinnamon	600 mg/kg FM	-87.9	*	-14.3	*	–	–	+28.7	*
Turan and Önenç (2018)	120 d		Fresh forage	–		–		–		–	
			Control	4.86		2.49		1.01		3.51	
		Cumin	300 mg/kg FM	-29.4	*	-100.0	*	-100.0	*	+76.1	*
			500 mg/kg FM	-22.6	*	-100.0	*	-100.0	*	+108.3	*

Δ% – Percentage variation relative to the control mean; LAB – lactic acid bacteria; FM – fresh matter; * – significant at P < 0.05; ns – not significant at P < 0.05

Tab. S4: Aerobic stability of alfalfa silage treated with essential oils

Reference	Storage length	Essential oil	Application rate	Δ% Aerobic stability	
Besharati et al. (2020b)	60 d		Control	77 h	
		Cinnamon	60 mg/kg FM	+29.0	*
		Flaxseed	60 mg/kg FM	+19.5	*
		Lemonseed	60 mg/kg FM	+25.5	*
		Mixture 1:1:1	180 mg/kg FM	+45.5	*

Δ% – Percentage variation relative to the control mean; LAB – lactic acid bacteria; FM – fresh matter; * – significant at P < 0.05; ns – not significant at P < 0.05

Tab. S5: Chemical composition of whole plant corn silage treated with essential oils.

Reference	Storage length	Essential oil	Application rate	Δ% DM		Δ% CP		Δ% NDF		Δ% WSC	
Önenç and Turgud (2019)	256 d	Blend (Thymol + Eugenol + Vanillin + Limonene	Fresh forage	289.9 g/kg FM		–		–		67.9 g/kg DM	
			Control	261.0 g/kg FM		–		–		23.4 g/kg DM	
			40 mg/kg FM	+1.1	ns	–	–	–	–	+14.1	ns
			80 mg/kg FM	−1.1	ns	–	–	–	–	−6.4	ns
Silva *et al.* (2024a)	60 d	Sweet orange	Fresh forage	323.2 g/kg FM		54.7 g/kg DM		554.0 g/kg DM		–	
			Control	293.4 g/kg FM		60.5 g/kg DM		546.6 g/kg DM		–	
			200 mg/kg FM	+2.3	*Q	+1.1	ns	+1.9	ns	–	–
			400 mg/kg FM	+5.8	*Q	+0.8	ns	−0.6	ns	–	–
			600 mg/kg FM	+3.5	*Q	+3.6	ns	−2.1	ns	–	–
Silva *et al.* (2024b)	60 d	Blend (*Eucalyptus staigeriana* : *Copaifera langsdorffii*) 500 mg/kg FM	Fresh forage	323 g/kg FM		54.7 g/kg DM		554 g/kg DM		–	
			Control	293 g/kg FM		60.5 g/kg DM		547 g/kg DM		–	
			1 : 3	+4.8	*	+4.6	ns	+0.7	ns	–	–
			1 : 1	+6.8	*	+0.5	ns	−5.1	ns	–	–
			3 : 1	+5.8	*	−3.8	ns	+0.5	ns	–	–

Reference	Storage length	Essential oil	Application rate	Δ% DM		Δ% CP		Δ% NDF		Δ% WSC	
Hodjatpanah-Montazeri *et al.* (2016)	45 d		Control	296.8 g/kg FM		74.7 g/kg DM		574.0 g/kg DM		–	
		Cinnamon	120 mg/kg DM	+12.9	*	+0.9	ns	+0.5	ns	–	–
			240 mg/kg DM	+8.1	*	+0.9	ns	−3.7	*	–	–
		Thyme	120 mg/kg DM	+5.0	ns	+0.7	ns	−4.2	*	–	–
			240 mg/kg DM	+1.0	ns	+2.4	ns	−0.7	ns	–	–
		Mint	120 mg/kg DM	−4.6	ns	+30.7	*	+10.1	*	–	–
			240 mg/kg DM	−4.3	ns	+34.5	*	+1.0	ns	–	–
		Oregano	120 mg/kg DM	−11.9	*	+21.3	*	+0.7	ns	–	–
			240 mg/kg DM	+2.7	ns	+37.3	*	+4.9	*	–	–
		Cumin	120 mg/kg DM	−0.5	ns	−4.0	ns	+1.2	ns	–	–
			240 mg/kg DM	+5.1	ns	+4.3	ns	−5.6	*	–	–
Maghsoudloo *et al.* (2017)	3 d		Control	295 g/kg FM		78.7 g/kg DM		560 g/kg DM		169.2 g/kg DM	
		Rosemary	125 µL/kg FM	+2.5	ns	0.0	ns	0.0	ns	+46.8	*
			250 µL/kg FM	+2.6	ns	+2.3	*	+7.1	*	+46.7	*
		Fennel	125 µL/kg FM	+2.0	ns	−1.5	*	+18.4	*	+72.0	*
			250 µL/kg FM	+4.6	ns	0.0	ns	−3.6	ns	+9.3	*
		Carum copticum	125 µL/kg FM	+3.3	ns	0.0	ns	−1.8	ns	+8.1	*
			250 µL/kg FM	+2.8	ns	0.0	ns	−5.4	ns	−1.9	*
Maghsoudloo *et al.* (2017)	3 d		Control	296.3 g/kg FM		78.7 g/kg DM		683.3 g/kg DM		156 g/kg DM	
		Rosemary	125 µL/kg FM	+2.5	ns	0.0	ns	0.0	ns	+46.8	*
			250 µL/kg FM	+2.6	ns	+2.3	*	+7.1	*	+46.7	*
		Fennel	125 µL/kg FM	+2.0	ns	−1.5	*	+18.4	*	+72.0	*
			250 µL/kg FM	+4.6	ns	0.0	ns	−3.6	ns	+9.3	*
		Carum copticum	125 µL/kg FM	+3.3	ns	0.0	ns	−1.8	ns	+8.1	*
			250 µL/kg FM	+2.8	ns	0.0	ns	−5.4	ns	−1.9	*
Maghsoudloo *et al.* (2017)	7 d		Control	296.3 g/kg FM		78.7 g/kg DM		683.3 g/kg DM		156 g/kg DM	
		Rosemary	125 µL/kg FM	+0.8	ns	0.0	ns	−3.4	ns	+31.0	*
			250 µL/kg FM	+1.6	ns	+11.2	*	+6.8	*	+41.0	*
		Fennel	125 µL/kg FM	+1.5	ns	+4.4	*	−3.4	ns	+63.1	*
			250 µL/kg FM	+4.0	ns	+2.3	*	−3.4	ns	+12.3	*
		Carum copticum	125 µL/kg FM	+2.4	ns	+8.9	*	−9.3	*	−19.6	*
			250 µL/kg FM	+2.1	ns	+6.7	*	−0.5	ns	+1.3	*
Maghsoudloo *et al.* (2017)	21 d		Control	300.1 g/kg FM		84 g/kg DM		613.3 g/kg DM		125.2 g/kg DM	
		Rosemary	125 µL/kg FM	−0.3	ns	−2.1	*	+10.9	*	+17.0	*
			250 µL/kg FM	−0.2	ns	+2.0	*	+1.1	ns	+67.9	*
		Fennel	125 µL/kg FM	+0.1	ns	+2.0	*	+19.0	*	+63.2	*
			250 µL/kg FM	+1.5	ns	−2.1	*	+3.3	ns	+18.6	*
		Carum copticum	125 µL/kg FM	+1.1	ns	−2.1	*	−1.7	ns	−20.0	*
			250 µL/kg FM	−0.7	ns	+2.0	*	+1.1	ns	+30.0	*

Reference	Storage length	Essential oil	Application rate	Δ% DM		Δ% CP		Δ% NDF		Δ% WSC	
Maghsoudloo et al. (2017)	45 d		Control	295.4 g/kg FM		85.7 g/kg DM		650 g/kg DM		90.5 g/kg DM	
		Rosemary	125 µL/kg FM	+0.9	ns	−2.0	*	−6.2	*	+18.9	*
			250 µL/kg FM	+0.5	ns	−4.1	*	−3.1	ns	+90.4	*
		Fennel	125 µL/kg FM	+0.8	ns	−6.1	*	−1.5	ns	+99.0	*
			250 µL/kg FM	+1.3	ns	−2.0	*	−13.8	*	+2.4	*
		Carum copticum	125 µL/kg FM	−0.1	ns	−12.3	*	0.0	ns	+9.5	*
			250 µL/kg FM	+2.2	*	+4.1	*	0.0	ns	+66.6	*

Δ% – Percentage variation relative to the control mean; CP – crude protein; DM – dry matter; FM – fresh matter; NDF – neutral detergent insoluble fiber; WSC – water soluble carbohydrates; * – significant at $P < 0.05$; ns – not significant at $P < 0.05$.

Tab. S6: Fermentative profile of whole plant corn silage treated with essential oils

Reference	Storage length	Essential oil	Application rate	Δ% DM losses		Δ% pH		Δ% NH_3–N		Δ% Lactic acid		Δ% Acetic acid	
Kung Jr. et al. (2018)	256 d	Blend (Thymol + Eugenol + Vanillin + Limonene	Fresh forage	–		5.38		0.82 g/kg DM		–		–	
			Control	101.0 g/kg		3.72		2.29 g/kg DM		55.0 g/kg DM		22.0 g/kg DM	
			40 mg/kg FM	+34.7	ns	−0.3	ns	−1.3	ns	−2.9	ns	−10.0	ns
			80 mg/kg FM	+12.9	ns	−1.6	ns	+8.3	ns	+3.6	ns	−2.7	ns
Silva et al. (2024a)	60 d		Fresh forage	–		–		–		–		–	
			Control	102.5 g/kg		3.62		0.94 g/kg CP		–		28.0 g/kg DM	
		Sweet orange	200 mg/kg FM	+1.3	*Q	−1.9	ns	−3.2	ns	–	–	+79.1	*Q
			400 mg/kg FM	−42.8	*Q	−0.8	ns	−7.4	ns	–	–	−28.3	*Q
			600 mg/kg FM	−26.8	*Q	−2.2	ns	−10.6	ns	–	–	+277.3	*Q
Silva et al. (2024b)	60 d	Blend (*Eucalyptus staigeriana* : *Copaifera langsdorffii*)	Control	44 g/kg		3.6		7.3 g/kg CP		–		28 g/kg DM	
			1 : 3	−2.3	ns	−2.8	ns	−8.2	ns	–	–	+118.2	*
			1 : 1	−2.3	ns	−2.8	ns	−13.7	ns	–	–	+133.2	*
			3 : 1	−6.8	ns	−5.6	ns	−13.7	ns	–	–	+51.1	*
Hodjatpanah–Montazeri *et al.* (2016)	45 d		Control	–		3.75		0.79 mg/dL		–		–	
		Cinnamon	120 mg/kg DM	–	–	+0.5	ns	+3.8	ns	–	–	–	–
			240 mg/kg DM	–	–	+1.6	*	−3.8	ns	–	–	–	–
		Thyme	120 mg/kg DM	–	–	−0.5	ns	−11.4	*	–	–	–	–
			240 mg/kg DM	–	–	+0.3	ns	−22.8	*	–	–	–	–
		Mint	120 mg/kg DM	–	–	+7.5	*	+13.9	*	–	–	–	–
			240 mg/kg DM	–	–	−3.2	*	+5.1	ns	–	–	–	–
		Oregano	120 mg/kg DM	–	–	+8.3	*	+34.2	*	–	–	–	–
			240 mg/kg DM	–	–	+10.1	*	+10.1	*	–	–	–	–
		Cumin	120 mg/kg DM	–	–	+3.7	*	0.0	ns	–	–	–	–
			240 mg/kg DM	–	–	+2.9	*	+25.3	*	–	–	–	–
Maghsoudloo *et al.* (2017	3 d		Control	–		3.72		3.37 mg/dL		–		–	
		Rosemary	125 µL/kg FM	–	–	−0.8	ns	−3.6	ns	–	–	–	–
			250 µL/kg FM	–	–	0.0	ns	−14.2	*	–	–	–	–
		Fennel	125 µL/kg FM	–	–	−1.1	ns	−6.2	*	–	–	–	–
			250 µL/kg FM	–	–	−0.8	ns	−7.7	*	–	–	–	–
		Carum copticum	125 µL/kg FM	–	–	−0.8	ns	−5.3	*	–	–	–	–
			250 µL/kg FM	–	–	0.0	ns	−8.6	*	–	–	–	–

Reference	Storage length	Essential oil	Application rate	Δ% DM losses		Δ% pH		Δ% NH_3–N		Δ% Lactic acid		Δ% Acetic acid	
Maghsoudloo *et al.* (2017	7 d		Control	–		3.54		2.99 mg/dL		–		–	
		Rosemary	125 µL/kg FM	–	–	+0.6	ns	+10.7	ns	–	–	–	–
			250 µL/kg FM	–	–	+0.6	ns	+24.7	*	–	–	–	–
		Fennel	125 µL/kg FM	–	–	−0.3	ns	+22.4	*	–	–	–	–
			250 µL/kg FM	–	–	+0.3	ns	+16.4	ns	–	–	–	–
		Carum copticum	125 µL/kg FM	–	–	−0.3	ns	+15.4	ns	–	–	–	–
			250 µL/kg FM	–	–	+0.6	ns	+5.4	ns	–	–	–	–
Maghsoudloo *et al.* (2017	21 d		Control	–		3.56		2.85 mg/dL		–		–	
		Rosemary	125 µL/kg FM	–	–	+0.6	ns	−3.2	ns	–	–	–	
			250 µL/kg FM	–	–	+0.6	ns	−4.2	ns	–	–	–	
		Fennel	125 µL/kg FM	–	–	+0.3	ns	−9.8	ns	–	–	–	
			250 µL/kg FM	–	–	+0.3	ns	−15.8	ns	–	–	–	
		Carum copticum	125 µL/kg FM	–	–	0.0	ns	−16.8	ns	–	–	–	
			250 µL/kg FM	–	–	+0.6	ns	−1.4	ns	–	–	–	
Maghsoudloo *et al.* (2017	45 d		Control	–		3.41		2.93 mg/dL		–		–	
		Rosemary	125 µL/kg FM	–	–	+0.3	ns	−8.5	ns	–	–	–	–
			250 µL/kg FM	–	–	+0.9	ns	−8.5	ns	–	–	–	–
		Fennel	125 µL/kg FM	–	–	+0.3	ns	−3.4	ns	–	–	–	–
			250 µL/kg FM	–	–	+1.8	ns	−9.6	ns	–	–	–	–
		Carum copticum	125 µL/kg FM	–	–	+0.6	ns	−9.6	ns	–	–	–	–
			250 µL/kg FM	–	–	+1.8	ns	−6.8	ns	–	–	–	–

Δ% – Percentage variation relative to the control mean; DM – dry matter; FM – fresh matter; NH_3-N – ammonia nitrogen; * – significant at $P < 0.05$; ns – not significant at $P < 0.05$.

Tab. S7: Microbial counts of whole plant corn silage treated with essential oils.

Reference	Storage length	Essential oil	Application rate	Δ% Yeasts (log cfu/g)		Δ% Molds (log cfu/g)		Δ% Enterobacteria (log cfu/g)		Δ% LAB (log cfu/g)	
Kung Jr. *et al.* (2018)	256 d	Blend (Thymol + Eugenol + Vanillin + Limonene)	Fresh forage	7.04		6.16		7.65		7.40	
			Control	5.16		4.47		–		–	
			40 mg/kg FM	+4.8	ns	+2.2	ns	–	ns	–	ns
			80 mg/kg FM	+0.4	ns	+0.9	ns	–	ns	–	ns
Silva *et al.* (2024a)	60 d		Fresh forage	–		–		–		–	
			Control	–		2.55		–		2.29	
		Sweet orange	200 mg/kg FM	–	–	+83.1	ns	–	–	−29.7	ns
			400 mg/kg FM	–	–	+12.5	ns	–	–	−3.9	ns
			600 mg/kg FM	–	–	−52.2	ns	–	–	−41.5	ns
Silva *et al.* (2024b)	60 d	Blend (*Eucalyptus staigeriana* : *Copaifera langsdorffii*)	Control	–		3.03		–		2.77	
			1 : 3	–	–	−89.1	*	–	–	−28.9	ns
			1 : 1	–	–	+43.6	*	–	–	+24.9	ns
			3 : 1	–	–	+27.4	*	–	–	−2.5	ns

Δ% – Percentage variation relative to the control mean; LAB – lactic acid bacteria; FM – fresh matter; * – significant at $P < 0.05$; ns – not significant at $P < 0.05$

Tab. S8: Aerobic stability of whole plant corn silage treated with essential oils

Reference	Storage length	Essential oil	Application rate	Δ% Aerobic stability	
Kung Jr. *et al.* (2018)	256 d	Blend (Thymol + Eugenol + Vanillin + Limonene)	Fresh forage	–	
			Control	47.5 h	
			40 mg/kg FM	−4.6	ns
			80 mg/kg FM	+4.2	ns
Silva *et al.* (2024a)	60 d		Fresh forage	–	
			Control	36 h	
		Sweet orange	200 mg/kg FM	0.0	ns
			400 mg/kg FM	+16.7	ns
			600 mg/kg FM	+66.7	*
Silva *et al.* (2024b)	60 d	Blend (*Eucalyptus staigeriana* : *Copaifera langsdorffii*)	Control	36 h	
			1 : 3	+66.7	*
			1 : 1	+83.3	*
			3 : 1	+66.7	*
Hodjatpanah-Montazeri *et al.* (2016)	45 d		Control	72 h	
		Cinnamon	120 mg/kg DM	+129.6	*
			240 mg/kg DM	+300.0	*
		Thyme	120 mg/kg DM	+300.0	*
			240 mg/kg DM	+44.4	*
		Mint	120 mg/kg DM	−20.8	ns
			240 mg/kg DM	−3.7	ns
		Oregano	120 mg/kg DM	+300.0	*
			240 mg/kg DM	+300.0	*
		Cumin	120 mg/kg DM	+300.0	*
				+300.0	*

Δ% – Percentage variation relative to the control mean; LAB – lactic acid bacteria; FM – fresh matter; * – significant at $P < 0.05$; ns – not significant at $P < 0.05$

Tab. S9: Chemical composition of other silages treated with essential oils

Reference	Silage	Essential oil	Application rate	Δ% DM		Δ% CP		Δ% NDF		Δ% WSC	
Cantoia Jr. *et al.* (2020)	Sugarcane 60 d		Fresh forage	249.0 g/kg FM		27.0 g/kg DM		542.0 g/kg DM		–	
			Control	222.0 g/kg FM		21.4 g/kg DM		621.0 g/kg DM		129 g/kg DM	
		Lemongrass	1 mL/kg FM	+14.4	*Q	+22.0	*Q	−15.6	*Q	+27.1	*Q
			2 mL/kg FM	+20.7	*Q	+16.8	*Q	−20.1	*Q	+40.3	*Q
			3 mL/kg FM	+14.4	*Q	+29.2	*Q	−15.3	*Q	+29.5	*Q
Zhou *et al.* (2024)	*Stylosanthes guianensis* 30 d		Fresh forage	363.0 g/kg FM		99.0 g/kg DM		701.0 g/kg DM		18.1 g/kg DM	
			Control	365.0 g/kg FM		85.0 g/kg DM		–		–	
		Neolamarkia cadamba leaf	5 g/kg FM	+1.9	ns	−0.1	ns	–	–	–	–
			10 g/kg FM	+2.7	ns	−5.9	ns	–	–	–	–
	Rice straw 30 d		Fresh forage	279.0 g/kg FM		72.6 g/kg DM		611.0 g/kg DM		21.6 g/kg DM	
			Control	271.0 g/kg FM		82.8 g/kg DM		–		–	
		Neolamarkia cadamba leaf	5 g/kg FM	+10.7	ns	−11.2	*	–	–	–	–
			10 g/kg FM	+9.6	ns	−8.7	*	–	–	–	–

Reference	Silage	Essential oil	Application rate	Δ% DM		Δ% CP		Δ% NDF		Δ% WSC	
Li *et al.* (2022)	*Paper mulberry* 60 d		Fresh forage	288.7 g/kg FM		156.7 g/kg DM		667.0 g/kg DM		20.7 g/kg DM	
			Control	258.3 g/kg FM		149.8 g/kg DM		312.1 g/kg DM		7.1 g/kg DM	
		Amomum villosum	1 mL/kg FM	+2.5	ns	+3.5	ns	+27.8	*	+63.4	*
Aniceto *et al.* (2024)	*Whole plant sorghum* 60 d		Fresh forage	363.8 g/kg FM		47.8 g/kg DM		685.3 g/kg DM		–	
			Control	345.0 g/kg FM		42.3 g/kg DM		726.2 g/kg DM		–	
		Rosemary (Ros)	1 g/kg FM	+5.9	ns	+26.5	*	−7.7	ns	–	–
		Tea tree (TT)	1 g/kg FM	+12.2	ns	+13.0	*	−5.3	ns	–	–
		Citronella (Cit)	1 g/kg FM	+17.2	ns	+16.2	*	−3.4	ns	–	–
		Ros:TT 1:1	1 g/kg FM	+6.6	ns	+18.5	*	+1.7	ns	–	–
		Ros:Cit 1:1	1 g/kg FM	+9.8	ns	+20.6	*	−2.3	ns	–	–
		TT:Cit 1:1	1 g/kg FM	−3.9	ns	+9.8	ns	+1.0	ns	–	–
		Ros:TT:Cit 1:1:1	1 g/kg FM	+2.3	ns	+17.3	*	−0.4	ns	–	–
Chaves *et al.* (2012)	Whole−plant barley 365 d		Control	349.3 g/kg FM		117.4 g/kg DM		487.1 g/kg DM		40.9 g/kg DM	
		Cinnamon leaf	125 mg/kg FM	+0.3	ns	+4.3	*	−4.7	ns	+33.3	*
			250 mg/kg FM	−4.5	ns	+5.6	*	−3.8	ns	+46.2	*
			400 mg/kg FM	−0.3	ns	+6.0	*	+5.1	ns	+0.5	ns
		Oregano	125 mg/kg FM	−1.8	ns	+0.3	ns	+1.8	ns	−19.6	ns
			250 mg/kg FM	−3.8	ns	+0.9	ns	+3.0	ns	+24.0	ns
			400 mg/kg FM	−1.5	ns	+3.3	ns	+1.2	ns	+13.4	ns
		Sweet orange	125 mg/kg FM	−1.8	ns	+5.4	*	+2.6	ns	−2.7	ns
			250 mg/kg FM	−3.5	ns	+5.4	*	+2.5	ns	−23.7	ns
			400 mg/kg FM	−4.4	ns	+10.2	*	+3.9	ns	+21.3	ns
Foskolos *et al.* (2016)	Ryegrass 35 d		Control	–		38.8 g/kg DM		–		–	
		Eugenol	50 mg/kg FM	–	–	+3.4	ns	–	–	–	–
			500 mg/kg FM	–	–	+0.5	ns	–	–	–	–
			2000 mg/kg FM	–	–	−2.3	ns	–	–	–	–
			Control	–		48.9 g/kg DM		–		–	
		Thymol	50 mg/kg FM	–	–	+1.8	ns	–	–	–	–
			500 mg/kg FM	–	–	−3.9	ns	–	–	–	–
			2000 mg/kg FM	–	–	−12.5	ns	–	–	–	–
			Control	–		42.0 g/kg DM		–		–	
		Cinnamaldehyde	50 mg/kg FM	–	–	−2.9	ns	–	–	–	–
			500 mg/kg FM	–	–	−5.7	ns	–	–	–	–
			2000 mg/kg FM	–	–	−4.0	ns	–	–	–	–
			Control	–		38.6 g/kg DM		–		–	
		Carvacrol	50 mg/kg FM	–	–	+2.6	ns	–	–	–	–
			500 mg/kg FM	–	–	−4.1	ns	–	–	–	–
			2000 mg/kg FM	–	–	−2.1	ns	–	–	–	–
Önenç *et al.* (2015)	Field pea 60 d		Fresh forage	311.7 g/kg FM		233.7 g/kg DM		399.5 g/kg DM		38.0 g/kg DM	
			Control	235.3 g/kg FM		223.4 g/kg DM		375.6 g/kg DM		11.5 g/kg DM	
		Oregano	400 mg/kg FM	−3.8	*	+4.3	*	+4.4	*	+33.0	*
		Cinnamom	400 mg/kg FM	+8.3	*	+8.6	*	+3.4	*	−18.6	*
		Mixture 1:1	400 mg/kg FM	+2.0	*	−3.2	*	+4.0	*	+4.7	ns

Reference	Silage	Essential oil	Application rate	Δ% DM		Δ% CP		Δ% NDF		Δ% WSC	
Önenç *et al.* (2017)	Field pea 120 d		Fresh forage	311.7 g/kg FM		233.7 g/kg DM		399.5 g/kg DM		38.0 g/kg DM	
			Control	227.9 g/kg FM		226.0 g/kg DM		376.7 g/kg DM		6.9 g/kg DM	
		Oregano	400 mg/kg FM	+10.0	*	−3.1	*	+8.1	*	+7.3	*
		Cinnamom	400 mg/kg FM	+12.7	*	−3.3	*	+10.2	*	+33.7	*
		Mixture 1:1	400 mg/kg FM	+9.8	*	−8.9	*	+8.8	*	+22.6	*
Akinci and Önenç (2021)	Vetch + Oat 70 d		Fresh forage	312.4 g/kg FM		96.1 g/kg DM		607.6 g/kg DM		40.7 g/kg DM	
			Control	282.2 g/kg FM		97.3 g/kg DM		642.5 g/kg DM		14.1 g/kg DM	
		Cumin	200 mg/kg FM	−0.1	ns	+3.2	*	+1.0	*	−35.4	*
			300 mg/kg FM	+7.8	*	+5.3	*	−1.3	*	−15.8	*
			500 mg/kg FM	+4.9	*	+5.3	*	−0.8	*	−20.5	*
Duru (2024)	Vetch + Wheat 75 d		Fresh forage	369.2 g/kg FM		137.2 g/kg DM		384.0 g/kg DM		–	
			Control	384.6 g/kg FM		137.4 g/kg DM		356.8 g/kg DM		–	
		Hypericum perforatum	20 mg/kg	+4.9	*	−1.5	ns	+1.6	ns	–	–
			40 mg/kg	+2.9	*	−6.9	ns	+4.3	ns	–	–
			80 mg/kg	−0.9	ns	−4.4	ns	+11.0	*	–	–
			160 mg/kg	−1.7	ns	−4.9	ns	+9.3	*	–	–
Xu *et al.* (2023)	*Broussonetia papyfera* 3 d	*Amomum villosum*	Control	263.3 g/kg FM		152.6 g/kg DM		661.6 g/kg DM		7.4 g/kg DM	
			1 mL/kg FM	+3.0	ns	+3.7	ns	−2.2	ns	+4.1	ns
	B. papyfera : *P. sinese* 7:3 3 d		Control	232.7 g/kg FM		149.8 g/kg DM		587.0 g/kg DM		12.6 g/kg DM	
			1 mL/kg FM	+2.4	*	+5.1	ns	−4.3	*	+15.9	*
	B. papyfera : *P. sinese* 1:1 3 d		Control	210.8 g/kg FM		140.5 g/kg DM		553.9 g/kg DM		15.1 g/kg DM	
			1 mL/kg FM	+2.4	ns	+11.1	*	−9.8	*	+2.6	ns
	B. papyfera : *P. sinese* 3:7 3 d		Control	182.4 g/kg FM		128.9 g/kg DM		473.7 g/kg DM		10.8 g/kg DM	
			1 mL/kg FM	+4.8	*	+14.0	*	−14.0	*	+26.9	*
	Pennisetum sinese 3 d		Control	142.1 g/kg FM		115.1 g/kg DM		417.8 g/kg DM		15.2 g/kg DM	
			1 mL/kg FM	+10.9	*	+5.2	ns	−7.7	*	−5.9	ns
Xu *et al.* (2023)	*Broussonetia papyfera* 30 d	*Amomum villosum*	Control	258.3 g/kg FM		152.3 g/kg DM		613.3 g/kg DM		5.6 g/kg DM	
			1 mL/kg FM	+2.5	ns	+1.8	ns	−2.4	ns	−1.8	ns
	B. papyfera : *P. sinese* 7:3 30 d		Control	205.0 g/kg FM		147.0 g/kg DM		523.2 g/kg DM		10.8 g/kg DM	
			1 mL/kg FM	+9.5	*	+2.4	ns	−5.1	*	+26.9	*
	B. papyfera : *P. sinese* 1:1 30 d		Control	187.2 g/kg FM		132.6 g/kg DM		509.3 g/kg DM		12.6 g/kg DM	
			1 mL/kg FM	+6.1	*	+5.2	*	−16.1	*	+18.3	*
	B. papyfera : *P. sinese* 3:7 30 d		Control	173.7 g/kg FM		120.7 g/kg DM		417.0 g/kg DM		11.7 g/kg DM	
			1 mL/kg FM	+2.1	ns	+3.5	ns	−14.7	*	+32.5	*
	Pennisetum sinese 30 d		Control	140.8 g/kg FM		105.4 g/kg DM		386.7 g/kg DM		11.8 g/kg DM	
			1 mL/kg FM	+3.8	ns	+12.4	ns	−19.3	*	−1.7	*
Andrade *et al.* (2024)	TRM 90 d		Fresh TMR	624.5 g/kg FM		181.0 g/kg DM		305.4 g/kg DM		–	
			Control	637.8 g/kg FM		165.8 g/kg DM		282.0 g/kg DM		–	
		Monensin	35 mg/kg DM	−2.9	*	+8.6	ns	+9.4	ns	–	–
			45 mg/kg DM	−2.0	*	+6.2	ns	+4.8	ns	–	–
		Limonene−rich essential oil	300 mg/kg DM	−2.6	*	+15.6	ns	+12.3	ns	–	–
			600 mg/kg DM	−2.9	*	+15.7	ns	+15.0	ns	–	–

Reference	Silage	Essential oil	Application rate	Δ% DM		Δ% CP		Δ% NDF		Δ% WSC	
Andrade *et al.* (2024)	TRM 90 d		Fresh TMR	410.0 g/kg FM		164.2 g/kg DM		395.3 g/kg DM		–	
			Control	362.8 g/kg FM		156.2 g/kg DM		412.1 g/kg DM		–	
		Monensin	35 mg/kg DM	+2.1	ns	+4.2	ns	−7.2	*	–	
			45 mg/kg DM	+0.2	ns	+7.9	ns	−7.8	*	–	
		Limonene–rich essential oil	300 mg/kg DM	−2.1	ns	+8.6	ns	−8.6	*	–	
			600 mg/kg DM	−2.0	ns	+5.2	ns	−10.0	*	–	

Δ% – Percentage variation relative to the control mean; CP – crude protein; DM – dry matter; FM – fresh matter; NDF – neutral detergent insoluble fiber; WSC – water soluble carbohydrate; * – significant at P < 0.05; ns – not significant at P < 0.05; Q – quadratic effect

Tab. S10: Fermentative profile of whole plant corn silage treated with essential oils

Reference	Silage	Essential oil	Application rate	Δ% DM losses		Δ% pH		Δ% NH_3–N		Δ% Lactic acid		Δ% Acetic acid	
Cantoia Jr. *et al.* (2020)	Sugarcane 60 d	Lemongrass	Fresh forage	–		–		–		–		–	
			Control	311.0 g/kg		3.89		146.0 g/kg N		23.3 g/kg DM		14.4 g/kg DM	
			1 mL/kg FM	−37.9	*Q	−2.1	*Q	+1.4	*L	−10.3	*L	−40.1	*Q
			2 mL/kg FM	−51.8	*Q	−1.3	*Q	+23.3	*L	−27.9	*L	−49.6	*Q
			3 mL/kg FM	−32.8	*Q	3.6	*Q	+41.1	*L	−43.8	*L	−33.3	*Q
Zhou *et al.* (2024)	*Stylosanthes guianensis* 30 d	*Neolamarkia cadamba* leaf	Control	–		5.66		86.8 g/kg N		5.29 g/kg DM		3.45 g/kg DM	
			5 g/kg FM	–	–	−5.3	*	−37.3	*	+54.8	*	ND	*
			10 g/kg FM	–	–	−9.4	*	−57.9	*	+100.4	*	ND	*
	Rice straw 30 d	*Neolamarkia cadamba* leaf	Control	–		5.18		98.2 g/kg N		4.44 g/kg DM		3.23 g/kg DM	
			5 g/kg FM	–	–	−14.5	*	−59.1	*	+74.8	*	+22.3	*
			10 g/kg FM	–	–	−14.5	*	−64.6	*	+95.7	*	+42.1	*
Aniceto *et al.* (2024)	Whole plant sorghum 60 d		Control	64.1 g/kg		4.41		0.65 g/kg CP		35.1 g/kg DM		58.0 g/kg DM	
		Rosemary (Ros)	1 g/kg FM	−9.8	ns	+3.4	ns	+36.9	ns	+86.3	ns	−5.0	ns
		Tea tree (TT)	1 g/kg FM	−10.0	ns	+4.3	ns	+4.6	ns	+15.9	ns	−77.2	*
		Citronella (Cit)	1 g/kg FM	−27.8	*	+2.3	ns	−10.8	ns	−39.3	ns	−50.5	*
		Ros:TT 1:1	1 g/kg FM	−2.3	ns	+0.2	ns	+23.1	ns	+75.9	ns	−37.4	*
		Ros:Cit 1:1	1 g/kg FM	−19.7	ns	+3.6	ns	−9.2	ns	+6.4	ns	−34.6	*
		TT:Cit 1:1	1 g/kg FM	−26.5	*	−0.5	ns	+3.1	ns	−10.5	ns	−79.5	*
		Ros:TT:Cit 1:1:1	1 g/kg FM	−25.3	ns	+0.5	ns	+13.8	ns	+19.7	ns	−34.5	*
Chaves *et al.* (2012)	Whole plant barley 365 d		Control	–		4.10		1.20 g/kg DM		69.8 g/kg DM		16.8 g/kg DM	
		Cinnamon leaf	125 mg/kg FM	–	–	−2.4	ns	0.0	ns	+10.6	ns	−1.8	ns
			250 mg/kg FM	–	–	−2.4	ns	0.0	ns	+19.1	ns	−23.8	*
			400 mg/kg FM	–	–	0.0	ns	+8.3	ns	+12.8	ns	+2.4	ns
		Oregano	125 mg/kg FM	–	–	0.0	ns	+8.3	ns	+2.6	ns	+7.1	ns
			250 mg/kg FM	–	–	−2.4	ns	−8.3	ns	+5.6	ns	−14.3	ns
			400 mg/kg FM	–	–	−2.4	ns	0.0	ns	+7.2	ns	−1.2	ns
		Sweet orange	125 mg/kg FM	–	–	−2.4	ns	+8.3	ns	+8.3	ns	+5.4	ns
			250 mg/kg FM	–	–	0.0	ns	+16.7	ns	+13.9	ns	+8.9	ns
			400 mg/kg FM	–	–	−2.4	ns	0.0	ns	+14.2	ns	−18.5	ns

Reference	Silage	Essential oil	Application rate	Δ% DM losses		Δ% pH		Δ% NH_3–N		Δ% Lactic acid		Δ% Acetic acid	
Foskolos *et al.* (2016)	Ryegrass 35 d	Eugenol	Control	–		5.81		1.74 g/kg DM		43.3 g/kg DM		–	
			50 mg/kg FM	–	–	−1.4	ns	−3.4	ns	+8.5	ns	–	–
			500 mg/kg FM	–	–	+2.4	ns	−17.8	ns	−18.2	ns	–	–
			2000 mg/kg FM	–	–	+11.5	ns	−44.8	*	−24.2	ns	–	–
		Thymol	Control	–		5.6		1.60 g/kg DM		39.9 g/kg DM		–	
			50 mg/kg FM	–	–	−2.5	ns	+6.3	ns	+4.3	ns	–	–
			500 mg/kg FM	–	–	+12.9	ns	+6.3	ns	−1.8	ns	–	–
			2000 mg/kg FM	–	–	+15.7	*	−53.1	*	−92.2	*	–	–
		Cynnamal-dehyde	Control	–		5.63		1.85 g/kg DM		43.7 g/kg DM		–	
			50 mg/kg FM	–	–	−0.7	ns	+5.4	ns	+28.4	ns	–	–
			500 mg/kg FM	–	–	+3.2	ns	−1.1	ns	+28.6	ns	–	–
			2000 mg/kg FM	–	–	+6.9	ns	−33.5	*	−17.4	ns	–	–
		Carvacrol	Control	–		5.77		1.81 g/kg DM		48.6 g/kg DM		–	
			50 mg/kg FM	–	–	+4.0	ns	−7.7	ns	−26.7	ns	–	–
			500 mg/kg FM	–	–	+12.0	*	−33.7	*	−39.5	*	–	–
			2000 mg/kg FM	–	–	+13.9	*	−40.3	*	−80.0	*	–	–
Önenç *et al.* (2015)	Field pea 60 d		Fresh forage	–		6.06		–		–		–	
			Control	15.2 g/kg		4.31		99.48 g/kg N		56.7 g/kg DM		23.8 g/kg DM	
		Oregano	400 mg/kg FM	+9.9	ns	+2.1	ns	+2.9	*	−17.6	*	−3.5	ns
		Cinnamom	400 mg/kg FM	−18.4	*	+3.7	*	−58.9	*	−54.1	*	−18.7	*
		Mixture 1:1	400 mg/kg FM	+19.7	*	+3.2	*	−31.4	*	−46.4	*	−27.6	*
Önenç *et al.* (2017)	Field pea 120 d		Fresh forage	–		6.06		–		–		–	
			Control	17.2 g/kg		5.18		99.08 g/kg N		45.3 g/kg DM		27.0 g/kg DM	
		Oregano	400 mg/kg FM	+98.3	*	+6.2	*	+32.4	*	−32.6	*	−27.2	*
		Cinnamom	400 mg/kg FM	+12.2	ns	+6.0	*	+38.1	*	−47.0	*	−14.9	*
		Mixture 1:1	400 mg/kg FM	+99.4	*	+6.4	*	+45.5	*	−37.9	*	−28.6	*
Li *et al.* (2022)	Paper mulberry 3 d	*Amomum villosum*	Control	–		5.77		–		44.9 g/kg DM		7.7 g/kg DM	
			1 mL/kg FM	–	–	−20.5	*	–	–	+23.6	ns	+83.1	*
	Paper mulberry 7 d		Control	–		5.51		–		58.4 g/kg DM		7.7 g/kg DM	
			1 mL/kg FM	–	–	−18.3	*	–	–	+3.1	*	+172.7	*
	Paper mulberry 15 d		Control	–		5.45		–		65.5 g/kg DM		10.9 g/kg DM	
			1 mL/kg FM	–	–	−18.0	*	–	–	−32.2	*	+149.5	*
	Paper mulberry 30 d		Control	–		5.32		–		31.4 g/kg DM		15.4 g/kg DM	
			1 mL/kg FM	–	–	−18.0	*	–	–	+140.8	*	+52.6	*
	Paper mulberry 60 d		Control	–		5.25		24.4 g/kg DM		13.0 g/kg DM		22.5 g/kg DM	
			1 mL/kg FM	–	–	−15.2	*	−8.6	*	+397.7	*	+42.7	*
Sousa *et al.* (2022)	Sorghum + *Urochloa brizantha* 15 d		Control	–		4.07		–		–		–	
		Lippia sidoides	400 mg/kg FM	–	–	−1.7	ns	–	–	–	–	–	–
		62% Thymol	400 mg/kg FM	–	–	−2.0	ns	–	–	–	–	–	–
		100% Thymol	400 mg/kg FM	–	–	+0.7	ns	–	–	–	–	–	–
	Sorghum + *Urochloa brizantha* 30 d		Control	–		3.99		–		–		–	
		Lippia sidoides	400 mg/kg FM	–	–	−0.8	ns	–	–	–	–	–	–
		62% Thymol	400 mg/kg FM	–	–	−1.3	ns	–	–	–	–	–	–
		100% Thymol	400 mg/kg FM	–	–	+3.5	*	–	–	–	–	–	–
	Sorghum + *Urochloa brizantha* 45 d		Control	–		3.99		–		–		–	
		Lippia sidoides	400 mg/kg FM	–	–	−0.8	ns	–	–	–	–	–	–
		62% Thymol	400 mg/kg FM	–	–	0.0	ns	–	–	–	–	–	–
		100% Thymol	400 mg/kg FM	–	–	+4.5	*	–	–	–	–	–	–

Reference	Silage	Essential oil	Application rate	Δ% DM losses		Δ% pH		Δ% NH_3–N		Δ% Lactic acid		Δ% Acetic acid	
Akinci and Önenç (2021)	Vetch + Oat 70 d	Cumin	Fresh forage	–		6.20		–		–		–	
			Control	11.4 g/kg		4.46		56.54 g/kg N		33.6 g/kg DM		–	
			200 mg/kg FM	−4.4	ns	−0.7	ns	−4.1	*	+20.3	*	–	
			300 mg/kg FM	−17.5	ns	−0.7	ns	−14.1	*	+15.4	*	–	
			500 mg/kg FM	−5.3	ns	−0.7	ns	−14.3	*	+2.5	*	–	
Duru (2024)	Vetch + Wheat 75 d	Hypericum perforatum	Control	–		4.71		–		8.1 g/kg DM		2.7 g/kg DM	
			20 mg/kg	–	–	−3.2	*	–	–	+4.9	ns	+7.4	ns
			40 mg/kg	–	–	−3.8	*	–	–	+4.9	ns	+25.9	ns
			80 mg/kg	–	–	−4.2	*	–	–	+9.9	ns	+33.3	ns
			160 mg/kg	–	–	−4.2	*	–	–	+6.2	ns	+22.2	ns
Xu *et al.* (2023)	*Broussonetia papyfera* 3 d	*Amomum villosum*	Control	–		5,77		103,5 g/kg N		19,2 g/kg DM		8,9 g/kg DM	
			1 mL/kg FM	–	–	−20,5	*	−4,6	ns	+64,1	*	−13,5	ns
	B. papyfera : *P. sinese* 7:3 3 d		Control	–		5,68		102,6 g/kg N		14,7 g/kg DM		13,3 g/kg DM	
			1 mL/kg FM	–	–	+1,1	*	−7,9	*	+23,1	*	+12,8	ns
	B. papyfera : *P. sinese* 1:1 3 d		Control	–		5,49		108,0 g/kg N		19,5 g/kg DM		15,3 g/kg DM	
			1 mL/kg FM	–	–	−1,3	*	−13,1	*	+46,7	*	+31,4	*
	B. papyfera : *P. sinese* 3:7 3 d		Control	–		5,31		116,6 g/kg N		10,4 g/kg DM		11,6 g/kg DM	
			1 mL/kg FM	–	–	0,0	ns	−21,9	*	+47,1	*	+83,6	*
	Pennisetum sinese 3 d		Control	–		5,29		108,3 g/kg N		17,7 g/kg DM		21,3 g/kg DM	
			1 mL/kg FM	–	–	0,0	ns	−8,2	ns	+33,9	ns	−11,3	ns
Xu *et al.* (2023)	*Broussonetia papyfera* 30 d	*Amomum villosum*	Control	–		5,25		93,2 g/kg N		25,6 g/kg DM		22,1 g/kg DM	
			1 mL/kg FM	–	–	−15,2	*	+1,5	ns	+39,1	*	+57,9	*
	B. papyfera : *P. sinese* 7:3 30 d		Control	–		5,21		102,0 g/kg N		17,9 g/kg DM		24,2 g/kg DM	
			1 mL/kg FM	–	–	+0,6	ns	−10,4	*	+39,7	*	+26,4	*
	B. papyfera : *P. sinese* 1:1 30 d		Control	–		5,10		92,4g/kg N		24,5 g/kg DM		33,5 g/kg DM	
			1 mL/kg FM	–	–	−1,0	*	−8,0	*	+38,4	*	+45,1	*
	B. papyfera : *P. sinese* 3:7 30 d		Control	–		5,04		99,2 g/kg N		17,8 g/kg DM		30,0 g/kg DM	
			1 mL/kg FM	–	–	0,0	ns	−13,5	*	+80,9	*	+67,0	*
	Pennisetum sinese 30 d		Control	–		4,95		104,8 g/kg N		26,4 g/kg DM		35,0 g/kg DM	
			1 mL/kg FM	–	–	−6,9	ns	−10,6	*	+32,2	*	+23,7	*
Andrade *et al.* (2024)	TMR 90 d		Control	26.2 g/kg		4.75		104.8 g/kg N		36.1 g/kg DM		7.6 g/kg DM	
		Monensin	35 mg/kg DM	−30.5	ns	0.0	ns	−15.6	*	+5.0	ns	+27.6	*
			45 mg/kg DM	−20.6	ns	+0.2	ns	−22.5	*	+16.1	ns	+38.2	*
		Limonene-rich essential oil	300 mg/kg DM	−21.8	ns	−0.2	*	−20.9	*	+40.2	*	+47.4	*
			600 mg/kg DM	−29.8	ns	−0.4	*	−26.0	*	+34.1	*	+42.1	*
Andrade *et al.* (2024)	TMR 90 d		Control	54.2 g/kg		4.01		84.6 g/kg N		55.0 g/kg DM		8.8 g/kg DM	
		Monensin	35 mg/kg DM	−13.1	*	+1.5	ns	+1.2	ns	+16.5	*	−12.5	*
			45 mg/kg DM	−25.3	*	+1.0	ns	+1.4	ns	+19.6	*	−13.6	*
		Limonene-rich essential oil	300 mg/kg DM	+9.6	ns	−1.2	ns	+1.4	ns	+30.7	*	+10.2	*
			600 mg/kg DM	+35.4	*	+4.0	*	+10.4	*	+5.3	*	+75.0	*

Tab. S11: Microbial counts of other silages treated with essential oils

Reference	Silage	Essential oil	Application rate	Δ% Ethanol		Δ% Yeasts (log cfu/g)		Δ% Molds (log cfu/g)		Δ% Enterobacteria / Clostridium (log cfu/g)		Δ% LAB (log cfu/g)	
Cantoia Jr. *et al.* (2020)	Sugarcane 60 d	Lemongrass	Fresh forage	–		–		–		–		–	
			Control	101.0 g/kg DM		4.87		–		–		14.4 g/kg DM	
			1 mL/kg FM	–67.0	*Q	+3.7	*L	–	–	–	–	–40.1	*Q
			2 mL/kg FM	–92.1	*Q	–21.4	*L	–	–	–	–	–49.6	*Q
			3 mL/kg FM	–83.1	*Q	–36.1	*L	–	–	–	–	–33.3	*Q
Önenç *et al.* (2015)	Field pea 60 d		Fresh forage	–		–		–		–		–	
			Control	–		0.33		ND		–		–	
		Oregano	400 mg/kg FM	–	–	+384.8	*	ND	–	–	–	–	–
		Cinnamom	400 mg/kg FM	–	–	+663.6	*	ND	–	–	–	–	–
		Mixture 1:1	400 mg/kg FM	–	–	+557.6	*	ND	–	–	–	–	–
Zhou *et al.* (2024)	*Stylosanthes guianensis* 30 d		Fresh forage	–		5.06		4.46		7.59		6.29	
			Control	–		< 2.00		< 2.00		4.20		7.78	
		Neolamarkia cadamba leaf	5 g/kg FM	–	–	< 2.00		< 2.00		< 3.00		–18.3	*
			10 g/kg FM	–	–	< 2.00		< 2.00		< 3.00		–44.0	*
	Rice straw 30 d		Fresh forage	–		4.33		3.56		6.40		6.18	
			Control	–		< 2.00		< 2.00		6.00		8.14	
		Neolamarkia cadamba leaf	5 g/kg FM	–	–	< 2.00		< 2.00		< 2.00		–10.6	*
			10 g/kg FM	–	–	< 2.00		< 2.00		< 2.00		–18.7	*
Chaves *et al.* (2012)	Whole plant barley 365 d		Control	4.7 g/kg DM		3.50		1.20		–		7.30	
		Cinnamon leaf	125 mg/kg FM	+44.7	*	–25.7	ns	–58.3	ns	–	–	–1.4	ns
			250 mg/kg FM	+21.3	ns	–11.4	ns	–8.3	ns	–	–	–16.4	ns
			400 mg/kg FM	+10.6	ns	–42.9	ns	–16.7	ns	–	–	–1.4	ns
		Oregano	125 mg/kg FM	+10.6	ns	+5.7	ns	+41.7	ns	–	–	0.0	ns
			250 mg/kg FM	+8.5	ns	+31.4	ns	+125.0	ns	–	–	–13.7	ns
			400 mg/kg FM	+2.1	ns	–82.9	ns	0.0	ns	–	–	–2.7	ns
		Sweet orange	125 mg/kg FM	+55.3	*	–22.9	ns	+58.3	ns	–	–	–4.1	ns
			250 mg/kg FM	+27.7	ns	–34.3	ns	+41.7	ns	–	–	0.0	ns
			400 mg/kg FM	+6.4	ns	–34.3	ns	–16.7	ns	–	–	–4.1	ns
Akinci and Önenç (2021)	Vetch + Oat 70 d	Cumin	Fresh forage	–		2.26		1.80		0.89		2.11	
			Control	–		1.87		1.87		0.85		3.46	
			200 mg/kg FM	–		–72.7	*	–81.3	*	–92.9	*	+82.1	*
			300 mg/kg FM	–		–84.0	*	–94.7	*	–92.9	*	+53.2	*
			500 mg/kg FM	–		–84.0	*	–94.7	*	–92.9	*	+48.8	*

Reference	Silage	Essential oil	Application rate	Δ% Ethanol		Δ% Yeasts (log cfu/g)		Δ% Molds (log cfu/g)		Δ% Enterobacteria / Clostridium (log cfu/g)		Δ% LAB (log cfu/g)	
Foskolos *et al.* (2016)	Ryegrass 35 d	Eugenol	Control	–		–		–		0.89		8.57	
			50 mg/kg FM	–	–	–	–	–	–	0.0	ns	–2.5	ns
			500 mg/kg FM	–	–	–	–	–	–	–21.3	ns	–2.7	ns
			2000 mg/kg FM	–	–	–	–	–	–	–13.5	ns	–7.9	*
		Thymol	Control	–		–		–		1.05		7.88	
			50 mg/kg FM	–	–	–	–	–	–	–15.2	ns	+2.2	ns
			500 mg/kg FM	–	–	–	–	–	–	+16.2	ns	+5.3	ns
			2000 mg/kg FM	–	–	–	–	–	–	–26.7	ns	–33.9	*
		Cynnamal-dehyde	Control	–		–		–		0.85		8.58	
			50 mg/kg FM	–	–	–	–	–	–	+70.6	ns	–1.7	ns
			500 mg/kg FM	–	–	–	–	–	–	+8.2	ns	–0.6	ns
			2000 mg/kg FM	–	–	–	–	–	–	+14.1	ns	–4.3	ns
		Carvacrol	Control	–		–		–		0.90		8.04	
			50 mg/kg FM	–	–	–	–	–	–	+6.1	ns	+3.1	ns
			500 mg/kg FM	–	–	–	–	–	–	–14	ns	+2.6	ns
			2000 mg/kg FM	–	–	–	–	–	–	+8.4	ns	–11.6	ns

Δ% – Percentage variation relative to the control mean; LAB – lactic acid bacteria; DM – dry matter; FM – fresh matter; * – significant at P < 0.05; ns – not significant at P < 0.05; L – linear effect; Q – quadratic effect; ND – bellow detection limit.

Tab. S12: Aerobic stability of other silages treated with essential oils

Reference	Silage	Essential oil	Application rate	Δ% Aerobic stability		Δ% pH after aerobic exposure		Δ% DM losses by aerobic exposure	
Cantoia Jr. *et al.* (2020)	Sugarcane 60 d		Control	52.2 h		4.24		14.1 g/kg/d	
		Lemongrass	1 mL/kg FM	+15.3	*L	–4.2	*Q	+51.8	ns
			2 mL/kg FM	+66.7	*L	–5.7	*Q	+39.0	ns
			3 mL/kg FM	+103.1	*L	–2.6	*Q	–3.5	ns
Chaves *et al.* (2012)	Whole-plant barley 365 d		Control	–		5.50		–	
		Cinnamon leaf	125 mg/kg FM	–	–	–23.6	ns	–	–
			250 mg/kg FM	–	–	–20.0	ns	–	–
			400 mg/kg FM	–	–	–25.5	ns	–	–
		Oregano	125 mg/kg FM	–	–	+1.8	ns	–	–
			250 mg/kg FM	–	–	–5.5	ns	–	–
			400 mg/kg FM	–	–	–25.5	ns	–	–
		Sweet orange	125 mg/kg FM	–	–	–5.5	ns	–	–
			250 mg/kg FM	–	–	–12.7	ns	–	–
			400 mg/kg FM	–	–	–1.8	ns	–	–

Reference	Silage	Essential oil	Application rate	Δ% Aerobic stability		Δ% pH after aerobic exposure		Δ% DM losses by aerobic exposure	
Sousa *et al.* (2022)	Sorghum + *Urochloa brizantha* 15 d		Control	30.2 h		–		–	
		Lippia sidoides	400 mg/kg FM	+21.9	ns	–	–	–	–
		62% Thymol	400 mg/kg FM	+27.2	ns	–	–	–	–
		100% Thymol	400 mg/kg FM	+74.2	*	–	–	–	–
	Sorghum + *Urochloa brizantha* 30 d		Control	31.0 h		–		–	
		Lippia sidoides	400 mg/kg FM	+36.1	ns	–	–	–	–
		62% Thymol	400 mg/kg FM	+61.9	*	–	–	–	–
		100% Thymol	400 mg/kg FM	+23.2	ns	–	–	–	–
	Sorghum + *Urochloa brizantha* 45 d		Control	40.6 h		–		–	
		Lippia sidoides	400 mg/kg FM	–0.5	ns	–	–	–	–
		62% Thymol	400 mg/kg FM	+29.6	*	–	–	–	–
		100% Thymol	400 mg/kg FM	0.0	ns	–	–	–	–
Akinci and Önenç (2021)	Vetch + Oat 70 d	Cumin	Control	–		8.07		–	
			200 mg/kg FM	–	–	–7.1	*	–	–
			300 mg/kg FM	–	–	–7.1	*	–	–
			500 mg/kg FM	–	–	–2.5	*	–	–
Andrade *et al.* (2024)	TMR 90 d		Control	213.7 h		–		–	
		Monensin	35 mg/kg DM	+21.4	*	–	–	–	–
			45 mg/kg DM	+13.7	*	–	–	–	–
		Limonene-rich essential oil	300 mg/kg DM	+15.0	*	–	–	–	–
			600 mg/kg DM	+39.4	*	–	–	–	–
Andrade *et al.* (2024)	TMR 90 d		Control	83.9 h		–		–	
		Monensin	35 mg/kg DM	+9.1	*	–	–	–	–
			45 mg/kg DM	+6.2	*	–	–	–	–
		Limonene-rich essential oil	300 mg/kg DM	+6.0	*	–	–	–	–
			600 mg/kg DM	+10.0	*	–	–	–	–

Δ% – Percentage variation relative to the control mean; DM – dry matter; FM – fresh matter; * – significant at $P < 0.05$; ns – not significant at $P < 0.05$; L – linear effect; Q – quadratic effect.

CARBON NEUTRALITY AND SUSTAINABLE DEVELOPMENT IN ANIMAL HUSBANDRY

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Abstract

Carbon neutrality has become a global consensus and a core trend in development. Many countries are accelerating the development of green and low-carbon agricultural and animal husbandry technologies, steadily advancing the realization of the 2050 carbon neutrality goal. This conference will focus on sharing a series of research results from our team in the field of carbon neutrality and sustainable development in agriculture and animal husbandry, showcasing technical pathways and practical breakthroughs.

Keywords: agriculture, animal husbandry, green and low-carbon, sustainable development, carbon neutrality

INTRODUCTION

Global warming has become one of the most severe environmental challenges faced by humanity. The chain reactions it triggers, such as glacier melting and the frequent occurrence of extreme weather, are fundamentally threatening the balance of natural ecosystems and the sustainable development path of human society. As significant contributors to greenhouse gas emissions, agriculture and animal husbandry also possess enormous carbon sequestration potential and emission reduction capacity, making them key areas that cannot be ignored in global climate governance. Against this backdrop, developing and promoting green and low-carbon feed-livestock production technologies, to curb greenhouse gas emissions throughout the entire agricultural and livestock production chain from the source, is not only a core approach to advancing the green transformation of agriculture but also an inevitable choice to ensure global food security and ecological safety.

Our team has long focused on core areas such as the efficient utilization of crop straw resources, the integration of low-cost and high-quality livestock production technologies, precise reduction of greenhouse gas emissions, the practical application of smart agriculture technologies, and the construction of carbon-neutral pathways for the entire life cycle of agriculture and animal husbandry. Through interdisciplinary collaborative innovation and deep integration of industry, academia, research, and application, we are committed to providing feasible technical solutions and practical support for the high-quality development of green and low-carbon agriculture and animal husbandry in China and even globally (Fig. 1).

MATERIAL AND METHODS

Systematically review the resource types, regional yield distribution, and traditional disposal methods of global staple crop straws such as wheat, maize, and rice, as well as typical agricultural by-products, and quantify the carbon emission intensity and mitigation potential of different processing pathways. Monitor greenhouse gas emissions throughout the full cycle of silage fermentation to identify core reduction targets and action pathways.

Using a multi-omics combined analysis strategy including metagenomics, transcriptomics, metabolomics, and proteomics, analyze the multi-dimensional mechanisms of microbial-enzyme synergy between exogenous lactic acid bacteria and cellulase from aspects such as gene expression, protein regulation, and metabolic flux changes. Targeted development of multifunctional composite microbial agents that integrate efficiency improvement, emission reduction and carbon sequestration enhancement, and gut probiotic benefits.

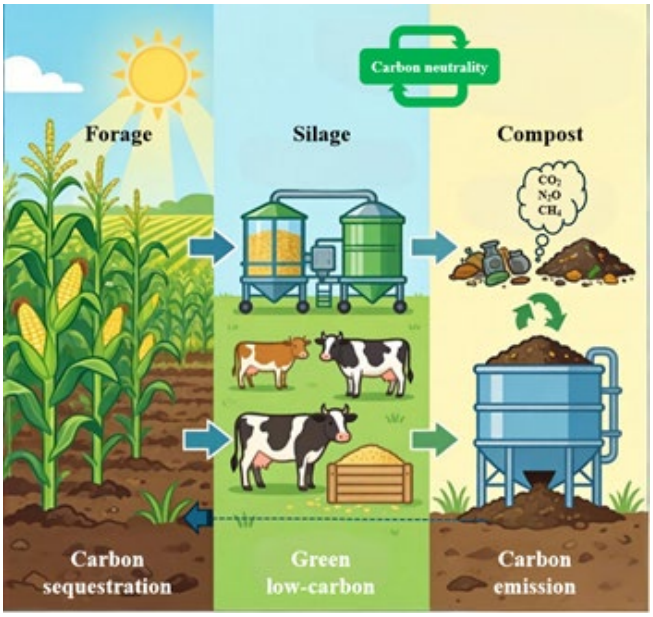


Fig. 1: Livestock production and carbon neutrality

Based on the fermentation characteristics of core silage feed types, greenhouse gas emission databases, and regional agricultural and livestock production parameters, construct a "Global Major Silage Feed Carbon Emission Dynamic Reduction Model" that couples resource endowment, production patterns, and mitigation technologies. Through multi-scenario simulation, optimize the combination of mitigation technologies to form a livestock and agricultural carbon neutrality technology pathway and implementation framework covering the entire chain from feed production and livestock farming to waste resource utilization.

RESULTS AND DISCUSSION

Silage fermentation regulation

By integrating third-generation sequencing microbiomics with untargeted metabolomics techniques, the dynamic microbial communities and metabolic regulatory networks during silage fermentation were systematically analyzed (Du *et al.* 2025). Based on indigenous wild lactic acid bacteria resources, a composite microbial preparation with synergistic effects of lactic acid bacteria and cellulase was developed, promoting the industrialization of scientific research results and upgrading silage feed into high value-added products with market circulation attributes. This preparation has been applied on a large

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Fig. 2: Livestock greenhouse gas monitoring System



Fig. 3: Collaborative Production Model of Crops and Livestock

scale for forage silage production in Japan, Southeast Asia, and tropical regions of Africa, significantly improving the fermentation stability and nutrient retention of silage feed, while also enhancing the daily weight gain and feed conversion efficiency of ruminants.

Eco-agriculture model of zero biological waste

Through collaborative efforts among industry, academia, and research, a feed processing system has been established using tea dregs, coffee grounds, vegetable residues, and fruit residues as raw materials. After standardized processing, the aforementioned food waste can be used as high-nutrition alternative feed for dairy and beef cattle, significantly improving feed resource utilization. At the same time, a closed-loop resource utilization system for food waste has been created, integrating 'food manufacturing factories-feed processing enterprises-large-scale farms,' promoting a biological resource recycling production model where food manufacturing factories achieve nearly zero food waste emissions. This system effectively supports environmentally friendly agricultural development and improves the self-sufficiency of feed raw materials and the production efficiency of dairy and beef cattle (Cai *et al.* 2025).

Monitoring and Reduction of Greenhouse Gas Emissions from Livestock

In Southeast Asia, a 'Real-time Monitoring System for Livestock Greenhouse Gas Emissions' (Fig. 2) has been established. Based on this monitoring system, a low-carbon feed production system has been developed, using regional crop straw and food waste as the main raw materials. In addition, precise feeding and rumen regulation technologies have been developed to significantly suppress methane (CH_4) emissions. This system notably reduces feed costs, increases daily weight gain of beef cattle, and lowers methane emission intensity per unit of livestock product, providing key technological support for the low-carbon transformation of livestock farming in Southeast Asia and regional climate mitigation goals.

Construction of a Crop-Livestock Integrated Production Model

Systematically promote the 'crop-livestock coordinated production model' among small-scale farmers (Fig. 3), establishing a four-in-one circular production system of ‚agricultural by-product resources-livestock feeding-compost return to fields-crop cultivation.' This model is based on the nutritional needs of ruminants, using agricultural by-products as the base material to formulate a full-price mixed fermented feed. The model effectively increases farmers' self-sufficiency in feed, reduces the cost of purchased protein feed, and simultaneously promotes annual milk production and carcass weight in dairy and beef cattle, providing a replicable practical model for smallholder farming systems in developing countries to achieve protein security and livelihood improvement.

CONCLUSION

Animal husbandry is a major source of greenhouse gas emissions. We should focus on knowledge innovation and develop green and low-carbon livestock production technologies. Carbon neutrality is key to controlling global warming, and reducing greenhouse gas emissions can slow the pace of climate change and protect the Earth's ecosystems. Achieving carbon neutrality requires balancing animal production and environmental protection, and promoting sustainable agricultural development.

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SEEING THE RIGHT MOMENT FOR SILAGE HARVEST: METHODS AND DECISION FRAMEWORKS

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Abstract

Finding the right time for maize silage harvest is one of the biggest practical challenges in forage production. Farmers must balance feed quality, yield, weather, and harvest logistics often within only a few days. In reality, harvest decisions are still mostly based on field experience and limited sampling, even though crop maturity can vary considerably within the same field

This paper presents a practical framework combining multispectral drone monitoring, laboratory analysis, and field observations to support harvest decisions under real farming conditions. The approach was tested on multiple maize hybrids and agronomic treatments, with monitoring from standing crop through harvest to final silage.

Part of the experiment was affected by unfavorable weather during harvest, which revealed the difference between theoretical prediction and practical farming reality. The results show that UAV monitoring can become a useful tool for silage management, especially when combined with agronomic experience and laboratory validation.

Keywords: maize silage, Earth Observation, UAV monitoring, dry matter, field heterogeneity, harvest timing

INTRODUCTION

In practice, the decision is rarely straightforward. Farmers must balance crop maturity, expected feed quality, weather uncertainty, field accessibility, and harvest logistics within a very limited time window. Harvest timing is therefore often based on field experience, visual crop assessment, calendar estimates, and limited laboratory sampling.

Months to Grow, Days to Harvest, Months to Feed

This imbalance between the length of crop production, the very short harvest window, and the long feeding period creates significant pressure on decision-making. Maize is cultivated for several months, harvested within approximately one to two weeks, and then used in cattle feeding for many additional months. The final harvest decision therefore represents an economic and organizational compromise under time pressure. Responsibilities on modern farms are often divided between agronomists, harvest contractors, nutrition specialists, and farm management, each working with different priorities and budgets. While the agronomist focuses on crop condition and yield, harvest logistics are constrained by machinery‘s availability and operational costs, and nutritionists evaluate the long-term impact on feed quality and animal performance. The overall farm profit is often distributed across separate operational budgets and therefore not immediately visible, as illustrated in Figure 1.

One Field, Multiple Crop Conditions

In reality, maize fields are rarely uniform. Different parts of the same field may develop differently due to soil conditions, water availability, hybrid characteristics, local stress factors, or previous agronomic interventions. Variability within the field often becomes even more visible during dry periods or under unstable weather conditions. As a result, point sampling can only describe a small portion of the field and may not fully reflect the actual crop condition across the entire harvested area. For practical harvest management, this creates a gap between laboratory precision and the spatial reality of the field.

The Hidden Cost of Silage Losses

An 18% silage loss may represent:

- approximately 100,000 EUR in additional silage purchase costs or
- around 130,000 EUR in unrealized electricity revenue from biogas production compared with a well-preserved silage loss of 5% (2,889 t × 35 EUR/t silage; 2,889 t × 343 kWh/t × 0.13 EUR/kWh) or
- approximately 1 500 000 EUR in potential milk production value (2,889 t × 1,300 l milk/t silage × 0.40 EUR/l milk).

These estimates are based on simplified calculation models and average market values. An actual economic impact depends on multiple factors including silage quality, digestibility, starch content, animal performance, storage conditions and energy conversion efficiency.

Monitoring the Reality of the Field

Understanding the crop variability under practical farming conditions requires more than a single field visit or a laboratory measurement. In this study, the UAV monitoring,



Fig. 1: A silage bunker at a biogas plant in South Moravia, the Czech Republic (2025, 2024). Comparison of silage preserved with and without the use of a preservative in consecutive years under practical farm conditions, illustrating the contrast between short-term agronomic cost reduction and long-term biomass utilization efficiency. The recorded biomass loss reached approximately 18%.

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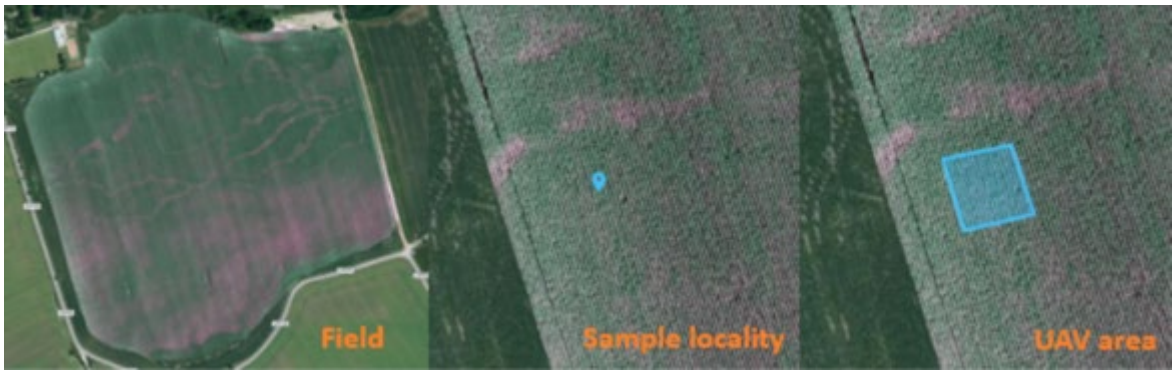


Fig. 2: Maize field in the Slovácko region, the Czech Republic (2024). UAV monitoring revealed differences in crop maturity and dry matter content across the field. Higher dry matter values were observed near the road, while the opposite side, inaccessible by car, remained at the milk stage of maturity. Laboratory analysis showed 27.52% DM compared with 30.34% estimated from UAV.

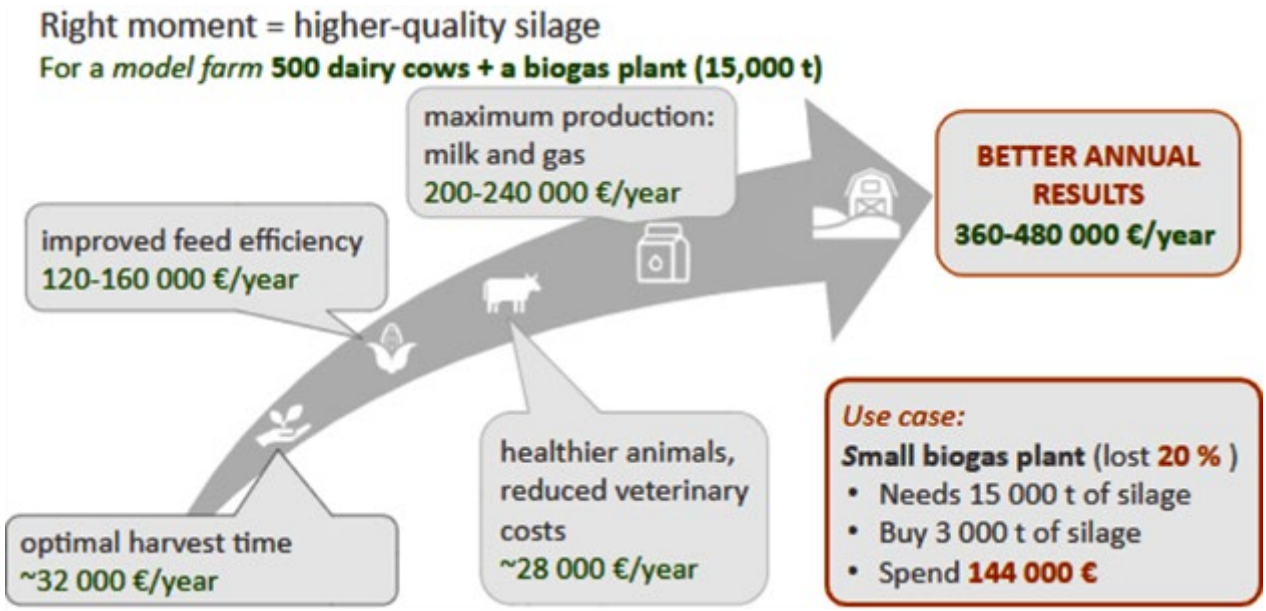


Fig. 3: Influence of individual operational budgets on the overall economic performance of dairy and biogas production under practical farm conditions

the laboratory analyses, and the field observations were combined throughout the vegetation period to capture the changes in crop maturity, dry matter development, and spatial variability across the field. Particular attention was given to the differences between the maize hybrids, the agronomic treatments, and the areas exposed to different water availability and stress conditions. The monitoring continued from the standing crop through the harvest and the final silage production, allowing the comparison of the theoretical predictions with the practical reality observed during the harvest and conservation process.

MATERIALS AND METHODS

The objective of this study was not to evaluate a single sensing technology under controlled experimental conditions, but to compare the practical role, limitations, and decision-making value of different methods currently used for silage harvest management. The presented approach therefore focuses on the integration of multiple information sources under real farming conditions rather than on the development of a specific predictive model.

Modern silage management increasingly combines multiple sources of information, each providing a different perspective on crop development and harvest readiness (Figure 4). Satellite monitoring is commonly used for large-scale observation of vegetation development during the season, while UAV monitoring enables a more detailed evaluation of spatial variability within individual fields. During harvest, onboard NIR sensors provide rapid real-time estimation of crop parameters directly on the harvester. Laboratory analyses remain the reference method for determining dry matter and nutritional quality, although they are limited by sampling frequency and processing time.

The practical challenge is no longer the availability of individual technologies, but the integration of these data sources into a single decision framework capable of supporting practical harvest decisions under real farming conditions. Particular attention was therefore given to how individual methods contribute to decision-making under conditions of crop variability, weather uncertainty, operational constraints, and differing economic priorities within the farm management system.

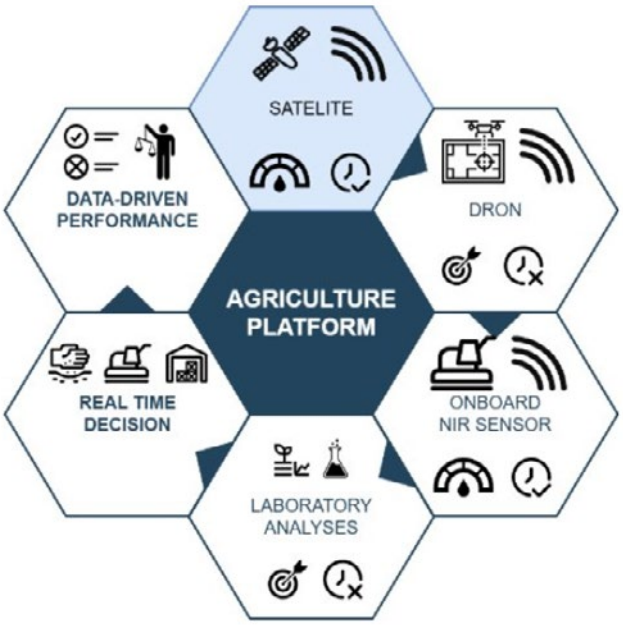


Fig. 4: Overview of the main data sources used for harvest decision support, combining satellite monitoring, UAV sensing, onboard NIR measurements, laboratory analyses, and real-time operational data into a unified agricultural decision framework.

Seeing Crop Development Over Time

One of the major advantages of modern remote sensing methods is their ability to monitor crop development repeatedly throughout the growing season without destroying the measured material. Both satellite and UAV monitoring are based on the principle of non-destructive sensing, where reflected electromagnetic radiation from the crop canopy is used to estimate vegetation condition, crop development, and spatial variability within the field.

Satellite monitoring provides a continuous large-scale overview of vegetation development during the season and allows the observation of long-term trends across extensive agricultural areas. Its main advantage lies in regular revisit times and the ability to monitor crop dynamics over time. However, the spatial resolution and cloud sensitivity may limit the detailed evaluation of variability during critical harvest periods. Another practical limitation is that publicly available satellite imagery is typically accessible with a delay of approximately one day after acquisition, reducing its suitability for immediate operational decisions during rapidly changing harvest conditions.

UAV monitoring enables a flexible and spatially detailed assessment of crop conditions directly before harvest. Multispectral UAV sensing allows the detection of within-field variability, including differences in crop maturity, stress conditions, and dry matter development. By providing high-resolution observations focused on specific fields and operational decision-making, UAV monitoring effectively complements satellite data.

Both technologies were considered complementary rather than competing approaches. Satellite monitoring provides continuous seasonal context, while UAV monitoring delivers detailed local information during critical decision periods. Together, they create a more synchronized view of crop development, combining long-term monitoring with field-scale operational evaluation under practical farming conditions.

Seeing Quality During Harvest

Both laboratory analyses and onboard NIR sensing aim to evaluate the nutritional and technological quality of the harvested crop. Their common objective is to estimate key parameters influencing silage preservation, feed quality, and harvest timing, particularly dry matter content.

Laboratory analyses remain the reference method due to their high analytical precision and the possibility of evaluating a wide range of nutritional parameters. However, the results are limited by sampling frequency, processing time, and the representativeness of the collected sample. Under rapidly changing harvest conditions, the analytical result may no longer fully reflect the actual field situation at the moment of harvest.

Onboard NIR sensing enables continuous real-time monitoring of crop quality parameters directly during harvesting, allowing the preservation of information about the harvested material flow without extensive manual sampling. However, NIR measurements are still based on calibration models and linear regression approaches and therefore represent operational indicators rather than absolute analytical values. While onboard sensing can reliably identify trends, variability, and minimum or maximum zones during harvest, laboratory analyses may still reveal a wider variability range and higher analytical precision within the harvested material.

Both methods were considered complementary rather than competing approaches. Laboratory analyses provide reference validation and nutritional interpretation, while onboard NIR sensing enables continuous monitoring of quality changes during harvest under practical farming conditions.

Fighting Drought: Practical Field Observations

Drought stress has become one of the major challenges influencing maize production and harvest stability under practical farming conditions. By combining Earth Observation methods with conventional laboratory analyses, it became possible to work with field heterogeneity in a way that is difficult to achieve using traditional point-based sampling alone. The following practical observations demonstrate how spatial monitoring introduced an additional perspective into the interpretation of otherwise conventional agronomic and laboratory data.

Hybrid Influence on Field Heterogeneity was evaluated under foothill conditions using eight maize hybrids intended for future field deployment. The objective was to compare hybrid behaviour under uneven water availability and to estimate their potential contribution to milk production. All nutritional parameters were determined from samples consisting of five consecutive maize plants, with grain and green biomass evaluated separately. Particular attention was given to spatial variability detected through Earth Observation methods, which revealed differences in crop development and field heterogeneity that were not fully visible during standard field inspections and conventional laboratory evaluation.

Hydrogel Application and Crop Development and field variability was evaluated under identical field conditions using the same maize hybrid and locality. The monitored treatment consisted of four rows with hydrogel applied beneath the seed together with fertilizer, compared with four control rows receiving fertilizer only. All nutritional parameters were determined from samples consisting of five consecutive maize plants, with grain and green biomass evaluated separately. Earth Observation methods were used to monitor the spatial development of the crop and to evaluate differences between the treated and untreated variants during the vegetation period.

RESULTS AND DISCUSSION

While the presented experiments demonstrated the practical value of combining laboratory analyses, Earth Observation methods, and onboard sensing, they also revealed several important limitations affecting harvest decision-making under real farming conditions. Field heterogeneity, rapidly changing weather conditions, sampling strategy, and the loss of spatial context during harvest may significantly influence the interpretation of otherwise accurate analytical data. In several situations, individual methods provided results that appeared technically correct but did not fully describe the actual biological and operational complexity of the crop. These observations highlighted that the practical challenge is often not the absence of data, but rather the correct interpretation of information obtained from different sources, scales, and time horizons. The following practical cases illustrate several situations where conventional interpretation approaches may lead to misleading conclusions if the broader spatial and agronomic context is not considered.

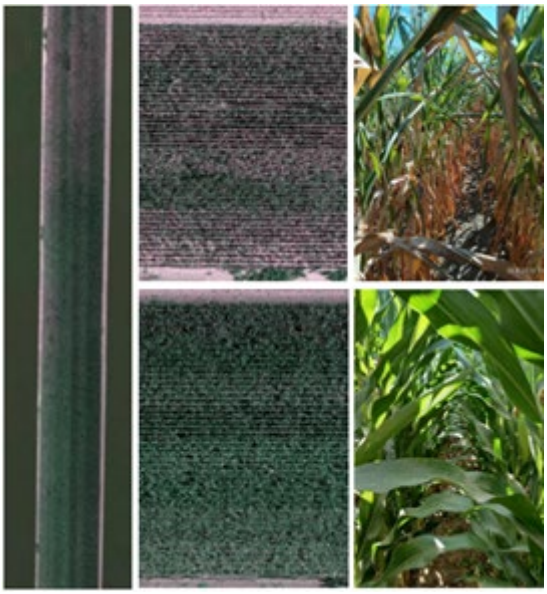


Fig. 5: Selection of maize hybrids in the foothill region of the Orlické Mountains, the Czech Republic (2024). Captured by UAV

Hybrid Influence on Field Heterogeneity

The experiment was located on a strongly heterogeneous field, where the influence of drought significantly affected the validity and interpretation of the trial. While the front part of the field (sampled) remained in relatively good condition, the rear part showed severe drought stress and partial crop desiccation.

Earth Observation monitoring revealed visible differences in crop maturity and canopy development between the evaluated hybrids. Individual hybrids were distinguishable not only through multispectral monitoring, but also by direct visual observation in the field, with each hybrid showing a different response to water stress and maturity development. Laboratory analyses indicated grain starch content ranging from 60 to 72%, green biomass dry matter from 30 to 36.5%, grain dry matter around 63.5%

Hydrogel Application and Crop Development

During the dry period in June and July, the hydrogel variants showed visibly stronger crop development and greater canopy stability.

However, wetter conditions during August and September reduced the visible differences before harvesting, and UAV monitoring no longer detected substantial heterogeneity between the variants. Despite the visually compact crop canopy, laboratory analyses revealed heavier and more developed cobs in the hydrogel-treated variants. Although the differences were not statistically significant due to field variability, the results indicated an approximately 5% higher yield potential.

Onboard NIRS sensing did not detect significant differences during harvest, while the final weighing of the harvested biomass confirmed an approximately 5% increase in yield in the hydrogel variants. Detailed UAV analysis is summarized in Table 1.

Practical Case of Failure

Precision without representativeness may lead to misleading harvest decisions even when laboratory analyses themselves are analytically correct. In one practical case, the agronomist regularly delivered a single maize plant to the laboratory every three days for precise dry matter determination. Although the analytical accuracy was high, the measured values fluctuated significantly over time due to natural field variability and inconsistent sample selection.

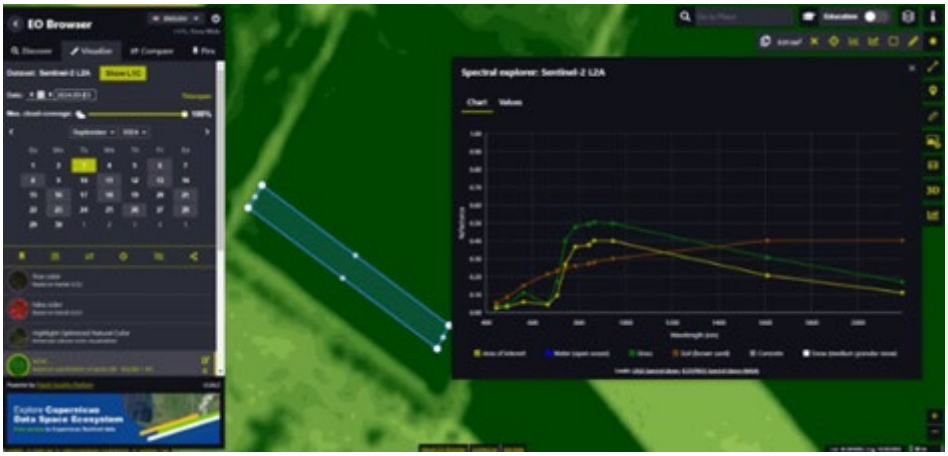


Fig. 6: Selection of maize hybrids in the foothill region of the Orlické Mountains, the Czech Republic (2024). Captured by Copernicus

Tab. 1: Comparison of UAV-derived dry matter parameters between maize variants with and without hydrogel application under practical field conditions.

UAV Parameters	Without hydrogel					With hydrogel				
	H1	H2	H3	H4	Avg.	H1	H2	H3	H4	Avg.
Avg Field DM (%)	34.4	37.5	32.0	32.0	34.0	36.0	33.2	32.1	31.9	33.3
Biomass DM (%)	35.0	37.4	31.7	31.5	33.9	35.8	32.8	31.6	31.2	31.5
Optimal Area (%)	**53.3**	**64.4**	**58.9**	**65.2**	**60.4**	**72.9**	**73.2**	**62.1**	**63.2**	**67.9**
Veg. Coverage (%)	99.1	99.5	100	100	99.6	100	100	100	99.8	99.9

This case illustrates that harvest decision-making cannot rely only on analytical precision if the sampling strategy itself remains random or operationally biased. Integrating Earth Observation data into the sampling process allows the identification of representative field zones and supports a more transparent and spatially informed selection of laboratory samples before harvest decisions are made.

False positives in Earth Observation may occur when crop spectral response no longer fully reflects the actual harvestable biomass condition. UAV-based monitoring can show very good predictive performance in well-developed and spatially uniform stands. However, in mountainous regions where maize reaches physiological maturity but produces limited biomass, remote sensing may falsely indicate harvest readiness despite insufficient silage yield.

A similar situation may occur during extremely dry weather conditions, when leaf senescence rapidly changes the spectral response of the canopy, while the stalk and cob still retain significant moisture. In such cases, remote sensing may indicate advanced maturity even though the actual dry matter development of the harvestable biomass remains incomplete.

These situations illustrate that Earth Observation methods should not be interpreted as isolated indicators, but rather as decision-support tools requiring agronomic context, field observations, and validation through complementary measurements.

Homogenization of harvested material is beneficial for silage stability and feed consistency, but it may simultaneously reduce the ability to evaluate the real performance and variability of individual field zones. During harvest, onboard NIR sensing continuously measures crop quality parameters across the harvested material flow. However, once the material becomes mixed during transport and storage, the direct connection between measured values and their original field location may be partially lost.

Uniform onboard harvest data do not necessarily guarantee uniform silage behavior during storage and feed-out. In practical conditions, NIR records from multiple fields may show similar dry matter values, for example within a range of 32–38 % with an average around 36 %, suggesting apparently homogeneous harvest quality. However, Earth Observation data may simultaneously reveal pronounced spatial differences in water availability, crop stress, and field moisture conditions.

These hidden differences can later influence fermentation dynamics and aerobic stability, even when the harvested material appears analytically similar during collection. Dry and stressed zones may behave differently during ensiling than wetter and physiologically active parts of the field, despite comparable average dry matter values.

CONCLUSION

The presented observations demonstrate that practical silage harvest decisions cannot rely on a single method alone. Laboratory analyses, Earth Observation methods, onboard NIR sensing, and agronomic experience each provide different spatial and temporal perspectives on crop development and harvest readiness. While laboratory analyses deliver high analytical precision, Earth Observation methods improve the understanding of field variability and crop maturity dynamics throughout the growing season.

The results further showed that similar average harvest values may still conceal important biological and spatial differences affecting silage quality, crop stability, and final utilization efficiency. Rather than replacing conventional agronomic approaches, the proposed framework highlights the value of combining complementary monitoring methods to support practical harvest decision-making under real farming conditions.

The future of practical silage management will likely depend not on replacing agronomic experience, but on improving the interpretation of increasingly complex spatial and temporal data under real farming conditions.

Conclusion of the Hybrid Observation

The hybrid comparison demonstrated that under heterogeneous foothill conditions affected by severe drought stress, the selection of suitable maize hybrids may significantly influence the final stability and homogeneity of the crop stand. Earth Observation monitoring revealed that some hybrids maintained a more balanced canopy development and maturity progression even under uneven water availability. The results suggest that selecting hybrids with stable performance under variable moisture conditions may help preserve harvest quality and improve crop uniformity during dry seasons.

Conclusion of the Hydrogel Observation

Although onboard NIR sensing did not detect statistically significant differences between the monitored variants, laboratory analyses and harvested biomass evaluation indicated a positive influence of hydrogel application on cob development and final yield. Earth Observation monitoring further revealed a higher proportion of the hydrogel-treated area within the optimal harvest interval, suggesting improved synchronization of crop maturity. The results indicate that hydrogel application may contribute to more uniform harvest conditions, improved cob maturation, and potentially higher starch accumulation under variable environmental conditions.

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PART 2

FORAGE PRODUCTION AND SILAGE FERMENTATION PROCESSES

EFFECTS OF APPLIED LACTIC ACID BACTERIA (LAB) ON FERMENTATION QUALITY OF GRASS SILAGES FROM AUSTRIAN DAIRY FARMS

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Abstract

Silage additives were evaluated using 600 Austrian grass silage samples from the LK Silage Project 2024. Homofermentative Lactic Acid Bacteria (LAB) improved fermentation by increasing lactic acid and reducing butyric acid and dry matter losses, while heterofermentative LAB increased acetic acid and aerobic stability. Economic evaluation showed that homofermentative LAB significantly reduced nutrient losses, highlighting the practical value of LAB additives in grass silage production.

Keywords: silage quality, fermentation losses, butyric acid, aerobic stability

INTRODUCTION

Ensiling of forage is a practical challenge to preserve the nutrients quantitatively and qualitatively for a longer period by inactivating microbial and feed-specific enzymes, i.e. to limit the decomposition processes to an unavoidable or desirable extent (Steinhöfel *et al*., 2023). According to Zhang *et al.* (2023), the application of homofermentative Lactic Acid Bacteria (LAB-homo), like *L. plantarum*, improves the fermentation process, while heterofermentative Species (LAB-hetero, i.e. *L. buchneri*) increase aerobic stability (ASTA) after the silo is opened (Kung *et al*., 2018). At Austrian dairy farms, the average amount of butyric acid (BA) of first cut grass silages is above 12 g/kg DM (SD = 10 g/kg DM) in the past ten years, due to low nitrate and too high clostridia-level in fresh grass (Resch, 2025). In the Austrian 'LK Silage Projects', a trend toward increased use of LAB silage additives was observed between 2003 and 2024. In the following evaluations, the fermentation success of the LAB treatment is assessed on the basis of the BA content and the formation of acetic acid (AA) or 1,2-Propanediol (1,2-PD) due to the activity of LAB-homo and LAB-hetero or a mixture of both types (LAB-Mix homo/hetero). In addition, the economic impact of successful LAB-homo fermentation is calculated, based on the results of the precise silage trial S-81 (Resch *et al*., 2025), using the example of a dairy farm to illustrate the economic significance of LAB application in practice.

MATERIAL AND METHODS

In 'LK Silage Project 2024', a total of 600 grass silage samples were taken from trained persons across Austria, mainly from dairy farms. Determination of DM, nutrients, pH-value and volatile organic compounds (VOC) was performed with wet chemistry methods (VDLUFA, 2012) at the Rosenau feed laboratory in lower Austria. DM and nutrient correction of the silages was carried out by calculations of Weißbach and Kuhla (1995). Data of silage management, especially the application of silage additives was surveyed in detail using a questionnaire covering product information, dosing methods and application details (Resch, 2025). The calculation of losses due to ensiling (DM, Protein, Energy) is based on the test results of Resch *et al.* (2025) and the balancing method of Köhler *et al.* (2019). The protein loss in the silage was determined by subtracting of the mass of NH_3-N from total N, as NH_3-N is formed during N-degradation. Statistical analysis was carried out by procedure GLM (Statgraphics Centurion XVII). The mean differences were evaluated using the Tukey-HSD method and a confidence level of 95%.

RESULTS AND DISCUSSION

In 2024, 34% of analysed grass silages were treated with additives to improve silage quality. The majority (31%) was treated with LAB. However, only 3% of them got chemical ingredients. The following fermentation quality results of grass silages in table 1 refer to GLM-adjusted mean values for Dry Matter (DM), aNDFom and crude ash (CA). In comparison to untreated grass silage (CON), application of LAB effectively lowered the pH value and reduced the BA content, especially LAB-homo. Chemical ingredients had no statistical influence on BA-reduction. There was no significant effect of additives on formation of lactic acid (LA), but LAB-homo increased LA up to 70 g/kg DM. Treatment with LAB-hetero significantly increased acetic acid (AA) to 22 g/kg DM. According to Kung *et al.* (2018), aerobic stability (ASTA) is improved by an increase in AA with LAB-hetero. The formation of 1,2-PD > 5 g/kg DM confirms sufficient activity of *L. buchneri* via LAB-hetero or LAB-Mix (homo/hetero). In silage treated with LAB-homo, AA and 1,2-PD were lower than in CON and LA/AA ratio was

Tab. 1: Effect of different silage additives on fermentation parameters of grass silage

forage treatment	samples		pH	LA	AA	PA	1,2-PD	BA	ETH	NH_3-N	WSC
	n	%		g	g	g	g	g	g	%	g
no additive (CON)	396	66.0	4.48^{b}	56.8^{a}	12.7^{a}	0.4^{a}	2.2^{a}	7.8^{b}	6.8^{a}	6.0^{b}	63.8^{b}
LAB-homo	28	4.7	4.27^{a}	70.0^{a}	9.8^{a}	0.3^{a}	1.9^{ab}	1.6^{a}	5.5^{a}	5.3^{ab}	55.8^{ab}
LAB-hetero	50	8.3	4.30^{a}	62.4^{a}	22.1^{b}	0.6^{a}	11.8^{c}	4.2^{ab}	6.3^{a}	6.2^{ab}	39.7^{a}
LAB-Mix (homo/ hetero)	107	17.8	4.34^{a}	59.5^{a}	14.1^{a}	0.3^{a}	6.3^{b}	3.7^{a}	5.6^{a}	5.1^{a}	50.4^{a}
chemical additive	19	3.2	4.38^{ab}	60.5^{a}	12.9^{ab}	0.5^{a}	2.6^{ab}	9.9^{ab}	12.6^{a}	6.0^{ab}	49.7^{ab}

Abbreviations: pH (pH-value), LA (lactic acid), AA (acetic acid), PA (propionic acid), 1,2-PD (1,2-Propanediol), BA (butyric acid), ETH (Ethanol), NH_3-N (Ammonia-N in % of total N), WSC (Water soluble carbohydrates)

GLM–adjusted means: DM = 378 g/kg FM; aNDFom = 414 g/kg DM; CA = 100 g/kg DM. Means with different superscript letters (a, b, ab) within a column differ significantly (P < 0.05).

https://doi.org/10.11118/978-80-7701-093-1-0074

above 3.0, so ASTA tends to decrease. N-Degradation to ammonia-N was reduced by LAB-Mix and we observed no effect of additives on ethanol content. With regard to water soluble carbohydrates (WSC), LAB-hetero significantly decreased WSC.

In silage trial S-81(Resch *et al*., 2025), LAB-homo reduced DM losses in wet grass silage (23.7% DM) during ensiling by 6.8% compared to CON (11.7%). Applied to an example on an agricultural scale with a harvest yield of 100 tons of dry matter, this corresponds to a gain of 6,800 kg of silage dry matter through the use of LAB-homo. This benefit of LAB-homo corresponds to the harvest yield of approx. 2.3 hectares of grassland forage or 680 feeding days for dairy cows with a daily grass silage DM intake of 10 kg DM/cow per day. In this trial, energy losses (ME) were reduced by 8.7% and protein quality losses by as much as 14% compared to CON, demonstrating that the use of LAB-homo can also be highly successful in terms of feed value.

CONCLUSION

In the application of LAB for grass silage, two successful effects have emerged in the 'LK Silage Project 2024' under practical conditions in Austria. On the one hand, LAB-homo improved lactic acid formation and the fermentation process, so that on average it was possible to reduce BA-formation significantly and thus also DM losses. The downside of this application is a tendency toward poorer ASTA. On the other hand, heterofermentative LAB (*L. buchneri*, etc.) were also able to reduce BA formation. However, this required more energy in the form of WSC. Nevertheless, LAB-hetero resulted in increased AA levels and thus improved ASTA, which is particularly necessary at higher air temperatures and deficits in compaction, as well as low feed consumption at the silo face. For farmers who cannot clearly decide between the two possibilities, LAB-MIX (homo/hetero) additives represent an alternative, but those are often less successful in terms of fermentation and ASTA effects.

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THE EFFECT OF DIFFERENT COMBINATIONS OF LACTIC ACID BACTERIA ON ESTER CONCENTRATIONS IN GRASS AND WHOLE-PLANT MAIZE SILAGE

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Abstract

Alcohol esters such as ethyl lactate and ethyl acetate are discussed as having a negative effect on silage palatability and intake. This study evaluated ester formation in grass and whole-plant maize silages treated with combinations of homo- and heterofermentative lactic acid bacteria. Concentrations of ethyl lactate and ethyl acetate were determined and compared with values estimated using a commonly applied ethanol-based prediction equation. Additive treatments influenced ester profiles, demonstrating treatment effects on fermentation pathways. However, under optimal ensiling conditions with limited air exposure and inoculation with heterofermentative lactic acid bacteria, ethanol concentration alone did not reliably predict ethyl lactate and ethyl acetate concentrations, and estimated values were generally higher than analytically determined concentrations. The results indicate that direct chemical determination of alcohol esters is necessary for accurate evaluation of silage fermentation characteristics, especially when silage quality or palatability is of concern.

Keywords: silage, alcohol esters, ethyl lactate, ethyl acetate, ethanol, lactic acid bacteria, fermentation quality, silage additives, VOCs

INTRODUCTION

Silages with elevated ethanol concentrations are frequently reported to exhibit reduced palatability. Weiss (2016) demonstrated a strong positive correlation between ethanol concentration and ethyl ester levels in silages, specifically ethyl lactate and ethyl acetate. These volatile organic compounds (VOCs) are often discussed as potential contributors to diminished feed intake and adverse effects on animal health. Owing to the labour-intensive and costly nature of VOC analyses, Weiss *et al.* (2017) proposed an estimation equation based on ethanol concentration.

The objective of the present study was to quantify alcohol esters in grass and maize silages treated with various silage additives and to evaluate their relationship to ethanol concentration.

MATERIAL AND METHODS

In experiment I, grass was ensiled in round bales at a dry matter (DM) content of 407 g/kg. The herbage originated from a field in northwestern Germany. During baling, grass was treated with three commercial silage inoculants. Nine bales were treated with a mixture containing only homofermentative lactic acid bacteria (HF; *L. plantarum*, *Enterococcus lactis*, and *Lactococcus lactis*). Eight bales were treated with an additive containing a mixture of two strains of homo- and heterofermentative lactic acid bacteria (LLLB; *Lactococcus lactis* and *L. buchneri*). Seven bales were treated with an additive containing a mixture of three strains of homo- and heterofermentative lactic acid bacteria (LBLDLP; *L. buchneri*, *L. diolivorans*, and *L. plantarum*). Bales were sampled after 100 days of ensiling.

In experiment II, whole-plant maize was ensiled in 1.7-liter volume mini-silos at a DM content of 339 g/kg. Five mini-silos per treatment were prepared. Control treatment (CON) was not inoculated. LLLB treatment was inoculated with a mixture of *Lactococcus lactis* and *L. buchneri*. LBLDLR treatment was inoculated with a mixture of *L. buchneri*, *L. diolivorans*, and *L. rhamnosus*. LBLP treatment was inoculated with a mixture of *L. buchneri* and *L. plantarum*. Mini-silos were stored for 109 days at room temperature before analysis.

Silages were analysed for proximate analysis, forage hygiene, and fermentation products, including ethyl lactate,

Tab. 1: Effect of treatment on the silage fermentation characteristics

	Grass silage			Whole-plant maize silage			
	HF	LLLB	LBLDLP	CON	LLLB	LBLDLR	LBLP
DM g/kg	388	407	371	347	342	344	347
pH	4.32^{c}	4.64^{a}	4.43^{b}	3.76^{d}	3.98^{a}	3.84^{a}	3.80^{c}
Lactic acid g/kg DM	68.3^{a}	36.6^{b}	38.0^{b}	46.6^{a}	29.6^{d}	33.7^{c}	42.0^{b}
Acetic acid g/kg DM	7.0^{c}	26.7^{b}	39.4^{a}	17.4^{b}	30.5^{a}	31.8^{a}	18.5^{b}
Propionic acid g/kg DM	nd^{c}	0.3^{b}	3.4^{a}	nd^{c}	1.3^{a}	0.4^{b}	0.4^{b}
Ethanol g/kg DM	5.7^{b}	10.5^{a}	9.8^{a}	9.8^{c}	12.8^{b}	9.0^{c}	14.9^{a}
Propanol g/kg DM	0.4^{c}	2.0^{b}	18.0^{a}	1.1^{c}	8.7^{b}	11.8^{a}	1.3^{c}
1,2-propanediol g/kg DM	1.5^{c}	18.7^{a}	8.1^{b}	3.9^{c}	5.2^{b}	nd^{d}	8.1^{a}
Methanol mg/kg DM	367^{a}	274^{b}	242^{b}	163^{b}	164^{b}	229^{a}	171^{b}
Ethyl acetate mg/kg DM	nd^{b}	68^{a}	83^{a}	44^{b}	133^{a}	70^{b}	54^{b}
Propyl acetate mg/kg DM	nd^{b}	20^{b}	232^{a}	nd^{c}	103^{b}	146^{a}	nd^{c}
Ethyl lactate mg/kg DM	72^{a}	24^{b}	54^{a}	123^{b}	102^{c}	78^{d}	152^{a}

HF = *L. plantarum*, *Enterococcus lactis*, and *Lactococcus lactis*; LLLB = *Lactococcus lactis* and *L. buchneri*; LBLDLP = *L. buchneri*, *L. diolivorans*, and *L. plantarum*; CON = untreated whole-plant maize silage, LBLDLR = *L. buchneri*, *L. diolivorans*, and *L. rhamnosus*; LBLP = *L. buchneri* and *L. plantarum*; DM = dry matter corrected for volatiles; nd = not detected, set as 0 for the statistical analysis. Values with different superscript letters within crop are significantly different ($P < 0.05$).

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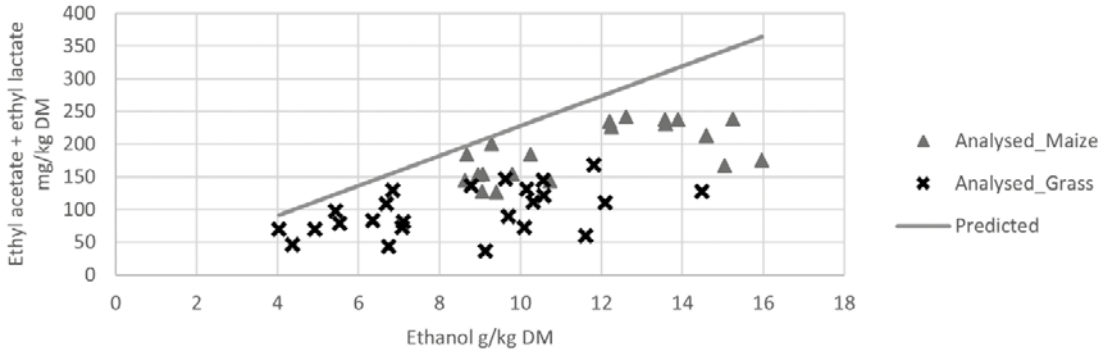


Fig. 1: Comparison between predicted as *Σ ethyl lactate + ethyl acetate (mg/kg DM) = ethanol (g/kg DM) × 114/5* (Weiss 2017) and analysed concentrations of ethyl acetate and ethyl lactate in grass and whole-plant maize silages

ethyl acetate, and propyl acetate. Predicted content of the sum of ethyl lactate and ethyl acetate was calculated with the equation of Weiss (2017). Data were analysed separately for each experiment as a completely randomized design using treatment as a fixed factor.

RESULTS AND DISCUSSION

The products LLLB and LBLDLP significantly increased acetic acid and ethanol in grass silage compared with HF treatment (Table 1). In experiment II, LBLP silage had the highest ethanol concentration, followed by LLLB.

The increased concentrations of acetate and ethanol in silages treated with products containing *L. buchneri* are consistent with the proposed pathway for anaerobic degradation of lactic acid into 1,2-propanediol, acetic acid, and trace amounts of ethanol (Oude Elfering *et al*., 2001). The predicted concentrations of ethyl acetate and ethyl lactate were higher than the chemically analysed values in all samples (Figure 1).

The weak correlation between ethyl esters and ethanol content in grass silages can be explained by the high pH (>4.25) observed in our study (Weiss 2017). Weiss (2022) reported that the regression lines for ethyl acetate and ethyl lactate with respect to ethanol content may exhibit different slopes depending on the practical silo type and sampling time. In our experiments, silages were prepared under optimal conditions: immediate wrapping of bales after grass baling and airtight sealing of mini-silos within 3 hours after maize harvest. The silages were not exposed to air before sampling. The counts of yeasts and moulds in silages were below the detection level of 500 cfu/g in all treatments. Weiss *et al*., (2022) confirmed that yeasts can produce ethyl acetate. Yeasts are also associated with increased ethanol content in silages and increased dry matter loss (McDonald *et al*, 1991). In our study, we used products containing *L. buchneri*. On one side, these bacteria convert lactic acid into acetic acid and trace amounts of ethanol. On another side, acetic acid produced by *L. buchneri* inhibits yeasts. The dry matter loss was below 5 % in all treatments (data not shown). It can explain the weaker correlation between ethanol content and the sum of ethyl esters in our study.

CONCLUSION

Previous studies demonstrated a strong correlation between ethanol content and ethyl esters, especially in conditions where high ethanol content was associated with greater dry matter losses in the presence of yeasts in silage. Our study demonstrated that under optimal ensiling conditions with minimal air exposure and silage inoculation with heterofermentative lactic acid bacteria, which reduces yeast counts, ethanol concentration alone cannot reliably predict ethyl lactate and ethyl acetate levels. Consequently, in situations where silage quality or palatability is of concern, direct chemical determination of alcoholic esters is recommended to ensure accurate evaluation.

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CHANGES IN LACTIC AND ACETIC ACID, PH, DRY MATTER AND TEMPERATURE DURING AEROBIC DETERIORATION

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Abstract

Oat whole crop silage was produced in glass jars with addition of homofermentative lactic acid bacteria, yeast and 1% or 2% of acetic acid. Jars were opened after and 49 days. On days 1 to 7 of exposure to air, lactic acid, acetic acid, pH and dry matter were measured daily. Temperature was measured continuously. The addition of acetic acid delayed aerobic spoilage. Dry matter, lactic acid and acetic acid decreased during aerobic deterioration, whereas temperature and pH increased. However, the rise in pH was delayed compared to the temperature rise. The drop in fermentation acid and dry matter can only be determined by destructive measures and are not suitable for routine spoilage control. Therefore, the rise in temperature remains a sensitive and early parameter for the detection of silage deterioration.

Keywords: oat silage, acetic acid, aerobic deterioration

INTRODUCTION

An aerobically stable, high-quality silage is essential for high milk-yield and animal health. Therefore, it is necessary to know the biological and chemical processes during aerobic deterioration. Frequently, a rise in temperature is being reported during aerobic deterioration, but other parameters like lactic, acetic acid, pH, and dry matter content are also affected. A high yeast count can have a negative impact on aerobic stability. Therefore, preservatives which inhibit yeasts are applied to avoid aerobic spoilage.

The objective of this study was to investigate changes in lactic acid, acetic acid, pH, temperature and dry matter as early predictors of spoilage in silage during aerobic deterioration.

MATERIAL AND METHODS

Oat whole crop silage was produced in 1.5 L glass jars. The forage was inoculated with lactic acid bacteria (LAB) composed of Lactiplantibacillus plantarum DSM 8862 and DSM 8866 at a rate of 3×10^5 CFU/g FM. (BIO-SIL®, Dr. Pieper Technologie- und Produktentwicklung GmbH, Germany). To increase the number of acid-degrading yeasts, Pichia kudriavzevii (YEA) were added (1×10^6 CFU/g FM). Acetic acid (AA) was added at 10 g or 20 g/kg FM (see Table 1).

Jars were opened after 10 and 49 d. On days 1 to 7 of exposure to air, lactic acid, acetic acid, pH, and dry matter were measured daily. Temperature was measured continuously (wireless).

RESULTS AND DISCUSSION

The nutrient content of the ensiled oat (*Avena sativa L.*) corresponds to a typical oat at the beginning of the dough-ripe stage (Jeroch *et al.*, 1993). The starting material was in the range of moderate to easy-to-ensile fodder with a high dry matter content (DLG, 2011). The nitrate content was below the optimum range (Table 2).

Dry matter, lactic acid and acetic acid decreased during aerobic deterioration, whereas temperature and pH increased. After 10 d of ensiling, the addition of yeast caused an accelerated aerobic deterioration by 0.8 days (CON vs CON + YEA) and 2.2 days (AA1% vs AA1% + YEA; data not shown) whereas after 49 d of ensiling, there was no effect of addition of yeast observed in any group (Figure 1). On day 49 of ensiling, the addition of 1% acetic acid to the forage delayed aerobic deterioration by 1.2 days. When adding 2% acetic acid to the forage, silages were stable over 7 days after 10 and 49 days of ensiling even when challenged with lactate assimilating yeasts.

In all cases of aerobic deterioration, lactic and acetic acid decreased at the same time as the rise in temperature. The increase of pH-value was delayed by 1 to 2 days.

Tab. 1: Ensiling of oat using lactic acid bacteria (LAB) composed of *L. plantarum* DSM 8862 and DSM 8866 and acetic acid (AA) as a chemical preservative. To group B, D, and F, lactate assimilating yeasts of the species P. kudriavzevii (YEA) were added. FM = fresh matter

Group	Name	Inoculation density LAB [CFU/g FM]	Inoculation density YEA [CFU/g FM]	Acetic Acid [g/kg FM]
A	CON	3×10^5	–	–
B	CON + YEA	3×10^5	1×10^6	–
C	AA1%	3×10^5	–	10
D	AA1% + YEA	3×10^5	1×10^6	10
E	AA2%	3×10^5	–	20
F	AA2% + YEA	3×10^5	1×10^6	20

Tab. 2: Forage characteristics of the fresh harvested oat (*Avena sativa L.*). BC = buffering capacity; FC = fermentation coefficient. FM = fresh matter, DM = dry matter)

Dry matter [g/kg FM]	Crude ash [g/kg DM]	Crude protein [g/kg DM]	Crude fibre [g/kg DM]	Crude fat [g/kg DM]	Sugar [g/kg DM]	Starch [g/kg DM]	BC [g/kg DM]	FC	NO_3 [g/kg DM]
380	57	59	235	40	57	218	17.1	64	1.1

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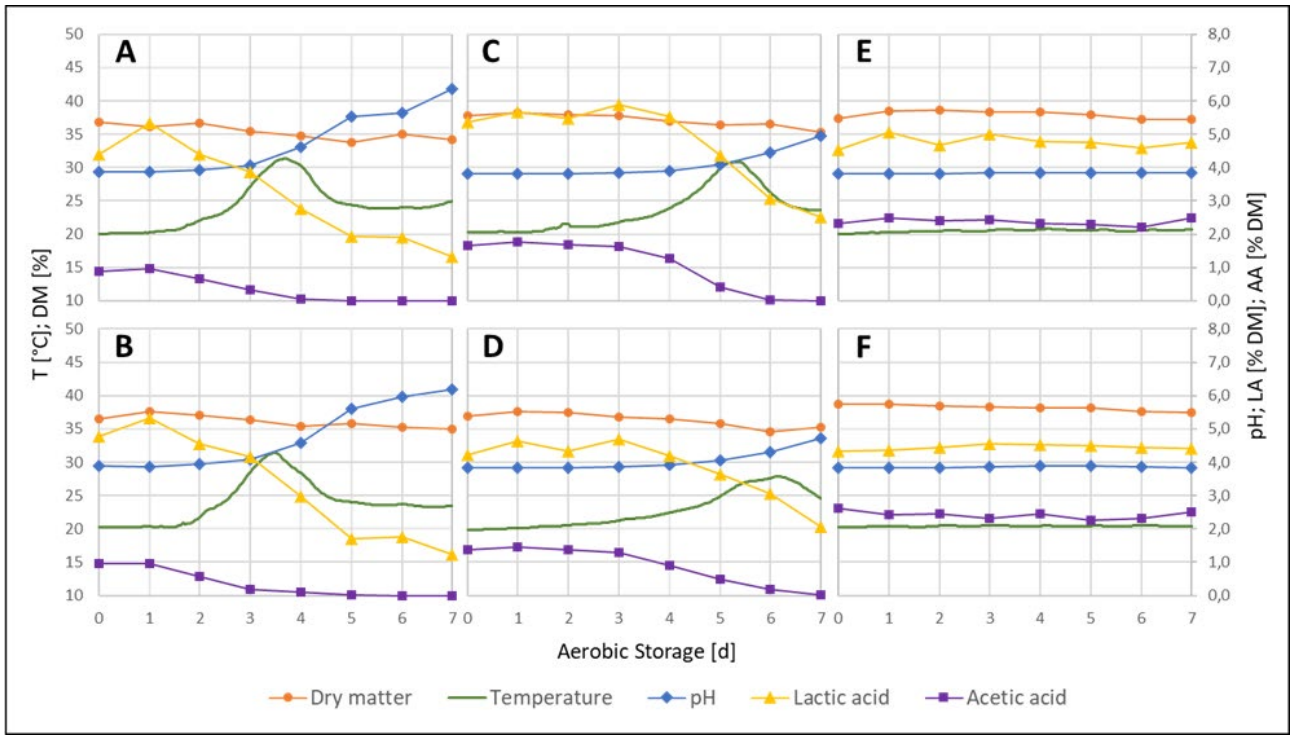


Fig. 1: Temperature and pH profile as well as dry matter (DM), lactic acid (LA), and acetic acid (AA) content during aerobic storage of oat silage inoculated with 3×10^5 CFU *L. plantarum*/g fresh matter (FM) after 49 days of ensiling. A and B: Without addition of AA; C and D: addition of 10 g AA/kg FM; E and F: addition of 20 g AA/kg FM. Upper row: without addition of lactate assimilating yeasts (YEA); bottom row: with addition of 10^6 YEA/g FM (*P. kudriavzevii*).

CONCLUSION

The drop in fermentation acid and dry matter can only be determined by destructive measures and are not suitable for routine spoilage control. Therefore, the rise in temperature remains a sensitive and early parameter for the detection of silage deterioration.

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EFFECTS OF *ARTEMISIA ARGYI* ACTIVE MATTER ON THE AEROBIC STABILITY OF WHOLE-CROP CORN SILAGE

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Abstract

In this study, whole-crop corn was fermented by adding eudesmol, essential oil, flavanone, and their mixture (group M) extracted from *Artemisia argyi* (AA), with a blank group and a group treated with *Lentilactobacillus buchneri* (LB) serving as controls. After 90 days (d) of ensiling, samples were exposed to oxygen for 0, 1, 3, 5, and 7 d to analyze changes in fermentation quality, chemical composition, and microbial populations. The results indicated that after 90 d of ensiling, group M exhibited the lowest pH value (4.63), the highest contents of acetic acid (82.61 g/kg DM) and water-soluble carbohydrates (WSC, 26.44 g/kg DM). During the aerobic exposure period, group A had a significantly lower pH value than the control group (CK), while groups J, H, and M had higher WSC content than the CK. Group M, in particular, had the highest dry matter, crude protein and WSC content, the lowest ammonia nitrogen content, and a relatively lower abundance of harmful microorganisms. In summary, the addition of AA active components effectively suppressed microbial metabolism and improved the fermentation quality of silage, providing a theoretical basis for the development of new feed supplements and the enhanced utilization of traditional Chinese herbal medicine resources.

Keywords: whole-crop corn, *Artemisia argyi*, active components, silage, aerobic exposure

INTRODUCTION

Whole-crop corn (WCC) silage, with its high nutritional value and excellent palatability, is the predominant roughage source for dairy cows and other ruminants (Pang *et al*., 2024). Silage is prone to aerobic microbial activity, causing pH rise and spoilage (Pang *et al*., 2011, Pang *et al*., 2023). Therefore, how to inhibit the proliferation of aerobic microorganisms and improve the quality of silage during ensiling has become a research focus in recent years. In this study, active components of *Artemisia argyi* (AA) were added to WCC at the milky stage for 90 days (d) of mixed ensiling, followed by aerobic exposure. By comparing the fermentation quality, chemical composition, and microbial communities of WCC supplemented with different active components of AA, this study aims to elucidate the effects of AA active components on silage quality during ensiling and aerobic exposure, thereby providing a theoretical basis for the development of novel feed additives.

MATERIAL AND METHODS

Whole-crop corn (WCC) at the milky stage (Xingyang, Henan Province); whole-plant AA (Anyang, Henan Province); LB provided by China Agricultural University; AA essential oil (purity: 990 g/kg) and crude flavonoids (purity: 150 g/kg) were purchased from Shaanxi Taihe Kangze Biotechnology Co., Ltd.; eucalyptol (purity: 990 g/kg) was purchased from Shanghai Macklin Biochemical Technology Co., Ltd.

The experimental design was as follows: based on the additive type, the treatment groups were designated as A, J, H, M, and LB, which received eucalyptol, essential oil, flavonoids, and a mixture of these three components derived from AA, respectively, at a rate of 600 g/kg of WCC. WCC (CK) and LB (6.00 lg cfu/g) were set as control groups. The moisture content was adjusted to 650 g/kg fresh matter. The mixture (300 g) was placed into fermentation bags and vacuum-sealed. Each group had 3 replicates and was fermented for 90 d, followed by aerobic exposure at room temperature (16-35 °C) for 0, 1, 3, 5, and 7 d. At each opening, the fermentation quality, chemical composition, and microbial communities were analyzed.

RESULTS AND DISCUSSION

After 90 d of ensiling, group M exhibited the lowest pH value, along with the highest lactic acid and water-soluble carbohydrate (WSC) contents, reaching 4.63, 82.61 g/kg DM, and 26.44 g/kg DM (dry matter), respectively. In contrast, group LB showed the lowest ammonia nitrogen and acetic acid contents, at 0.13 g/kg DM and 28.11 g/kg DM, respectively. With With prolonged aerobic exposure, the pH value of each treatment group increased significantly, while the concentrations of WSC, lactic acid and acetic acid decreased significantly. After 7 d of aerobic exposure, group A had the lowest pH value and the highest lactic acid and acetic acid contents, whereas group M had the highest WSC content and the lowest ammonia nitrogen content.

For chemical composition, after 90 d of ensiling, Group M had the highest dry matter (DM) and crude protein (CP) contents, reaching 409.0 g/kg and 74.88 g/kg DM, respectively, while the contents of acid detergent fiber (ADF) and neutral detergent fiber (NDF) were the lowest, at 298.00 g/kg DM and 436.33 g/kg DM, respectively. As aerobic exposure time increases, DM content in all treatment groups continued to rise, ADF and NDF contents gradually decreased, while CP content exhibited dynamic changes. After 7 d of aerobic exposure, group J achieved the highest DM content, group M had the lowest ADF and NDF contents, and group H showed the highest CP content.

In this study, the Chao1 index and Shannon index were used as key diversity indicators to evaluate the species richness and diversity of microbial communities under different treatment conditions. For bacterial communities, the Shannon index of the H-90 group was significantly higher than that of the other groups, indicating that this group had the highest bacterial community diversity. After 3 days of aerobic exposure, the A-90-3d group showed the lowest Shannon index, suggesting that different treatments had a significant impact on bacterial diversity. For fungal communities, Shannon index analysis showed that the fungal community diversity was highest in the WCC group and lowest in the J-90 group, with extremely significant differences among treatments

At the bacterial phylum level, after 90 d of ensiling, the dominant phylum in all treatment groups was Firmicutes. After 3 d of aerobic exposure, the relative abundance of Firmicutes decreased in all treatment groups except the M-90 group, where it increased. This may be attributed to the inhibition of Firmicutes bacteria, such as lactic acid bacteria, during aerobic exposure, along with the proliferation of aerobic microorganisms and the rise in pH, which is unfavorable for the growth of Firmicutes. At the genus level, for bacteria (Figure 2a), after 90 d of ensiling, the dominant genus in the A-90, H-90, and M-90

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groups was *Lactiplantibacillus*. After 3 d of aerobic exposure, the A-90-3, J-90-3, and M-90-3 groups remained dominated by *Lactiplantibacillus*, with relative abundances of 0.2573, 0.1869, and 0.2973, respectively. At the fungal phylum level, after 90 d of ensiling and 3 d of aerobic exposure, the fungal communities in all treatment groups were dominated by Ascomycota, with no significant differences observed. At the fungal genus level (Figure 2b), *Candida* was predominant in the H-90 and M-90 groups, while varying proportions of *Candida* were detected in the other treatment groups.

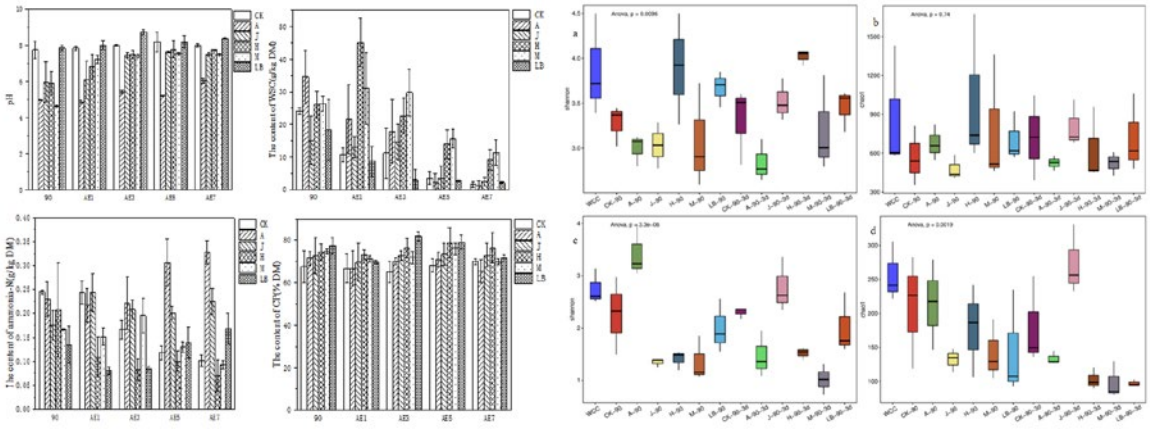


Fig. 1: Fermentation quality and chemical composition of silage, and Alpha diversity at the OTU level (a, b and c, d: Shannon, Chao1 index of bacterial and fungal community, respectively)

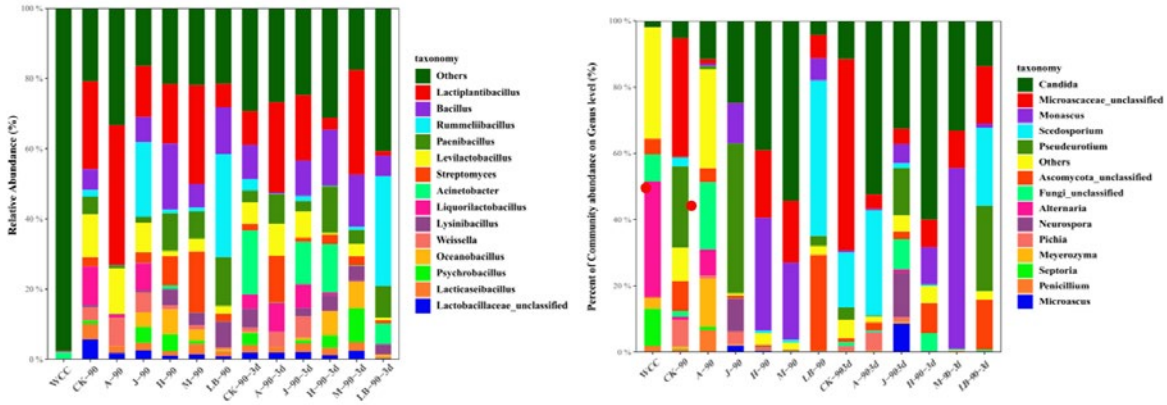


Fig. 2: Bacterial (a) and fungal (b) community composition of WCC at the genus levels

CONCLUSION

In this study, WCC at the milky stage was ensiled with the addition of active components derived from AA (eucalyptol, essential oil, and crude flavonoids). After 90 d of ensiling, group M exhibited the lowest pH value, along with the highest lactic acid, WSC contents, DM and CP contents. As the duration of aerobic exposure increased, the treatment groups supplemented with AA active components demonstrated better stability, characterized by a slower increase in pH and reduced losses of WSC and organic acids. This indicates that the addition of AA active components can effectively delay aerobic spoilage and maintain silage quality. In summary, the addition of AA active components significantly improves the fermentation quality and aerobic stability of WCC silage, inhibits the growth of harmful microorganisms, reduces nutrient loss, and thereby enhances the preservation capability and feeding value of the silage.

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EFFECTS OF *ARTEMISIA ARGYI* ADDITION ON FERMENTATION QUALITY AND GREENHOUSE GAS EMISSIONS OF CORN STOVER SILAGE

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ABSTRACT

Although silage can efficiently utilize forage, its fermentation process produces greenhouse gases, resulting in nutrient loss and environmental pollution. In this study, corn stover was used as the raw material and *Artemisia argyi* (AA) as an additive for mixed silage preparation, and the variations of microbial community, fermentation quality, chemical composition and greenhouse gas emissions during ensiling were analyzed. The results demonstrated that compared with the single corn stover silage, silage mixed with AA could enriched beneficial bacteria such as Lactiplantibacillus and inhibited harmful bacteria including Enterobacter, lowered the pH value, increased lactic acid content, and reduced emissions of greenhouse gas containing CO_2, CH_4, and N_2O more than 65%. This study offers reference for additive development, straw silage optimization, and the realization of the carbon neutralization targets in animal husbandry.

Keywords: forage preservation, delayed ensiling, bacterial strains, aerobic spoilage

INTRODUCTION

Corn stover is characterized by high crude fiber content, low nutritional value, insufficient moisture and soluble carbohydrates, and a low abundance of epiphytic lactic acid bacteria (LAB) on its surface, which makes natural ensiling difficult (Zhou *et al*., 2025). Meanwhile, greenhouse gases (GHGs) are generated during the silage fermentation process. When *Artemisia argyi* (AA) is used as an additive for corn fermentation, it can improve feed quality, reduce mycotoxin levels and enhance aerobic stability. At present, safe Chinese herbal medicines are rarely mentioned in the research on silage additives. In the present study, *Artemisia argyi* (AA) was utilized as a silage additive (Wang *et al*., 2023). This research is expected to provide a reference for the development of novel additives for straw silage, facilitate the efficient utilization of straw resources, and establish technical systems for GHGs emission mitigation in agriculture and animal husbandry

MATERIAL AND METHODS

TCorn stover and whole plant of *Artemisia argyi* (AA) were obtained from Henan, China in Sep., 2024. Four experimental treatments were set up as follows: CS (corn stover), 30% AA (corn stover supplemented with 30% *Artemisia argyi*), 60% AA, and AA (pure *Artemisia argyi* as the control group). Microbial populations were analysed via the dilution plate spreading method. Samples were filtered through four layers of gauze to determine pH values and LA concentrations. Samples were dried at 65 °C for 48 h and weighed to establish dry matter (DM) content. Thereafter, the dried samples were ground and sieved, followed by the determination of water-soluble carbohydrate (WSC), neutral detergent fibre (NDF), acid detergent fibre (ADF), and CP concentrations. The contents and concentrations of CO_2, CH_4 and N_2O were determined by the water displacement method combined with gas chromatography. Determination of microbial community was analyzed by high-throughput sequencing. Metabolic components were measured by liquid chromatography-tandem mass spectrometry (LC-MS/MS).

RESULTS AND DISCUSSION

Sampling and analysis of corn stover silage across different fermentation durations revealed that the groups supplemented with 30% and 60% AA maintained consistently lower pH values and higher CP contents throughout ensiling, while reducing ammonia nitrogen and NDF and ADF concentrations, thereby optimising overall silage quality.

At the phylum level (Figure 1a), the dominant flora shifted from Proteobacteria to Firmicutes on day 3 of fermentation. The relative abundance of Firmicutes in the 60% AA group further increased on day 30 compared with that on day (Figure 1b).

Among all treatments, the 60% supplementation ratio demonstrated the most pronounced improvement in the fermentation quality of corn stover silage. Analysis on the dynamic variations of greenhouse gas emissions (Figure 2) indicated that the 30% and 60% AA groups exhibited a pronounced inhibitory effect on the production of CO_2 and CH_4. AA treatment enriches beneficial microorganisms such as LAB and inhibits the proliferation of harmful bacteria. By regulating pivotal metabolic pathways including amino acid biosynthesis and dicarboxylic acid metabolism, it ultimately suppresses the production of greenhouse gases such as N_2O, CO_2 and CH_4 (Figure 3).

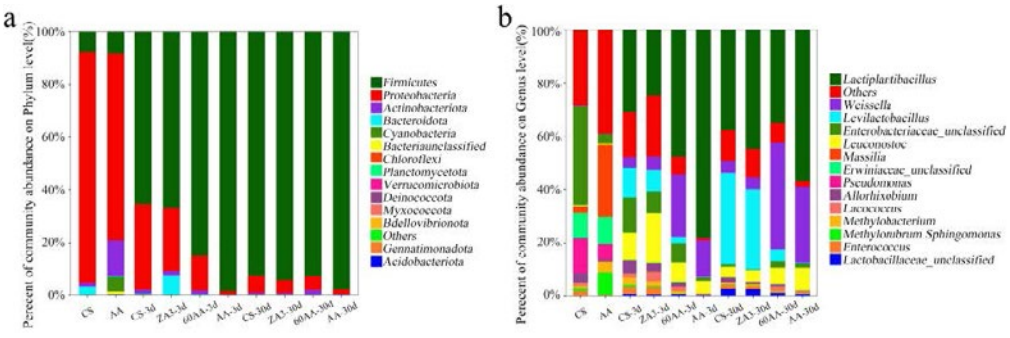


Fig. 1: Microbial community at the phylum level of corn stover silage after 30 days of ensiling

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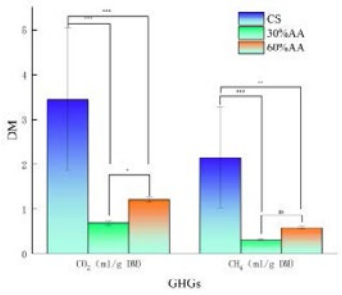


Fig. 2: Greenhouse gas variations

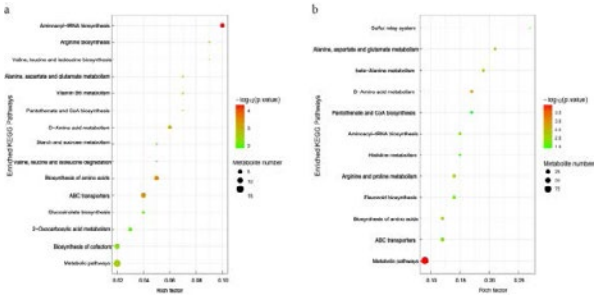


Fig. 3: KEGG functional annotation after 30 days of ensiling

CONCLUSION

Appropriate supplementation of AA reduces greenhouse gas emissions, pH values and crude fiber content of silage, improves the efficiency of lactic acid fermentation, and increases water-soluble carbohydrate levels, thereby enhancing the quality of straw forage. The optimal application ratio is 60% AA addition to corn stover silage.

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EFFECTS OF *LACTIPLANTIBACILLUS PLANTARUM* AND *PEDIOCOCCUS PENTOSACEUS* ON THE FERMENTATION CHARACTERISTICS AND AEROBIC STABILITY OF GRASS/LEGUME SILAGE

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ABSTRACT

This study evaluated the effect of an inoculant (containing *Lactiplantibacillus plantarum* and *Pediococcus pentosaceus* in combination with xylanase enzyme) on the chemical composition, fermentation characteristics, and aerobic stability of grass/legume silage. The experiment was conducted using an in vitro mini-silo system in accordance with DLG guidelines, with silages analyzed after 90 days of fermentation. Compared with the untreated control, inoculated silage showed significantly higher contents of dry matter (DM), DM corrected for volatiles (DMc), crude protein (CP), and water-soluble carbohydrates (WSC), as well as reduced overall DM losses during storage. The inoculant significantly improved fermentation quality, as indicated by lower pH values, higher total acid concentrations, and increased levels of individual organic acids, particularly lactic acid. In addition, concentrations of undesirable fermentation products, including ammonia-N, alcohols, and butyric acid, were significantly reduced. Microbiological analysis showed higher lactic acid bacteria counts and reduced populations of yeasts, moulds, and clostridia. During the aerobic stability test, inoculated silage maintained lower pH, lower weight losses, and reduced spoilage microorganisms, resulting in significantly longer aerobic stability. Overall, inoculant application improved fermentation efficiency, preserved nutrients, suppressed undesirable microorganisms, and enhanced the aerobic stability of grass/legume silage.

Keywords: aerobic stability, grass/legume silage, bacterial strains, fermentation, dry matter losses

INTRODUCTION

Grass/legume mixtures are increasingly used in sustainable livestock production systems because they combine the high energy yield of grasses with the protein-rich biomass of legumes. Nevertheless, these mixtures may present challenges during ensiling due to differences in fermentability and buffering capacity (Muck *et al*., 2021; Gao *et al*., 2024). The application of additives is an effective strategy to reduce the risk of undesirable fermentation, thereby limiting dry matter (DM) losses and aerobic spoilage in silage. Therefore, the use of live lactic acid bacteria (LAB) may play an important role in optimizing fermentation processes and improving the aerobic stability of mixed forages. Xylanase enzymes can be included in some silage inoculants to hydrolyze hemicellulosic components of the plant cell wall, thereby increasing the release of fermentable sugars available for LAB. The combination of LAB and enzymes is known for rapid lactic acid production and the ability to accelerate pH decline during the early stages of fermentation (Ávila and Carvalho, 2020; Kung *et al*., 2022). In addition to fermentation quality, the aerobic stability of silage during feed-out is an important factor influencing feed hygiene and nutrient retention (Wilkinson and Davies, 2019). The objective of this study was to evaluate the effects of *Lactiplantibacillus plantarum* and *Pediococcus pentosaceus* in combination with a xylanase enzyme on the fermentation characteristics and aerobic stability of perennial ryegrass/red clover/timothy silage.

MATERIAL AND METHODS

The tested additive was Josilac Classic (Josera GmbH, Germany), contained *Lactobacillus plantarum* strains (NCIMB 30083, NCIMB 30084) and *Pediococcus pentosaceus strains* (DSM 23689, DSM 23688) with a concentration of 1×10^{11} CFU g^{-1}, xylanase enzyme (43,000 HET g^{-1}), and dextrose as a carrier. The application rate was 6×10^5 CFU g^{-1} forage. The product was stored below 5 °C until use. A first-cut mixture of perennial ryegrass, red clover and timothy was harvested, wilted for 8–12 h to >30% dry matter (DM), and chopped to 2–3 cm length. Glass mini-silos (3 L) with gas-venting caps were used as experimental units. The inoculant was diluted in unchlorinated water to 6×10^8 CFU ml^{-1} and applied at 4 ml kg^{-1} forage to obtain a final dose of 6×10^5 CFU g^{-1} forage (JC). Control (C) forage received 4 ml kg^{-1} sterile unchlorinated water. Each 3-L silo was filled with 1.68 kg forage (36.6% DM) to reach a density of 1 kg DM per 5 L volume. Silos were stored at 20–21 °C for 90 days. After 90 days, silos were opened and analyzed for fresh matter and DM losses, pH, lactic acid, volatile fatty acids (acetic, propionic, butyric), ammonia-N, and alcohols. Counts of lactic acid bacteria, yeasts, and molds were determined at ensiling and at silo opening. After 90 days of storage, silages were exposed to air for 14 days. Aerobic stability was defined as the time required for silage temperature to increase by more than 3 °C above ambient temperature. Data were analyzed using one-way ANOVA in a randomized complete block design (5 replications per treatment) with SAS Proc GLM (version 8.02).

RESULTS AND DISCUSSION

Table 1 shows that the inoculant treatment (JC) significantly affected the fermentation quality, microbial characteristics, and aerobic stability of grass/legume silage compared with the control (C). The DM and volatile-corrected DM content was by 2.6% and by 2.1%, respectively, higher ($p < 0.01$) in JC than in C silage. Crude protein was by 3.9% higher ($p < 0.01$) in JC silage, whereas crude fibre concentration decreased by 15.1% ($p < 0.01$) compared with C ($p < 0.01$). Xylanase can promote a faster decline in pH, enhance fiber degradation, and potentially improve nutrient preservation and aerobic stability during ensiling (Muck *et al*., 2021). The WSC concentration retained by 76.3% higher ($p < 0.01$) in the inoculated silage compare with non-inoculated C silage. Total fermentation acids were 17.9% higher in JC ($p < 0.01$), mainly due to a 25.4% increase in lactic acid ($p < 0.01$). Butyric acid decreased by 2.6-fold in JC ($p < 0.01$), whereas acetic and propionic acids were not significantly affected by the inoculant treatment.

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Tab. 1: Effect of inoculant treatment on the fermentation and microbial characteristics, and aerobic stability of ensiled grass/legume

Variables	C	JC	LSD0.05	EMS	P
DM, g/kg	336.3b	345.2a	5.170	12.567	0.004
DM*, g/kg	348.3b	355.7a	5.118	12.314	0.010
Crude protein, g/kg DM	151.1b	157.0a	4.633	10.093	0.019
Crude fibre, g/kg DM	207.6a	176.2b	13.881	90.581	0.001
WSC, g/kg DM	17.7b	31.2a	9.771	44.885	0.013
Total acids, g/kg DM	72.45b	85.42a	7.046	23.337	0.003
Lactic acid, g/kg DM	56.52b	70.89a	6.327	18.821	0.001
Acetic acid, g/kg DM	14.99a	13.89a	2.248	2.376	0.292
Butyric acid, g/kg DM	0.47a	0.18b	0.131	0.008	0.001
Alcohols, g/kg DM	11.36a	7.04b	1.356	0.865	<0.0001
N-NH3, g/kg total N	46.28a	32.51b	5.710	15.326	0.0005
pH after 3 d.	4.82a	4.65b	0.068	0.002	0.0004
pH after 90 d.	4.40a	4.27b	0.059	0.002	0.0007
DM loss, g/kg	64.95a	42.33b	8.936	37.543	0.0004
Yeast, log10 CFU/g	1.99a	1.06b	0.157	0.011	<0.0001
Moulds, log10 CFU/g	1.74a	1.00b	0.115	0.006	<0.0001
Aerobic stability test					
pH at the end of AST (14 d.)	8.64a	4.94b	0.554	0.144	<0.0001
Fresh weight loss during AST, %	4.18a	2.74b	0.619	0.180	0.0007
Yeast after AST, log10 CFU/g	7.34a	4.85b	0.542	0.138	<0.0001
Moulds after AST, log10 CFU/g	9.00a	5.45b	0.762	0.273	<0.0001
Aerobic stability, hours	186b	256b	43.311	1743.00	0.001

*Dry matter corrected for volatiles; [a, b]Means with different superscript letters in a line indicate significant differences of $p < 0.01$

Alcohols and ammonia- N concentrations were by 38.0% and by 23.9%, respectively ($p < 0.01$) in JC silages compared with C silage. The inoculant treatment reduced silages pH values, decreasing by 0.17 units and by 0.13 units ($p < 0.01$) after 3 and after 90 days fermentation, respectively. Inoculant treatment reduced DM losses by 34.8% ($p < 0.01$). Regarding microbial counts, yeasts and moulds were reduced by 46.7% and 42.5%, respectively ($p < 0.01$). During the aerobic stability test, JC silage showed markedly mitigated aerobic deterioration: pH at the end of AST was 42.8% lower ($p < 0.01$), fresh weight losses decreased by 34.4% ($p < 0.01$), yeasts and moulds after AST were reduced by 33.9% and 39.4%, respectively ($p < 0.01$). Aerobic stability increased by 37.6% (256 vs. 186 h; $p < 0.01$).

CONCLUSION

The combined inoculant treatment significantly improved silage fermentation quality and microbial profile. It increased DM, crude protein, WSC, lactic acid, and LAB counts, while reducing crude fibre, butyric acid, alcohols, ammonia-N, DM losses, yeasts, moulds, and clostridia. In addition, the inoculated silage showed markedly reduced silage aerobic deterioration, with lower pH, reduced spoilage microorganisms, and longer stability time.

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EFFICASY STUDY OF PRESERVATIVE CONTAINING PROPIONIC ACID, SODIUM BENZOATE AND SODIUM PROPIONATE (1A700) FOR CONTROLLING MOULDS IN DIFFERENT MOIST GRAINS

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ABSTRACT

One of the principal challenges in preserving high-moisture grains is the extensive development of moulds, which significantly compromises storage stability. This study investigated the efficacy of the feed additive 1a700 containing propionic acid, sodium benzoate and sodium propionate (commercial product: KofaGrain pH5) in controlling mould growth in moist wheat, barley, and maize during extended storage. Wheat, barley, and maize obtained from commercial farms were adjusted to defined moisture levels and treated with different application rates of 1a700 and with water for a control. Treated and untreated grains were stored under controlled conditions at 25 °C and analysed after 6, 12, and up to 24 months of storage. Mould counts were determined according to VDLUFA methods at multiple storage intervals (VDLUFA, 2012). Untreated controls showed a strong, time-dependent increase in mould contamination, frequently exceeding 10^6 CFU/g. In contrast, 1a700 significantly reduced mould development in all grain types and at all storage periods ($p = 0.05$). Higher application rates consistently resulted in greater mould reduction, demonstrating a clear dose–response effect. The results confirm that 1a700 is an effective preservative for improving the storage stability of moist grains under conditions unsuitable for long-term storage.

Keywords: high-moisture grains, moulds, preservation, feed additive 1a700

INTRODUCTION

Preservation of high-moisture cereal grains represents a significant post-harvest challenge because elevated moisture levels create conditions that are highly conducive to fungal proliferation, leading to rapid spoilage, diminished nutritional quality, and increased risks of mycotoxin contamination. Propionic acid and its salts are widely recognized for their antifungal properties – they inhibit mold growth by penetrating and acidifying microbial cells, thereby disrupting metabolic functions that are critical for proliferation (Lückstädt, 2007). However, direct application of propionic acid can present practical challenges – including corrosiveness, volatility, and potential handling hazards – which has led to interest in buffered salts such as sodium propionate that offer comparable antifungal effects with improved handling characteristics (Rutenberg *et al.* 2018). In addition to propionates, sodium benzoate serves as antifungal preservatives by lowering pH and inhibiting metabolic pathways in fungi and yeasts. Although sodium benzoate is most effective under acidic conditions and its incorporation into grain preservation strategies can complement propionate-based approaches by broadening the spectrum of antifungal activity (Sofos, 2008).

Despite the widespread use of these preservatives, their effectiveness depends on multiple factors, including moisture level, application rate, storage duration, and microbial load. In order to validate the efficacy for controlling moulds of a non-corrosive preservative based on propionic acid, sodium benzoate and sodium propionate (KofaGrain pH5) a trial was conducted.

MATERIAL AND METHODS

The grains used in this study came from commercial farms close to the ADDCON plant in Bitterfeld, Germany. The study was started in July of 2023 and finished in March of 2025. The grain moisture for wheat and barley was analysed and re-moisten according to the attended grain moisture of 18 and 22 %. The necessary amount of water was sprayed onto the grain in a rotating material mixer. After spraying the grain remained in the mixer for 30 minutes. Afterwards the moisture was measured. If the grain was still to dry this procedure was repeated. In case of the corn grains, the natural given moisture was used. For each grain species and moisture level 5 kg were treated with 22.5 and 45.0 ml 1a700 for 4.5 and 9.0 l/t, respectively. The corn grains were treated with 60 and 75 ml for 12 and 15 litres per ton, respectively. The control was treated with the amount of 4.5 ml water for wheat and barley and 70 ml water for maize per 5 kg grain respectively. The grains were placed into a food processor, and the feed additive was added with a hand sprayer continuously during the mixing. The grain material was stored in 1.5 litre jars containing holes in the bottom and the cover. With cotton pads the holes were slightly closed. The grains of the different moisture levels were stored in different climate chambers at 25 °C. From the delivered grains and the grains after the storage time of 6, 12 and 24 month we took subsamples for analysing the mould counts. This was done according VDLUFA (2012) via plate counting on agar plates. For this report we used the different moisture levels (18 and 22 % DM, 3 rep. each) as a data set with one moisture and 6 replicates.

RESULTS AND DISCUSSION

All grain materials showed an increase in mould counts. But the counts were strongly affected by the different treatments (Tab. 1-3). Possibly caused by the rougher testa of the barley we found with 5.95×10^4 CFU/g higher mould counts in the raw material of that specie (Tab. 2). For wheat we found with 7.50×10^3 CFU/g a clear lower mould load. Contrarily, the wet maize kernels showed a load of 1.62×10^5 CFU/g.

The 12- or 24-month observation period showed more visible differences between the treatments. The untreated control samples of all grains experienced high mould populations, with numbers soaring past one million CFU/g – a level that poses significant challenges for grain safety and quality. In stark contrast, grains treated with the preservative 1a700, particularly at the recommended application rate of 9 litres per ton (calculated based on the initial grain moisture content), demonstrated a substantial suppression of mould growth. This effect was consistent across both wheat and barley and observed at all three monitored time points during storage.

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Tab. 1: Effect of different 1a700 application amounts on mould counts (CFU/g) over time in wet wheat grain

	Storage length (month)			
		6	12	24
	Initial mould count	Moisture (%)		
	7.50 x 10^3 CFU/g	18 / 21	18 / 22	17 / 19
Treatment	Dosage (l/t)	CFU/g		
control	0	$1.09 \times 10^{7\,a}$	$2.67 \times 10^{7\,a}$	$5.17 \times 10^{7\,a}$
1a700	4.5	$2.8 \times 10^{2\,b}$	$1.53 \times 10^{6\,b}$	$1.47 \times 10^{6\,b}$
1a700	9	$3.2 \times 10^{2\,b}$	$9.01 \times 10^{2\,c}$	$1.91 \times 10^{3\,c}$

*different letters show significant differences within a column ($p < 0.05$)

Tab. 2: Effect of different 1a700 application amounts on mould counts (CFU/g) over time in wet barley grain

	Storage length (month)			
		6	12	24
	Initial mould count	Moisture (%)		
	5.95×10^4 CFU/g	17 / 21	18 / 23	17 / 20
Treatment	Dosage (l/t)	CFU/g		
control	0	$4.97 \times 10^{6\,a}$	$2.71 \times 10^{8\,a}$	$6.25 \times 10^{7\,a}$
1a700	4.5	$3.73 \times 10^{4\,b}$	$1.09 \times 10^{5\,b}$	$2.48 \times 10^{5\,b}$
1a700	9	$5.84 \times 10^{2\,c}$	$1.40 \times 10^{3\,c}$	$3.50 \times 10^{3\,c}$

*different letters show significant differences within a column ($p < 0.05$)

Tab. 3: Effect of different 1a700 application amounts on mould counts (CFU/g) over time in wet corn grain

	Storage length (month)		
		6	12
	Initial mould count	Moisture (%)	
	1.62×10^5 CFU/g	31	19
Treatment	Dosage (l/t)	CFU/g	
control	0	$1.0 \times 10^{8\,a}$	$1.5 \times 10^{8\,a}$
1a700	12	$2.4 \times 10^{2\,b}$	$1.3 \times 10^{5\,b}$
1a700	15	$4.0 \times 10^{2\,b}$	$1.45 \times 10^{4\,c}$

*different letters show significant differences within a column ($p < 0.05$)

Corn grain also responded positively to preservative treatments, with the extent of mould reduction closely tied to the applied dosage (Tab. 3). This dose-response relationship was evident at both the 6- and 12-month storage intervals, underscoring the importance of proper dosing for effective mould control in higher-risk grains like maize. For maize the application of the highest preservative dose resulted in the most pronounced and statistically significant decline in mould counts after 12 months of storage (Table 3). This finding reinforces the critical role of adequate preservative dosage in managing mould risks in grains prone to high initial contamination.

CONCLUSION

Grain with a moisture level which is too high for a long-time storage gets rapidly and very moulded. This effect is in relation to the storage time as well. Preserving the wet grains via the additive 1a700 is decreasing the moulding significantly with increasing application rates and at all analysed storage periods. In all tested grains the highest dosage of 1a700 resulted in the highest reduction of moulds and was always significant against the control.

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THE EFFECT OF PLASMA-ACTIVATED WATER TREATMENT ON THE FERMENTATION QUALITY OF WHEAT STRAW SILAGE

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Abstract

Plasma-activated water (PAW) is a novel green functional water rich in reactive oxygen and nitrogen, while with no residues, and it has been widely used in food preservation and other fields. This study apply PAW to the pretreatment of wheat straw ensiling to investigate its effect on silage. In the research, PAW-treated and untreated control groups were set up, and the fermentation quality, chemical composition and microbial population at different ensile stages were measured. The results showed that after ensiling, PAW treatment could improve fermentation characteristics of wheat straw silage, with promoting lactic acid accumulation, optimizing nutritional composition, and reducing crude fiber loss; it also regulated microbial population dynamics by inhibiting the proliferation of molds and spoilage bacteria, and drove the microbial community towards targeted succession by silage-functional bacteria such as lactobacilli. This study confirmed the significant effect of PAW in enhancing the quality of straw silage, providing a new technical pathway for the green innovation and upgrading of silage fermentation technology.

Keywords: plasma-activated water, fermentation quality, silage

INTRODUCTION

Straw, as an agricultural by-product with an annual output of over 1 billion tons, has significant importance for sustainable agricultural development through efficient feed utilization. PAW, as a novel green treatment technology, shows broad application prospects in agriculture and the food sector. This study applied PAW to the pretreatment of wheat straw silage innovatively, revealing its effects on silage fermentation and nutrient retention by comparing key indicators at different fermentation stages and aerobic exposure stages. It provides referencer for developing low-cost, green, and efficient silage regulation technologies.

MATERIAL AND METHODS

Wheat straw irrigated with plasma-activated water (PAW) and ordinary water (CK) was selected as experimental material. The moisture content of each group was adjusted to about 65%. They were placed in fermentation plastic bags and sealed with a vacuum sealer. Each treatment was prepared in triplicate. Fermentation was carried out at room temperature (16~35 °C) for 3 and 30 days (d), followed by aerobic exposure for 3, 5, and 7 d after 30 d of fermentation. After each opening, the samples were tested for fermentation quality, chemical composition, and other related indicators.

RESULTS AND DISCUSSION

The experimental results showed that at 3 d of ensiling, the PAW group had acetic acid, DM, and CP of 23.00 g/kg DM, 0.37%, and 35.50 g/kg DM, respectively, all higher than the CK group‘s 21.96 g/kg DM, 0.34%, and 31.90 g/kg DM, while NDF and ADF decreased to 556.7 and 493.3 g/kg DM, lower than the CK group, indicating that PAW treatment is beneficial for nutrient preservation and fiber composition improvement at the early stage of fermentation. At 30 d of ensiling, the NH_3-N in the PAW group decreased to 0.07 g/kg DM, significantly lower than the CK group‘s

0.29 g/kg DM; meanwhile, WSC, acetic acid, and CP contents increased to 11.01, 30.53, and 29.21 g/kg DM, respectively, and NDF and ADF further decreased to 540.0 and 487.4 g/kg DM.indicating that PAW treatment can improve fermentation characteristics and optimize nutritional composition.

Microbial analysis results show that PAW pretreatment has little effect on bacterial communities, only maintaining higher community diversity and richness during fermentation. In contrast, it has a significant impact on fungal communities, markedly changing their initial composition, reducing species richness in the later stages of fermentation, optimizing community structure, and improving feed aerobic stability.

Tab. 1: Dynamic changes of fermentation quality during wheat straw silage

Item		Treatment	Days of ensiling (day, d)					SEM	P value		
			3	30	30–3	30–5	30–7		T	E	T×E
pH		CK	7.05^{aA}	5.38^{aCD}	5.32^{aD}	5.44^{aC}	5.71^{aB}	0.010	<0.01	0.039	0.043
		PAW	7.03^{aA}	5.37^{aD}	5.48^{bC}	5.44^{aCD}	5.81^{aB}				
WSC (g/kg DM)		CK	12.13^{aA}	7.30^{aA}	8.23^{aA}	8.78^{aA}	9.35^{aA}	0.439	0.029	0.559	0.194
		PAW	11.25^{aA}	11.01^{bA}	7.80^{aB}	5.81^{aB}	8.76^{aAB}				
NH_3-N (g/kg DM)		CK	0.13^{aA}	0.29^{aA}	0.17^{aA}	0.13^{aA}	0.25^{aA}	0.023	0.764	0.210	0.415
		PAW	0.16^{aA}	0.07^{aA}	0.09^{aA}	0.17^{aA}	0.19^{aA}				
Organic acid	Lactic acid	CK	7.74^{aB}	78.48^{aA}	39.09^{aAB}	40.34^{aAB}	58.33^{aA}	1.687	<0.01	0.909	<0.01
		PAW	6.94^{aC}	151.40^{bA}	38.61^{aB}	22.35^{aBC}	8.39^{bC}				
(g/kg DM)	Acetic acid	CK	21.96^{aB}	17.75^{aBC}	45.82^{aA}	ND	12.07^{aBC}	1.361	<0.01	0.224	<0.01
		PAW	23.00^{aB}	30.53^{aA}	8.58^{bC}	8.87^{bc}	9.53^{aC}				

Indices with different superscript letters indicate significant differences (P<0.05).

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Tab. 2: Dynamic changes of chemical composition during wheat straw silage

Item	Treatment	Days of ensiling (day, d)					SEM	P value		
		3	30	30–3	30–5	30–7		T	E	T×E
DM	CK	0.34^{aD}	0.37^{aD}	0.42^{aC}	0.53^{aB}	0.68^{aA}	0.004	<0.01	<0.01	<0.01
	PAW	0.37^{bD}	0.35^{aD}	0.47^{aC}	0.56^{aB}	0.80^{bA}				
NDF (g/kg DM)	CK	597.3^{aA}	577.7^{aA}	612.0^{aA}	652.3^{aA}	561.0^{aA}	4.447	0.075	0.199	0.363
	PAW	$556.7b^{AB}$	540.0^{aB}	611.3^{aA}	596.0^{aAB}	595.0^{aAB}				
ADF (g/kg DM)	CK	531.0^{aA}	523.0^{aA}	643.3^{aA}	542.0^{aA}	510.7^{aA}	4.764	0.064	0.161	0.362
	PAW	493.3^{bB}	484.7^{aB}	542.3^{aA}	537.7^{aA}	522.7^{aAB}				
CP (g/kg DM)	CK	31.90^{aA}	21.60^{aC}	25.12^{aB}	21.61^{aC}	21.45^{aC}	1.687	<0.01	<0.01	0.738
	PAW	35.50^{bA}	29.21^{bAB}	28.89^{bAB}	25.24^{aB}	25.63^{bB}				

Indices with different superscript letters indicate significant differences (P<0.05).

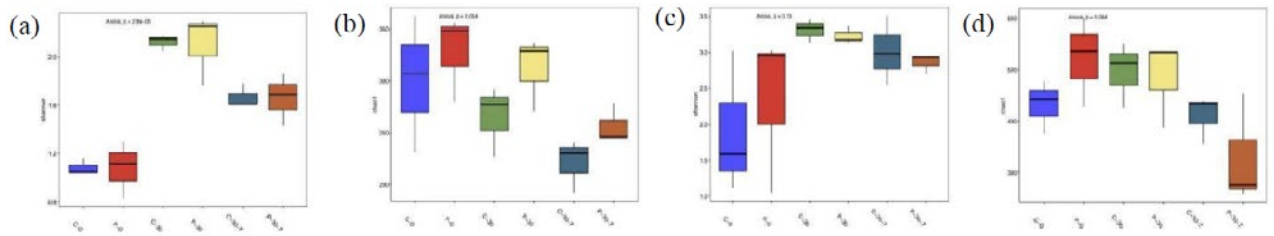


Fig. 1: The alpha-diversities at the OTU level (a and b, Shannon and Chao1 indexes of bacterial community, respectively; c and d, Shannon and Chao1 indexes of fungi community, respectively)

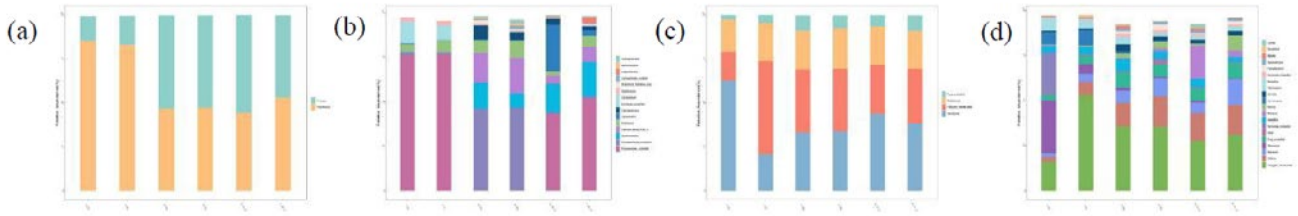


Fig. 2: The structure of the bacterial community at the phylum (a) and genus levels (b) and the structure of the fungi community at the phylum (c) and genus levels (d)

CONCLUSION

PAW treatment can effectively improve the silage quality of wheat straw, with higher acetic acid, DM and CP content, lower NDF and ADF content, NH3-N levels, and better aerobic stability and Microbial community regulation ability. Overall, PAW has good potential for improving the silage quality and aerobic stability of wheat straw.

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INFLUENCE OF *LACTOBACILLUS PLANTARUM* AND DANDELION ON FERMENTATION QUALITY, BIOACTIVE RETENTION AND MICROBIAL COMMUNITY OF MULBERRY LEAF SILAGE IN CHINA

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INTRODUCTION

Mulberry leaves are recognized for their high crude protein content (18–25% DM) and excellent digestibility, making them a promising and sustainable feed resource for ruminants. However, their high moisture content and strong buffering capacity often impair proper silage fermentation. This study aimed to address these limitations by applying Lactobacillus plantarum (LP) inoculation and dandelion (D) supplementation, either individually or in combination (LP-D). It was hypothesized that these additives would enhance fermentation efficiency by lowering pH and increasing lactic acid production, while also promoting the preservation and enrichment of bioactive compounds, reducing anti-nutritional constituents, and stabilizing the microbial community during ensiling and subsequent aerobic exposure. The objective was to evaluate the effects of LP and D on the fermentation characteristics, bioactive compound retention, and microbial dynamics of mulberry-leaf silage after 30 and 45 days of ensiling followed by 4 days of aerobic exposure.

MATERIAL AND METHODS

Fresh mulberry leaves containing approximately 50% moisture were divided into four treatment groups: control (CK), Lactobacillus plantarum (LP; 5% inoculum, 10^8 CFU·mL^{-1}), dandelion (D; 5% hydrated dandelion), and a combined treatment of LP and D (LP-D), with three replicate bags per treatment. The samples were ensiled for 30 and 45 days and subsequently subjected to 4 days of aerobic exposure. Parameters evaluated included pH, ammonia nitrogen (mg·kg^{-1} DM), dry matter (DM) loss (%), water-soluble carbohydrates (WSC), crude protein (CP), and organic acids determined through HPLC. Bioactive components such as tartaric acid, flavonoids, polyphenols, polysaccharides, alkaloids, and antioxidant activity (DPPH) were also quantified. Structural and anti-nutritional constituents including cellulose, hemicellulose, lignin, phytates, and tannins were measured. Microbial community composition was assessed through 16S rRNA gene sequencing and quantitative PCR analysis. Statistical evaluation was performed using one-way ANOVA followed by Fisher's least significant difference test in SPSS, with significance accepted at $P < 0.05$.

RESULTS

Both LP and LP-D treatments significantly improved fermentation quality compared with CK ($P < 0.001$). The LP-D combination produced the lowest pH (4.21 vs. 4.68 in CK; $P < 0.001$), lowest ammonia-N concentration (47.3 vs. 72.4 mg·kg^{-1} DM; $P < 0.001$), and lowest dry matter loss (8.6% vs. 12.5%; $P < 0.001$). Lactic acid concentration was highest in LP-D silage (5.82 g·kg^{-1} FM), exceeding LP and D treatments ($P < 0.01$), while butyric acid remained undetectable across treatments ($P > 0.05$). Dandelion supplementation significantly increased flavonoids (3.85 mg·g^{-1}), polyphenols (6.12 mg·g^{-1}), and antioxidant capacity (72.3%) compared with CK ($P < 0.01$). Anti-nutritional constituents including phytates and tannins decreased significantly in LP-D silage after 30 days ($P < 0.05$). During aerobic exposure, Enterobacter counts increased in CK but remained significantly lower in LP and LP-D treatments ($P < 0.01$). Microbial sequencing showed that LP and LP-D reduced α-diversity indices (Shannon index; $P < 0.05$) and increased Lactobacillus abundance ($P < 0.001$). Lactobacillus abundance was negatively correlated with pH and anti-nutritional factors ($P < 0.01$) and positively correlated with lactic acid concentration ($P < 0.01$).

CONCLUSION

It was concluded that the use of LP, especially in combination with dandelion, effectively improved the fermentation quality of mulberry-leaf silage by promoting LAB dominance, elevating lactic acid concentration, minimizing proteolysis and dry matter loss, preserving and enhancing antioxidant bioactive compounds, and lowering anti-nutritional components, thereby improving overall digestibility and nutritive value. Dandelion functioned as a beneficial phytogenic additive that contributed to improved aerobic stability. The most suitable practice was LP-D treatment with approximately 30 days of ensiling for optimal silage quality and bioactive preservation. These results encourage the use of combined microbial and plant-based additives to produce high-quality preserved feed, while further research is recommended to evaluate animal performance, in-vitro rumen fermentation responses, and economic feasibility under farm conditions.

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PRODUCTION AND FORAGE QUALITY OF GRASS STRAW

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Abstract

Data from the Czech Republic show that straw yields vary by species, ranging from about 2.0 to 12.1 t·ha^{-1}, with values comparable to hay. Grass straw is high in fibre (typically over 650 g·kg^{-1} NDF) and low in crude protein (approximately 26–92 g·kg^{-1} DM), with digestibility limited by lignin content.Despite this, it is a useful feed for ruminants and horses, mainly as a source of structural fibre that supports rumen function. Compared to cereal straw, it generally has higher nutritional value and represents a practical feed resource and additional income for producers.

Keywords: perennial ryegrass, Italian ryegrass, crude protein, dry matter content, NDF, ADF

INTRODUCTION

In past grass straw was commonly used as a fodder for cattle and horses feeding. In the worldwide largest grass seed production area in the Pacific Northwest USA, field burning of grass straw was a common practice until the middle of 1990s. This method was advantageous for farmers as it was cheap and solved sanitary problems with diseases, pests and weeds, recycled mineral nutrients and supported autumn tillering of the grasses. Due to air pollution and a hazard of fires spreading to nature, this management is banned. Farmers had to find another way of straw disposal. The most important market was found as a source of roughage for ruminants and horses. After dry years, there is limited supply of hay and its price rises and especially beef and horse farmers replace lower quality hay by grass straw, if available. Unfortunately, there is very limited number of data available concerning grass straw yield and quality evaluation.

MATERIAL AND METHODS

This study aims to review available information on grass straw yields and the quality from the point of view livestock nutrition. Most published data on grass straw comes from Oregon (USA) where grass seed multiplying is concentrated (e.g. Younberg and Yough, 1977). Data presented in this paper comes from unpublished research of authors in the area of Czech Republic. The most comprehensive data comes from Italian/annual and perennial ryegrass which are the most commonly cultivated species for seed production in Europe. Results come from Forage research station of Mendel University in Vatín (49.52N, 15.97E) and from Grass research station of Oseva PRO in Zubří (49.47N, 18.07E) (Hejduk and Macháč, 2019, unpublished data). Plot area harvested by combine was 10 m^2. Some data were obtained from farm fields where samples were taken from an area of 1 m^2 in three repetitions.

Immediately after harvest, the sample of grass straw was taken from fields for dry matter (DM) content and DM yield evaluation. For a nutrient content, samples of dry straw were homogenised by a laboratory mill with openings size 1.0 mm. Three samples from each species, location and year were used, crude protein (CP), neutral detergent fibre (NDF), acid detergent fibre (ADF). Ash content was measured after ignition at the temperature 550 °C for 6 hours. NDF and ADF concentration by using the method ANCOM (2017a, 2017b). The Kjeldahl method was used to determine N concentration (AOAC, 1984); crude protein was calculated as N content multiplied by factor 6.25.

RESULTS AND DISCUSSION

As the DM content is below 50% (immediately after direct combining) it is necessary to cure the straw before baling. Some farmers use two stages / windrow harvest and in this case, straw is usually dry enough after the threshing. Straw yield depends namely on species, cultivar and year and the values are similar as for grassland hay (Table 1).

The quality of grass straw is comparable to late-harvested meadow hay. However, there are significant differences between individual species related to the content of leaves. The highest proportion of crude protein is found in the straw of Kentucky bluegrass, while the lowest is found in meadow and red fescues. Next to species, the content of crude protein also depends on the dose of nitrogen fertilizers, yield (with high values, nitrogen is diluted) and the weather at harvest (leaching by rain).

Tab. 1: Production of dry straw at different grass species (trials in the Czech Republic, 2016-2017)

Species	Range of yield (t.ha-1)
Timothy	8.1 – 12.1
Perennial Ryegrass	3.3 – 11,4
Italian Ryegrass	3.3 – 11.3
Red Fescue	2.0 – 7,8
Meadow Fescue	5.3 – 9.1
Kentucky Bluegrass	6,9 – 7,8

Tab. 2: Mean nutrients content in grass straw (g.kg^{-1} DM) (trials 2017, Czech Republic)

Species	Crude protein	NDF	ADF	Ash
Timothy	81	629	412	71
Perennial Ryegrass	64	656	442	68
Italian Ryegrass	74	680	442	68
Red Fescue	26	762	523	41
Meadow Fescue	26	671	452	37
Kentucky Bluegrass	92	672	410	60

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Roughly 60–70% of grass straw is cellulose and hemicellulose. Together they should make up an excellent energy source for ruminants. The inability of ruminants to avail themselves of the energy from these carbohydrate sources is explained by high lignin content which acts as an inert barrier between the carbohydrate and the digesting enzyme, then by highly crystalline cellulose which hampers quick enzyme action and finally by silica content which inhibits carbohydrate digestibility. Guggolz *et al*. (1971) found out that steam and hydroxide treatment improved digestibility of the straw significantly.

From a nutritional point of view, straw is characterized by a high fibre content (mostly over 65% NDF) and, conversely, a low nitrogen content and energy. Therefore, its use in cattle nutrition is specific. It serves primarily as a bulk feed with the function of „diluting" energy-rich feed rations and as a source of structural fiber supporting proper rumen function. A sufficient supply of fibre stimulates rumination and salivation, which helps stabilize rumen pH and prevent metabolic disorders (Havekes *et al*., 2020). Grass straw could serve as a superior alternative to the commonly used cereal straw in cattle diets.

CONCLUSIONS

This paper examines the yield and quality of grass straw after the seeds harvest. Straw from perennial grasses is currently little used for animal feeding. However, it represents a valuable reserve, especially in dry years when sufficient hay cannot be harvested from grasslands. For horses and beef cattle, it can represent a significant proportion of the diet and for seed grass growers it can provide a side income in addition to the sale of seeds. When the grass straw is used for feeding purposes its quality is higher compared with cereal straw in terms of higher crude protein content and lower NDF and ADF concentration.

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IMPACT OF AN OXYGEN BARRIER FILM ON THE PRESERVATION AND PREDICTED FEEDING VALUE OF WHOLE-PLANT CORN SILAGE

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Abstract

This study evaluated the effects of a high-performance oxygen barrier (oxygen transmission rate < 0.4 $cm^3/m^2/24$ h; DIN 53380-3) on nutritive value, and estimated milk production of whole-plant corn silage produced on a commercial dairy farm in Brazil. Corn was harvested in early February (DM, CP, NDF, ADF, starch and ash concentrations; 349, 82.9, 407, 224, 271 and 41.3 g kg^{-1}, respectively) and ensiled in two bunker silos (60 × 12 × 1.8 m; length × width × height), which were sealed using a standard polyethylene (PE) and an oxygen barrier (OB) + PE cover. Samples were collected at 64, 103, 164, and 178 days after ensiling from three locations on the silo face: 25 cm below the surface under each cover type and from the center of the silo at 100 cm from the floor. Samples were analyzed for chemical composition, and the estimated milk yield/ton of silage was calculated. Data were analyzed using the MIXED procedure of SAS. Silage sealed with the OB showed greater dry matter and starch concentrations and lower fiber fractions (neutral detergent fiber, acid detergent fiber, and lignin) compared with PE-sealed silage ($P < 0.05$). Furthermore, predicted milk production, estimated using the Milk2006 and Milk2024 models, was greater for OB than for PE silages (1641.4 vs. 1598.3 kg/ton and 1589 vs. 1543 kg/ton; $P < 0.05$). These results demonstrate that the use of a high-performance oxygen barrier improves preservation of whole-plant corn silage nutritive value and enhances predicted milk yield per ton of silage.

Keywords: Commercial farm, High-performance oxygen barrier film, Milk2024

INTRODUCTION

Forages are the backbone of most ruminant livestock operations, and preserving forage yield and quality in the form of silage is essential to ensure a consistent feed supply in such systems. As the product of a fermentation process, silage requires a strict anaerobic environment to occur efficiently, preserving ensiled dry matter (DM) and forage quality. Although oxygen barrier films (OB) have been tested and used for the past 25 years (Degano, 1999), conventional polyethylene films are still widely used by farmers around the world due to their availability and low cost. Their relatively high oxygen permeability can compromise silage quality by promoting aerobic spoilage, which leads to dry matter (DM) losses, reduced nutrient preservation, and consequently lower animal performance. Oxygen barrier films are designed to significantly reduce oxygen transmission rates (OTR), creating an environment more conducive to optimal fermentation, reducing dry matter (DM) losses and extending aerobic stability (Wilkinson & Fenlon, 2014). A recent study has shown the benefits of high-performance oxygen barriers in experimental-scale silos (Amaro *et al.*, 2025), but few have evaluated these novel technologies under large-scale commercial conditions. Thus, our objective was to compare two sealing systems in large-scale silos on a commercial dairy farm in Brazil on nutrient preservation and predicted animal performance.

MATERIAL AND METHODS

Whole-plant corn (*Zea mays*) was harvested in early February after approximately 112 days after sowing (DM, CP, NDFom, ADF, starch and ash concentrations; 349, 82.9, 407, 224, 271 and 41.3 g kg^{-1}, respectively) on a commercial dairy farm in Brazil and ensiled in two bunker silos (60 × 12 × 1.8 m; length × width × height). Each silo was sealed using a standard polyethylene (PE, white-on-back 150 µ) and a high-performance oxygen barrier film (OB; Passion Yellow, 45 µ, OTR < 0.4 $cm^3/m^2/24$ h; DIN 53380-3) + PE cover. Samples were collected over time at 64, 103, 164, and 178 days after ensiling from three locations on the silo face: 25 cm below the surface under each cover type and from the center of the silo at 100 cm from the floor. Samples were analyzed for chemical composition using NIRS, and the estimated milk yield/ton of silage DM was calculated. Data were analyzed using the MIXED procedure of SAS.

RESULTS AND DISCUSSION

Preserving silage yield and digestible nutrients is essential for achieving greater animal performance. High-performance oxygen barriers can reduce oxygen ingress into the silo and, consequently, decrease aerobic losses, which are often most pronounced in the top layer of silos. Thus, we evaluated two silage sealing strategies to determine nutrient preservation and estimate animal performance under real farm conditions. Sealing strategy did not affect the fermentation profile of silages (pH = 3.89, lactic acid = 48.5 g kg^{-1}, acetic acid = 37.7 g kg^{-1}, butyric acid = not detected, and ammonia-N = 99.5 g kg^{-1}; $P > 0.10$). Treatment means for silage chemical composition are presented on Table 1, while Table 2 presents the estimated milk yield using two prediction equations. Silage sealed with OB showed greater dry matter concentration when compared to PE (327 vs. 341 g kg^{-1}, $P = 0.05$), suggesting improved DM preservation. This is consistent with the fact that aerobic deterioration leads to the oxidation of nutrients, producing CO_2, water, and heat, which reduces overall silage DM content (McDonald *et al.*, 1991) and the lower ash concentration observed in OB sealed silage compared to PE (39.1 vs. 36.0 g kg^{-1}, $P < 0.05$). Greater fiber fractions (NDFom, neutral detergent fiber; ADF, acid detergent fiber; and lignin) were observed in PE compared with OB-sealed silage ($P < 0.05$), possibly due to the greater loss of more digestible nutrients, such as starch, during aerobic deterioration. Starch concentrations were greater for OB when compared to PE (299 vs. 281 g kg^{-1}; $P = 0.05$) indicating reduced deterioration of this nutrient. Furthermore, predicted milk yield per ton of silage DM, estimated using the Milk2006 and Milk2024 models, were greater for OB than PE silages, regardless of the model used (1641.4 vs. 1598.3 kg/ton and 1589 vs. 1543 kg/ton; $P < 0.05$). The increased milk yield is likely due to the greater concentration of more digestible nutrients, such as starch, and the lower fiber content observed in OB sealed silage. Overall, Core samples showed similar chemical composition and estimated milk yield to OB-treated silage. These results demonstrate that the use of a high-performance oxygen barrier improves the preservation of whole-plant corn silage nutritive value and enhances predicted milk yield per ton of silage compared with a PE system.

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Tab. 1: Chemical composition of whole-plant corn silage sealed with conventional polyethylene film or a high-performance oxygen barrier in a commercial dairy farm

Item	Core	PE	Combo	SE	P-value
Dry matter, g kg^{-1} fresh weight	348^{a}	327^{b}	341^{a}	13.6	0.05
Starch, g kg^{-1} DM	296^{ab}	281^{b}	299^{a}	25.7	0.05
Crude protein, g kg^{-1} DM	80.6	85.2	81.5	2.45	0.15
NDFom, g kg^{-1} DM	373^{b}	385^{a}	360^{c}	22.8	0.01
ADF, g kg^{-1} DM	224^{b}	231^{a}	210^{c}	16.3	0.03
Lignin, g kg^{-1} DM	38.6^{ab}	40.8^{b}	37.1^{a}	2.24	0.02
Ether extract, g kg^{-1} DM	31.4	31.1	30.2	1.43	0.74
Ash, g kg^{-1} DM	38.2^{ab}	39.1^{a}	36.0^{b}	1.14	0.04

Means with different superscript letters within a line differ significantly (P < 0.05).

Tab. 2: Estimated milk yield of whole-plant corn silage sealed with conventional polyethylene film or a high-performance oxygen barrier in a commercial dairy farm

Item	Core	PE	Combo	SE	P-value
Estimated milk yield using Milk2006, kg ton^{-1}	1653.5^{a}	1598.3^{b}	1641.4^{a}	16.5	0.04
Estimated milk yield using Milk2024, kg ton^{-1}	1566.1^{ab}	1543.0^{b}	1589.0^{a}	23.4	0.01

Means with different superscript letters within a line differ significantly (P < 0.05).

CONCLUSION

In conclusion, the use of a high-performance oxygen barrier is an effective strategy to improve silage preservation under real farm conditions. By reducing oxygen ingress, OB films help minimize dry matter and nutrient losses, resulting in corn silages with higher starch content and and greater feeding value. This translates into improved efficiency of milk production per ton of silage, making OB sealing a valuable tool for enhancing both silage quality and overall dairy farm performance.

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ENSILING WITH A NOVEL *LENTILACTOBACILLUS BUCHNERI* SUBSP. *SILAGEI* STRAIN 30673 ENHANCES AEROBIC STABILITY OF MAIZE SILAGE THROUGH YEAST SUPPRESSION

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ABSTRACT

Maize silage is susceptible to aerobic spoilage during feed-out due to its high starch and residual sugar content leading to DM and feed quality losses. Heterofermentative lactic acid bacteria can be employed to improve silage stability during air exposure. This study evaluated the effects of a novel *Lentilactobacillus buchneri* subsp. *silagei* strain NCIMB 30673, on fermentation characteristics and aerobic stability of maize silage. Whole-crop maize at 37% DM was ensiled either untreated or inoculated at 1.0×10^5 CFU/g and stored for 28 or 99 days. At 28 days, both treatments achieved stable pH. The inoculated silage showed enhanced fermentation with higher lactic and acetic acid concentrations however both treatments exhibited high yeast levels, resulting in lower aerobic stability. After 99 days, treated silage exhibited a more pronounced impact on fermentation, characterised by increased acetic acid concentration, reduced yeast populations, and significantly improved aerobic stability compared with the control. These results demonstrate that *L. buchneri* subsp. *silagei* NCIMB 30673 effectively enhances aerobic stability of whole plant maize silage through yeast suppression, making it a promising candidate as silage inoculant.

Keywords: aerobic stability, inoculant, *Lentilactobacillus buchneri* subsp. *silagei*, yeast suppression

INTRODUCTION

Lentilactobacillus buchneri is an obligately heterofermentative lactic acid bacterium (LAB) widely used in silage making (Pahlow *et al*., 2003). In contrast to homofermentative LAB, which primarily promote rapid acidification through lactic acid (LA) production, *L. buchneri* is distinguished by its ability to anaerobically convert LA into acetic acid (AA) and 1,2-propanediol (1,2-PDOL) (Oude Elferink *et al*., 2001). Acetic acid functions as a potent antifungal compound, inhibiting the lactate-assimilating yeasts responsible for initiating aerobic spoilage. (Danner *et al*., 2003). Maize silage, owing to its high starch and residual sugar content, is particularly susceptible to aerobic deterioration during the feed-out phase, resulting in dry matter (DM) losses and reduced feed quality (Tabacco *et al*., 2011). Consequently, *L. buchneri* is widely employed as a silage inoculant to improve aerobic stability after silo opening through enhanced acetic acid production. Recent taxonomic advancements have identified two distinct subspecies within the *L. buchneri* group: *L. buchneri* subsp. *buchneri* and the newly characterised *L. buchneri* subsp. *silagei* originally isolated from rice grain silage (Tanizawa *et al*., 2020). *Lentilactobacillus buchneri* subsp. *silagei* strain NCIMB 30673 was isolated from aerobically stable whole-crop barley silage. This study aimed to evaluate the effectiveness of this strain in improving fermentation characteristics and enhancing the aerobic stability of maize silage.

MATERIAL AND METHODS

Whole plant maize was harvested and chopped in field and transported to the laboratory for ensiling. Forage (37% DM) was either left untreated (CON) or inoculated with *Lentilactobacillus buchneri* subsp. *silagei* strain NCIMB 30673 (LB) at a target application rate of 1.0×10^5 CFU/g of fresh forage. Samples were ensiled using 1.8 L glass jars at a target packing density of 180kg DM/m^3 and subsequently stored for 28 and 99 days at a constant 20 ± 1 °C. Samples were ensiled with three (day 28) or five (day 99) replicate silos. Upon opening, silages were homogenized, and representative subsamples were analysed for DM (oven drying at 60°C until constant weight), microbial populations, pH, and fermentation profiles. Volatile fatty acids and alcohols were quantified via high-performance liquid chromatography (HPLC) equipped with a refractive index detector. After silo opening, aerobic stability was assessed at 20 ± 1 °C by recording the internal temperature at 15-minute intervals using Squirrel data loggers (Grant). Stability was defined as the duration required for the silage temperature to rise 2 °C above ambient. Statistical analyses were performed in R (version 4.3.1) using ANOVA followed by Tukey's Honestly Significant Difference (HSD) test, with significance defined at $P < 0.05$.

RESULTS AND DISCUSSION

By day 28, both CON and LB- silages achieved a stable pH of 3.81, however LB silages exhibited more extensive fermentation characterized by higher total fermentable acids and alcohols products (TFP) ($P = 0.039$; Table 1). *L. buchneri*-treated silage had significantly higher LAB population compared to CON ($P < 0.001$; Table 1), which resulted in elevated levels of LA ($P = 0.053$; Table 1). After 28 days of fermentation AA concentrations remained relatively low across both treatments (11.07 in CON and 15.03 g/kg DM in LB) but were significantly higher in treated silages ($P = 0.037$; Table 1). The accumulated levels of AA, however, were insufficient to inhibit the undesirable yeasts which remained high in both CON and LB silages ($P = 0.926$; Table 1). Consequently, both treatments showed lower aerobic stability, with spoilage occurring within 1.15 days for CON and 1.23 days for LB-treated silages ($P = 0.864$; Table 1).

By day 99, silages treated with LB displayed more pronounced impact on fermentation. While the population of LAB remained higher than in CON ($P < 0.001$; Table 1), the levels of LA declined and were significantly lower than in untreated maize ($P < 0.001$; Table 1). In contrast, the concentration of AA in LB silages more than doubled relative to those at day 28, reaching 35.01 g/kg DM whereas CON silages showed minimal increase (14.86 g/kg DM, $P < 0.001$; Table 1). The increased AA concentration in the LB-treated silages was likely responsible for the lower yeast numbers recorded relative to the untreated control (0.66 log10 compared to 4.04 log10 CFU/g in LB and CON respectively; $P < 0.001$; Table 1). Consequently, this resulted in a significant improvement in aerobic stability ($P < 0.001$) and a significant reduction in aerobic losses ($P < 0.001$; Table 1) in LB relative to CON.

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Tab. 1: Fermentation parameters, microbial groups and aerobic stability in maize silages

Parameter	Day 28				Day 99			
	CON	LB	SEM	P-value	CON	LB	SEM	P-value
pH	3.81	3.81	0.01	1	3.84	4.02	0.01	<0.001
DM, g/kg	329.08	325.26	6.60	0.703	328.60	316.49	3.32	0.012
DM loss, %	4.8	5.41	0.14	0.044	4.48	5.43	0.07	<0.001
LA, g/kg DM	44.66	58.69	3.64	0.053	60.8	41.46	1.30	<0.001
AA, g/kg DM	11.07	15.03	0.91	0.037	14.86	35.01	0.78	<0.001
1,2-PDOL, g/kg DM	<LOD	0.37	0.03	<0.001	0.58	27.86	0.80	<0.001
Ethanol, g/kg DM	6.36	8.64	0.44	0.022	7.74	9.11	0.31	0.020
TFP, g/kg DM	62.78	83.91	4.95	0.039	85.64	113.78	2.65	<0.001
LAB, log10 CFU/g	7.77	8.43	0.03	<0.001	7.98	9.00	0.08	<0.001
Yeasts, log10 CFU/g	6.29	6.23	0.41	0.926	4.05	0.66	0.25	<0.001
Moulds, log10 CFU/g	0.60	0.60	–	–	0.60	0.66	0.04	0.347
AS, days	1.15	1.23	0.31	0.864	2.11	6.88	0.35	<0.001
Aerobic losses, % FM	2.29	2.35	0.20	0.824	2.57	1.76	0.10	<0.001

CONCLUSION

The application of LB significantly altered the fermentation profile and aerobic stability of maize silage. The greatest impact was realised after 99 days of fermentation where the metabolic conversion of LA to AA resulted in a successful suppression of yeasts, thereby enhancing aerobic stability relative to CON. These findings demonstrate that LB is a promising candidate as silage inoculant for improving aerobic stability.

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THE EFFECTS OF DIFFERENT TREATMENTS ON FERMENTATION QUALITY AND GREENHOUSE GAS EMISSIONS OF ENSILED WHEAT STRAW

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ABSTRACT

Wheat straw, a globally abundant agricultural by-product, faces challenges in ensiling and feed utilization due to its low moisture and carbohydrate content. To address this, in this research, a microbial-enzyme synergistic system was developed using *Lactiplantibacillus* (L.) strains ZA3 and FG1 (commercial strain) with cellulase to enhance the feed quality and aerobic stability of wheat straw silage. Results showed that all treatments improved fermentation quality over the control, with lower pH value, ammonia nitrogen content and harmful microbes counts, higher lactic acid bacteria counts and lactic acid content, and *Lactobacillus* and *Weissella* dominating throughout ensiling and aerobic exposure stages. Among which, *L. plantarum* subsp. *plantarum* ZA3 treated group performed best. Morover, these treatments also reduced emission of greenhouse gas including CO_2, CH_4 and N_2O. Aerobic exposure tests also confirmed that added LAB can significantly extended feed preservation. This study provides theoretical and technical support for utilizing wheat straw as silage feed.

Keywords: wheat straw, silage, lactic acid bacteria, feed quality, greenhouse gases

INTRODUCTION

The rapid expansion of the global livestock industry has increased demand for feed resources (Pang, *et al.*, 2024). Wheat (*Triticum aestivum* L.) produces up to 1 billion tonnes of straw annually, representing an abundant and accessible roughage source rich in water soluble carbohydrates (WSC) and crude fibre (Wang, *et al.*, 2023). Silage is an effective strategy for nutrient preservation and palatability improvement via organic acid production, thereby increasing feed intake (Pang, *et al.*, 2024). In this study, wheat straw was ensiled with lactic acid bacteria (LAB) and cellulase to optimise fermentation quality, enhance aerobic stability, and reduce greenhouse gas emissions. This work provides a theoretical and technical basis for improving feed quality and promoting the efficient and sustainable utilization of wheat straw in ruminant production.

MATERIAL AND METHODS

Wheat straw (cv. Bainong 207) was obtained from Henan, China. Two *Lactiplantibacillus plantarum* strains (ZA3 and FG1) and a commercial cellulase were used as additives. Six treatments were established: WS (control), ZA3, FG1, CEL (cellulase), ZC (ZA3 + cellulase), and FC (FG1 + cellulase). After 3 and 30 days (d) of ensiling, samples were exposed to aerobic conditions for 1 and 7 d, respectively. Fermentation characteristics, chemical composition, greenhouse gas emissions, and microbial parameters were determined after each open.

RESULTS AND DISCUSSION

During ensiling, pH declined while WSC, ammoniacal nitrogen (NH_3-N), and lactic acid (LA) increased across all treatments; notably, the ZA3 group exhibited the lowest pH and highest LA ($P < 0.05$). Upon aerobic exposure, pH and NH_3-N increased, whereas organic acids decreased; ZA3 consistently maintained superior fermentation characteristics ($P < 0.05$). At 3 d, pH and NH_3-N reached minimum levels, while WSC peaked at 30 d. Silage fermented for 30 d showed smaller fluctuations in pH and LA during aerobic exposure, indicating greater aerobic stability.

Crude protein (CP) decreased with fermentation time ($P < 0.05$) but remained higher in treated groups than in the control, suggesting improved protein preservation. Neutral detergent fibre (NDF) and acid detergent fibre (ADF) decreased in all treatments; ZA3 most effectively reduced NDF, while FG1 showed the greatest ADF degradation ($P < 0.05$). During aerobic exposure, CP proportion increased, likely due to losses of other components, while NDF and ADF rose but remained lower than in the control.

Silage production significantly suppresses greenhouse gas emissions, with CO_2 emissions being the highest throughout the silage process, followed by CH_4. With the exception of CH_4 emissions in the FG1 group at 15 d of fermentation and N_2O emissions in the CEL group at 3 d of fermentation, the suppression of gas emissions in all treatment groups was significantly greater than that in the control group ($P < 0.05$). This may be due to the acidic environment during ensilage inhibiting

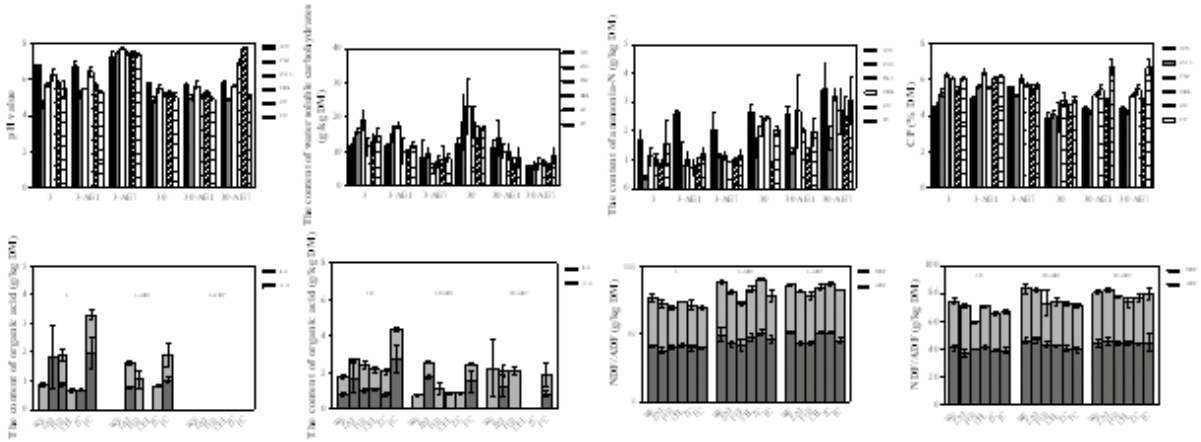


Fig. 1: Fermentation quality and chemical composition of silage

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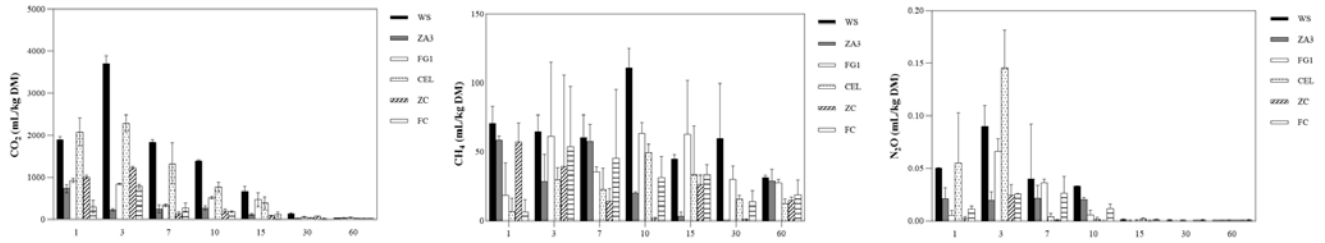


Fig. 2: Dynamics of greenhouse gas emissions from silage

the proliferation of gas-producing bacteria and reducing the degradation of organic matter.

During the silage process, the number of lactic acid bacteria in all treatment groups was significantly higher than that in the control group ($P < 0.05$), whilst the numbers of aerobic bacteria, fungi and other microorganisms were significantly reduced ($P < 0.05$). During aerobic exposure, this microbial trend was reversed. At the community level, *Lactiplantibacillus* became dominant after ensiling and remained so during aerobic exposure, contributing to pH stability, while aerobic genera such as *Sporolactobacillus* increased post-exposure. Fungal composition shifted from increased *Candida* and reduced plant pathogens (e.g., *Alternaria*) during ensiling to dominance of *Wickerhamomyces* and *Pichia* under aerobic conditions.

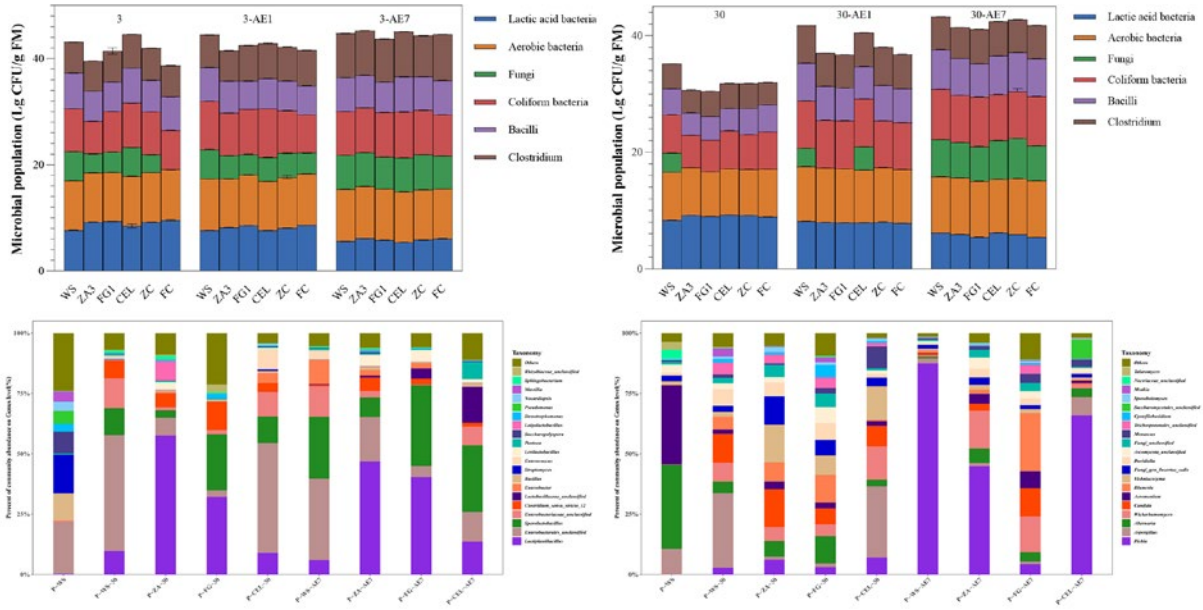


Fig. 3: Changes in microbial population (above) and community composition

CONCLUSION

This study systematically evaluated the effects of additive treatments on fermentation quality and greenhouse gas emissions during the ensiling and aerobic exposure of wheat straw. By regulating microbial community structure, all treatments lowered pH, increased beneficial LAB abundance, and suppressed harmful microorganisms, thereby improving fermentation quality and aerobic stability. LAB dominance inhibited gas-producing microbes and associated metabolic pathways, contributing to reduced greenhouse gas emissions. Among treatments, ZA3 showed the best overall fermentation performance. Microbial succession analysis highlighted the central role of LAB in maintaining pH stability and aerobic stability, while reduced fungal pathogens improved silage safety. These findings provide referenceer for improving feed utilization efficiency of straw.

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STRATEGIES FOR PRODUCTION OF ALFALFA SILAGE

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ABSTRACT

Production of high-quality alfalfa silage is challenging due to the crop's high buffering capacity and low sugar content, which hinder effective fermentation. While organic acids like formic acid reduce pH, they sometimes fail to ensure long-term microbiological stability, risking late spoilage. Homofermentative lactic acid bacteria (LAB) lower pH only until sugars are depleted, potentially leaving silage unstable. Combining homofermentative LAB with molasses or buffered formic acid improves silage stability and quality. Practical dosing equipment enables on-farm application of these additive combinations.

Keywords: alfalfa, buffered formic acid, homofermentative lactic acid bacteria, molasses, biogenic amines

INTRODUCTION

Alfalfa silage production faces persistent challenges due to the crop's inherent high buffering capacity and limited sugar availability, complicating effective ensiling. Mixed forage stands with alfalfa and high sugar grasses are difficult to manage and therefore, pure alfalfa stands are more practical and more common. Traditional methods, such as the use of organic acids or inoculants alone, often fall short in ensuring both rapid pH reduction and long-term stability, leading to risks of spoilage and reduced feed quality.

The objective of the studies was to assess the effectiveness of homofermentative lactic acid bacteria (LAB), buffered formic acid (BFA), and a combination of LAB with BFA and LAB with molasses as additives in low dry matter alfalfa silages.

MATERIALS AND METHODS

Two ensilage studies with alfalfa (18.8% DM and 25.5% DM) have been conducted in Schleswig-Holstein Chamber of Agriculture and Agricultural Centre Baden-Württemberg, respectively, in 2007. The studies compared homofermentative LAB ($3*10^5$ CFU/g FM, BIO-SIL®, Dr. Pieper Technologie- und Produktentwicklung GmbH, Germany), LAB with molasses (35 kg/t FM), BFA, and LAB with BFA (3 to 5 L/t FM) to control silages. Silage trials were conducted over 90 days.

RESULTS AND DISCUSSION

Dry matter losses, NH_3-N of total N, biogenic amines and butyric acid were lowest when combining BFA with LAB (Table 1). Good results were also achieved by BFA alone or the combination of molasses and LAB. LAB alone were not effectively reducing dry matter losses, NH_3-N or butyric acid production in low dry matter alfalfa silage. Results indicate that the combination of homofermentative LAB with BFA achieves a better silage quality compared to single treatments.

Tab. 1: Characteristics of alfalfa silage from two different trials over 90 days.

Parameter	Control	LAB	BFA	LAB + BFA	LAB + Molasses
Trail 1 (18.8% DM, FC 23)					
DM loss (%)	20	16.1	7.0	5.9	8.6
pH	5.4	5.7	4.1	4.1	4.2
NH_3-N / total N (%)	26	21	5	3	5
Lactic acid (g/kg DM)	16.5	9.3	44.9	44.9	106.7
Acetic acid (g/kg DM)	52.5	24.5	13.9	6.4	23.2
Butyric acid (g/kg DM)	52.1	51.7	0.5	0.5	0.5
Biogenic amines (g/kg DM)	7.96	6.79	0.86	0.27	1.14
Trail 2 (25.5% DM, FC 30)					
DM loss (%)	3.9	3.3	1.1	0.6	2.7
pH	4.4	4.4	4.2	4.2	4.2
NH_3-N / total N (%)	10.4	10.1	6.5	5	6.3
Lactic acid (g/kg DM)	15.4	15.3	9.0	7.7	73.5
Acetic acid (g/kg DM)	6.2	6.0	1.7	0.7	14.3
Butyric acid (g/kg DM)	0	0	0	0	0
Biogenic amines (g/kg DM)	2.83	2.51	0.97	0.21	0.7

LAB = homofermentative lactic acid bacteria (BIO-SIL®, Dr. Pieper Technologie- und Produktentwicklung GmbH, Germany); BFA = buffered formic acid, DM = dry matter, FC = fermentation coefficient

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CONCLUSION

The integration of homofermentative LAB with sugar sources or BFA presents a viable solution for producing stable, high-quality alfalfa silage. This approach enhances fermentation efficiency, reduces the risk of late spoilage, and improves overall silage stability and nutritional quality. The availability of practical dosing equipment further supports the adoption of these additive combinations at the farm level.

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PART 3

NUTRITIONAL VALUE OF SILAGES AND PRECISION FEEDING (NIR TECHNOLOGY)

EFFECTS OF ADDITIVES AND MOISTURE LEVEL ON CRIMPED ENSILED FABA BEAN COMPOSITION AND DEGRADATION OF ANTINUTRITIONAL FACTORS

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ABSTRACT

Wider use of crimping and ensiling as a preservation method of grain legume seeds could increase local production of protein feeds and improve the resilience of European livestock production. This experiment evaluated the effects of moisture level and formic acid addition on fermentation quality and vicine & convicine degradation of crimped ensiled faba beans. In addition, a by-product from grass protein extraction, grass whey or brown juice, was used as a novel component to improve fermentation by adding moisture and nutrients to the faba beans. All samples showed good preservation quality with limited protein breakdown and no presence of butyric acid. Higher moisture content promoted lactic acid production and decreased pH of the samples. On average 60% of the original vicine & convicine was degraded during ensiling indicating that the preservation method can be used to improve the feeding value. Use of grass whey showed potential as a feed component included in crimped ensiled seeds that can promote fermentation and recycle the nutrients in the whey back to the food chain.

Keywords: *Vicia faba* L., fermentation quality, formic acid, green biorefinery by-product, vicine, convicine

INTRODUCTION

Europe is heavily dependent on imported protein feed supplements, which poses a threat to food security. Grain legumes such as faba beans and peas can replace imported soya bean and rapeseed protein, and their use should be encouraged. The lack of suitable protein supplements is particularly high in organic pig and poultry production, where the antinutritional factors of faba bean limit the use of it. Crimping and ensiling is a method commonly used for grain preservation with benefits related to economics, work efficiency and smaller weather dependency. The fermentation process may also positively affect the digestibility of nutrients (Soares *et al.* 2026) and degrade antinutritional factors (Rinne *et al.* 2020). The aim of the current experiment was to evaluate the effect of moisture content and additive application on the fermentation quality and degradation of vicine & convicine in crimped ensiled faba bean seeds. A by-product from grass protein extraction, grass whey or brown juice, was used as a novel component to improve fermentation by adding moisture and nutrients to the faba beans.

MATERIAL AND METHODS

The faba beans (variety Sampo) were combine harvested at Jokioinen Finland on 24 September 2024, and crimped using a farm scale crimper mill (Murska VMAX, Aimo Kortteen Konepaja, Ylivieska, Finland). The grass wheys were obtained from another experiment (Ayanfe *et al.* 2026) where an organically produced red clover grass was separated by screw-pressing into liquid and solid fractions. The true protein from the juice was precipitated in buckets either without any additive or using a formic acid based additive (FA, AIV 2 Plus Na, Eastman, Oulu, Finland). The liquid on the top, or grass whey as we call it, was collected from the buckets to yield WheyC (without additive) and WheyFA (with additive) that were used in the current trial. The crimped faba beans were ensiled in laboratory scale using the following treatments: water, WheyC, WheyFA and formic acid (application rate 3 litres per ton), all at two levels of liquid (7.5 and 15 %). In addition, freshly harvested beans without any additive were used resulting in 9 treatments in total. Each 3 kg replicate (4 per treatment) received the additive individually and was sealed into a vacuum plastic bag for 3 months. After opening, the samples were analysed using routine laboratory methods for fermentation quality, and for vicine & convicine concentrations. The statistical analysis was conducted using SAS statistical package, and treatment effects were evaluated using contrasts as well as using Tukey's test.

RESULTS AND DISCUSSION

The faba bean raw material had a moisture content of 218 g/kg, and vicine & convicine concentrations were 6.80 and 5.49 g/kg DM, respectively. The DM concentrations of WheyC and WheyFA were 39 and 43 g/kg. WheyC contained 100 g lactic acid/kg DM but in WheyFA the fermentation had been restricted (2 g lactic acid/kg DM). The FA content in WheyFA was 5.4 g/kg so that the application of FA in it was negligible. All samples showed good preservation quality (Table 1) with limited protein breakdown and no presence of propionic or butyric acid. Higher moisture content promoted lactic acid production and decreased pH of the samples. At similar moisture level, grass wheys promoted fermentation compared to water only while FA efficiently restricted fermentation. On average, 60% of the original vicine & convicine was degraded during ensiling indicating that fermentation during ensiling can act as a means to improve the feeding value.

CONCLUSION

Under humid harvesting conditions, crimping and ensiling can be used as a low-cost flexible feed preservation method compared to drying, with additional benefits in feed quality. This approach could be of particular use in increasing the protein self-sufficiency of organic farms when including grain legumes in their crop rotations, and their use could be extended from the ruminant sector to diets of monogastric farm animals. Use of grass whey showed potential as a feed component included in crimped ensiled seeds that can promote fermentation and recycle the nutrients in the whey back to the food chain.

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Tab. 1: Composition of the crimped ensiled faba bean seeds

	Moisture, g/kg	pH	Ammonia-N, g/kg N	In dry matter, g/kg					
				Ethanol	Lactic acid	Acetic acid	Propionic acid	Vicine	Convicine
Treatment[1]									
1. Water0	210^{g}	6.09^{a}	4^{d}	0^{e}	0.2^{e}	0.1^{e}	0.2	2.65^{c}	2.20^{de}
2. Water1	261^{e}	5.73^{b}	12^{c}	9.5^{ab}	6.7^{e}	1.7^{de}	0.5	3.77^{b}	2.93^{b}
3. Water2	302^{b}	4.98^{d}	25^{b}	10.6^{a}	21.5^{d}	3.1^{cd}	0.3	2.32^{cd}	2.82^{bc}
4. WheyC1	253^{f}	4.67^{e}	25^{b}	4.0^{cd}	30.4^{c}	4.1^{c}	0.4	1.76^{d}	2.50^{bcd}
5. WheyC2	290^{c}	4.41^{f}	35^{a}	4.3^{cd}	54.6^{a}	5.8^{ab}	0.1	0.66^{e}	1.70^{e}
6. WheyFA1	250^{f}	4.84^{de}	22^{b}	6.4^{abc}	27.8^{cd}	4.5^{bc}	0.2	1.71^{d}	2.29^{cd}
7. WheyFA2	291^{c}	4.39^{f}	27^{b}	6.1^{bc}	45.0^{b}	6.2^{a}	0.3	0.82^{e}	2.27^{cd}
8. FA1	266^{d}	5.42^{c}	8^{cd}	0.6^{de}	0.3^{e}	0.3^{e}	0.2	5.82^{a}	4.09^{a}
9. FA2	312^{a}	5.28^{c}	10^{c}	1.1^{de}	1.3^{e}	0.6^{e}	0.1	2.15^{cd}	2.20^{de}
SEM	1.2	0.052	1.4	0.96	1.70	0.37	0.17	0.187	0.129
P-values for contrasts[2]									
Water Lin	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.444	0.198	0.001
Moisture 1 vs 2	<0.001	<0.001	<0.001	0.534	<0.001	<0.001	0.526	<0.001	<0.001
Whey C vs FA	0.383	0.128	<0.001	0.018	<0.001	0.200	0.980	0.762	0.116
Water vs Wheys	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.285	<0.001	<0.001
Water vs FA	<0.001	0.827	<0.001	<0.001	<0.001	<0.001	0.123	<0.001	0.027

[1]Treatments: Water0, 1 and 2: Addition of tap water at 0, 7.5 or 15 %; WheyC1 and 2: Addition of Grass whey without additive at 7.5 and 15 %; WheyFA1 and 2: Addition of Grass whey with formic acid at 7.5 and 15 %; FA1 and 2: Addition of formic acid with tap water at 7.5 and 15 %.

[2]Contrasts: Water Lin = 1 vs 3; Moisture 1 vs 2: 2, 4, 6, 8 vs 3, 5, 7, 9; Whey C vs FA: 4, 5 vs 6, 7; Water vs Wheys: 2, 3 vs 4, 5, 6, 7; Water vs FA; 2, 3 vs 8, 9.

Means in columns with different superscript letters differ statistically significantly (P<0.05).

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EFFECT OF DIETARY N-BUTYRIC AND ACETIC ACIDS ON THE FEED INTAKE AND MILK QUALITY OF DAIRY COWS

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ABSTRACT

The aim of this study was to examine the effects of n-butyric and acetic acids on feed intake and milk quality of dairy cows. Adding 10.0 g/kg of n-butyric acid or 16.5 g/kg of acetic acid to TMR (DM basis) decreased the first meal duration, but did not affect total eating time, DMI, and performance of late lactating dairy cows.

Keywords: acetic acid, butyric acid, intake, volatile fatty acids

INTRODUCTION

Well-preserved silages are virtually free of n-butyric acid. Compared with acetic acid, whose formation in silage can be promoted by inoculation with heterofermentative lactic acid bacteria aiming to improve the aerobic stability (Arriola *et al*., 2021), the n-butyric acid has a greater antifungal potential. Experiments with addition of pure n-butyric acid to animal diet are rare in the literature, and its effects on DMI and performance are equivocal. In animal tissues, n-butyric acid is converted to ketone bodies (β-OH-butyrate, acetoacetate and acetone), which may diffuse from the blood to the rumen digesta. In the rumen, acetone can be converted to isopropanol by rumen microorganisms. Isopropanol is then absorbed and partially imparted to milk (Bragatto *et al*., 2022). Therefore, the objective of this study was to examine the effects of dietary n-butyric and acetic acids on feed intake, performance and milk quality of dairy cows.

MATERIAL AND METHODS

Nine late lactating dairy Holstein cows (554 ± 45 BW; 25.4 ± 3.9 kg/d of milk) were assigned to three 3 × 3 Latin squares, with 18-d periods (14-d adaptation and 4-d sampling), to receive one of three dietary treatments: 1) CON: basal diet containing (DM basis) 400 g/kg chopped Bermuda grass hay and 600 g/kg concentrates; 2) BUT: basal diet added with 10 g/kg DM of n-butyric acid; and 3) ACE: basal diet added with 16.5 g/kg DM of acetic acid. The basal diet was formulated with dry (non-fermented) ingredients, so the effect of the organic acids can be evaluated without bias. Water was added to all TMR to reach 550 g/kg of DM content. The TMR ingredients were mixed by hand twice daily, immediately before each feeding at 0800 and 1330 h. Cows were milked in a milk parlor twice daily at 0630 and 1530 h. Data were analyzed using the Mixed procedure of SAS (v. 9.4, SAS Inst. Inc., Cary, NC). Significance was declared at $P \leq 0.05$ and tendencies at $0.05 < P \leq 0.10$.

RESULTS AND DISCUSSION

Eating time, meal frequency, meal size, meal duration, and eating rate were unaffected by treatment (Table 1). However, BUT increased ruminating time and, consequently, increased chewing time (min/d) and tended to increase chewing per kg DM. All TMR had a similar physical structure, with no difference in TMR sorting index at the feed bunk (data not shown). The mechanism underlying the increased rumination observed with BUT supplementation warrants further investigation; however, effects of BUT on rumen papillae development and the clearance of volatile fatty acids, as well as rumen motility, may be involved. Additionally, ACE decreased and BUT tended to decrease the duration of the first meal, likely due to the pungent odor of acetic and butyric acids, which may alter intake pattern via olfaction (Daniel *et al*., 2013).

Several studies have reported negative relationships among n-butyric or acetic acid and DMI by multivariate or correlation analysis. However, the occurrence of collinearity among variables prevents the establishment of cause and effect (e.g., high concentrations of n-butyric and acetic acids in poorly fermented silages containing other potentially hypophagic compounds). In studies where pure n-butyric acid was supplemented its effect on DMI was equivocal, whereas the sole effect of acetic acid on DMI depends on its concentration in diet. Gerlach *et al.* (2021) demonstrated in a meta-analysis that concentrations of acetic acid greater than 17.3 g/kg of diet DM depressed DMI, whereas lower concentrations had negligible effect. In the current study, adding 10.0 g/kg of n-butyric acid or 16.5 g/kg of acetic

Tab. 1: Feeding behavior of dairy cows fed TMR added with n-butyric and acetic acid

Item	Treatments[1]			SEM[2]	P-value
	CON	BUT	ACE		
Eating, min/d	302	301	299	13.7	0.983
Ruminating, min/d	537^{b}	577^{a}	538^{b}	9.0	0.011
Chewing, min/d	838^{y}	877^{x}	837^{y}	13.9	0.043
Chewing, min/kg DM	39.9^{xy}	41.4^{x}	39.7^{y}	0.83	0.060
Eating rate, g DM/min	70.0	70.4	70.8	2.75	0.966
Meal frequency, /d	6.56	7.22	7.11	0.517	0.526
Meal size, kg DM/meal	3.33	3.04	3.16	0.205	0.617
Meal duration, min	48.6	42.7	44.7	2.80	0.332
First meal duration, min	108^{a}	94.4^{ab}	90.0^{b}	6.67	0.045

[1]CON: control; BUT: 10.0 g/kg DM of n-butyric acid; ACE: 16.5 g/kg DM of acetic acid. [2]Standard error of the mean. ^{a,b}Tukey test (α = 0.05). ^{x,y}Tukey test (α = 0.10)

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acid to TMR (DM basis) did not affect the DMI (Table 2). Milk yield, feed efficiency, milk fat, lactose, and MUN did not differ among treatments, whereas BUT slightly increased milk protein concentration. The greater rumination time may have improved the ruminal environment, thereby increasing the intestinal flux of microbial amino acids that were subsequently utilized for milk protein synthesis. Further studies are needed to confirm this hypothesis. As expected, blood β-OH-butyrate tended to increase in cows fed BUT compared to CON or ACE, due to the conversion of butyrate to β-OH-butyrate mainly in the rumen epithelium (Kristensen and Harmon, 2004). All milk samples tested negative for the presence of alcohol.

Tab. 2: Performance of dairy cows fed TMR added with n-butyric and acetic acid

Item	Treatments1			SEM[2]	P-value
	CON	BUT	ACE		
DMI[3], kg/d	21.1	21.7	21.2	0.59	0.421
ECM[4], kg/d	24.2	25.4	25.6	1.33	0.139
Fat, g/kg	41.5	43.3	42.1	1.92	0.412
Protein, g/kg	31.9b	32.8a	32.7a	0.36	0.027
Lactose, g/kg	45.6	45.7	45.7	0.41	0.976
MUN[5], mg/dL	15.1	14.9	14.7	0.62	0.681
ECM/DMI	1.15	1.18	1.20	0.054	0.206
Milk alcohol test, %	0	0	0	–	–
Blood β-OH-butyrate, mmol/L	0.644[y]	0.744[x]	0.622[y]	0.0449	0.098

[1]CON: control; BUT: 10.0 g/kg DM of n-butyric acid; ACE: 16.5 g/kg DM of acetic acid. [2]Standard error of the mean. [3]Dry matter intake. [4]Energy-corrected milk. [5]Milk urea nitrogen. [a,b]Tukey test (α = 0.05). [x,y]Tukey test (α = 0.10)

CONCLUSION

Adding 16.5 g/kg of acetic acid or 10.0 g/kg of n-butyric acid to TMR (DM basis) did not affect total DMI and performance of late lactating cows.

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THE EFFECT OF FEEDING POOR-QUALITY SILAGE ON RUMEN FERMENTATION PROCESSES, METABOLIC PROFILE, AND HEALTH STATUS OF DAIRY COWS

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INTRODUCTION

Nutrition of dairy cows is considered the most important environmental factor determining milk production, fertility, animal health, and enabling the realization of the genetic potential of both individuals and the entire herd.

Silages form the basic component of dairy cow rations. Their quality determines the nutritional value and palatability of the diet and thus the total dry matter intake. High-quality maize silage has a high energy value, is well accepted by animals, and stabilizes fermentation processes in the rumen. In most dairy herds, it forms the basis of total mixed rations (TMR) throughout the year and is an important energy source during early and peak lactation. It influences milk yield and quality, animal health, and fertility. In combination with conserved protein forages and concentrate feeds, it creates optimal conditions for formulating TMRs with a high nutrient concentration required for high-producing dairy cows, while maintaining appropriate dietary effects and minimizing digestive disorders.

High-quality conserved forage is therefore indispensable for high-producing dairy cows. A major advantage of maize silage is that maize starch, compared to wheat and barley starch, is digested more slowly and partially escapes rumen fermentation. Its digestion in the intestine provides a direct source of glucose for dairy cows during early and peak lactation when they are in negative energy balance, thus helping to prevent ketosis.

Silage quality is influenced by many factors throughout all stages of production, storage, and feeding. Conserved forages are often not of the required quality and may pose health risks. Even well-produced silages that meet quality standards can quickly become problematic or hygienically unsuitable feeds. This situation is frequently observed in many herds during spring and summer when secondary aerobic fermentation occurs.

Aerobic fermentation of silage (also referred to as secondary fermentation or spontaneous heating) is caused by yeasts, bacteria, and fungi, with yeasts playing the most significant role. Yeasts are highly acid-tolerant, survive within a wide temperature range (8–35 °C), and persist in silage for long periods. Similarly, mold spores and some bacteria can survive. Under suitable conditions, adequate oxygen, moisture, and temperatures of approximately 12–35 °C, yeasts multiply rapidly and disrupt the preserved feed.

Yeasts intensively metabolize lactic acid and residual soluble sugars, producing heat, carbon dioxide, and water. As a result, the energy value of the silage decreases, and dry matter losses occur. Degradation of lactic acid alters the pH of the silage, creating favourable conditions for the growth of bacteria and fungi. Consequently, products of putrefaction, mycotoxins, and various toxic biogenic amines (such as cadaverine, tyramine, putrescine, spermidine, tryptamine, and histamine) accumulate. The number of bacteria such as *Escherichia coli*, Klebsiella, *Clostridia*, and *Listeria* increases. Heating of the silage mass leads to the formation of indigestible compounds. Such aerobically deteriorated feed has low nutritional value and is hygienically unsuitable, negatively affecting animal health, production, and reproduction.

Aerobically damaged forage reduces the value of the entire TMR. Yeasts rapidly degrade sugars added via molasses or feed sugar, fungi proliferate and produce large amounts of mycotoxins, proteins are degraded, and pathogenic bacteria multiply. These undesirable processes continue until the feed is consumed or removed.

Metabolites formed during aerobic fermentation reduce feed palatability, leading to a significant decrease in dry matter intake. Serious changes also occur in the rumen. Optimal rumen fermentation requires adequate nutrient intake; however, aerobic deterioration reduces soluble carbohydrates and protein digestibility, and ingested bacteria negatively affect rumen microflora and protozoa. Mycotoxins also have a highly detrimental effect on rumen fermentation.

As a result, digestive disorders such as simple indigestion, alkalosis, and putrefaction of rumen contents may develop. Typical clinical signs associated with feeding aerobically deteriorated forage include reduced appetite, decreased milk yield, changes in milk composition and quality, increased incidence of mastitis, diarrhoea, hoof diseases, and a high prevalence of purulent endometritis. The health status of cows is also reflected in the health of newborn calves.

Objective

The aim of this study was to evaluate the effect of feeding spoilt maize silage (secondary aerobic fermentation) on rumen fermentation processes, metabolic profile, and overall health status of dairy cows.

METHODS

At the request of the farmer, dairy cows were examined to determine the cause of a sudden and significant decrease in milk yield and an increase in somatic cell count. Daily milk production decreased by 3.5 L (from 38.2 L to 34.7 L). Somatic cell count in bulk milk increased from 180,000 to 320,000 cells/mL. Reduced feed intake was observed.

Rumen fluid and blood samples were collected from selected cows. Laboratory analyses were performed using standard methods at the Clinical Laboratory for Large Animals. In maize silage, concentrations of mycotoxins DON, T-2 toxin, and zearalenone were determined and found to exceed permissible limits.

As the results listed in Table 1 show, the feeding of spoilt silage negatively affected rumen fermentation processes. Total volatile fatty acid (VFA) concentration was only 62.96 ± 21.31 mmol/L compared to the physiological range of 80–120 mmol/L. The number of protozoa was also significantly reduced, indicating an unfavourable rumen environment. The proportion of individual VFAs was not significantly altered. Lactic acid concentration was below 1 mmol/L.

The blood metabolic profile indicated liver damage. High creatine kinase (CK) activity suggested weight loss and muscle catabolism. Significant changes were also observed in calcium, zinc, and vitamin A levels. Impaired rumen fermentation led to reduced absorption of calcium and zinc, resulting in hypocalcaemia and hypozincaemia. Decreased vitamin A levels were associated with impaired liver function and reduced conversion of beta-carotene.

Feeding spoilt maize silage resulted in reduced TMR intake, impaired rumen fermentation, liver disorders, decreased milk production, weight loss in dairy cows, and an increased incidence of lameness.

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CONCLUSION

Feeding aerobically deteriorated maize silage had a markedly negative impact on rumen fermentation, metabolic status, and overall health of dairy cows. The observed reduction in total volatile fatty acid concentration and protozoal counts indicates impaired rumen functionality, likely driven by decreased nutrient availability and the presence of undesirable microbial metabolites.

Metabolic profiling further revealed signs of hepatic impairment and mineral imbalance, particularly hypocalcaemia and hypozincaemia, together with reduced vitamin A status, suggesting compromised nutrient absorption and altered metabolic homeostasis.

These disturbances were associated with reduced feed intake, decreased milk yield, increased somatic cell count, and clinical signs including weight loss and lameness.

Overall, the findings highlight that even short-term feeding of poor-quality silage can substantially disrupt rumen ecology and systemic metabolism, leading to significant production losses and health deterioration in dairy cows. Ensuring high silage quality and preventing aerobic spoilage is therefore critical for maintaining performance and animal health in dairy systems.

Tab. 1: Metabolic profile

Serum	Unit	Mean	SD
Total protein	g/L	72,94	2,55
Albumin	g/L	34,44	3,46
Bilirubin	μmol/L	4,78	2,71
BHB	mmol/L	0,83	0,23
NEFA	mmol/L	1,05	0,23
AST	μkat/L	2,62	0,87
GGT	μkat/L	0,52	0,21
CK	μkat/L	18,8	13,3
Na	mmol/L	145,64	3
K	mmol/L	4,67	0,91
Ca	mmol/L	2,1	0,14
P	mmol/L	1,43	0,36
Mg	mmol/L	1	0,12
Zn	μmol/L	6,63	2,66
Cu	μmol/L	15,38	0,93
Vitamin A	μmol/L	0,73	0,5
Vitamin E	μmol/L	7,96	1,95
Beta-carotene	μmol/L	5,86	1,67
Rumen fluid			
Rumen pH		6,78	0,36
Total VFA	mmol/L	62,96	21,31
Acetic acid	mol%	63,82	1,12
Propionic acid	mol%	19,74	1,78
Butyric acid	mol%	12,96	1,93
Valeric acid	mol%	3,74	0,62
Protozoa	thous./mL	163,2	73,43
Ammonia	mmol/L	6,76	4,99

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RECLAMATION OF ARABLE LAND DEGRADED BY GRAVEL MINING BY COMPLEX OF COMPOSTING MICROORGANISMS TO RECOVERY FORAGE PRODUCTION

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ABSTRACT

The aim of the work was to reclaim the degraded area using waste sludge obtained from washing excavated gravel and long-term stored (and therefore biologically minimally active) topsoil treated with compost activated by Bacteriolite. In this way, we aimed to return the anthropogenically physically degraded area to a productive state so that, after reclamation, the land (Anthrozem) would achieve a production capacity similar to the arable Fluvisol on the adjacent control plot, with the potential to apply this reclamation method to other degraded locations if successful. Results showed that after one year of remediation, the total carbon content in Anthrozem increased only slightly but significantly; therefore, it is necessary to continue biological measures to increase the organic matter content in the soil. Maize, sown in the area a year after reclamation, achieved a grain yield of 5.1 t ha^{-1}, which nearly reached the three-year average yield in Slovakia for 2022–2024 (6.5 t ha^{-1}).

Keywords: composting microorganisms, forage production, organic carbon, soil remediation, soil structure

INTRODUCTION

A section of arable land in Horná Seč, covering an area of 3,800 m^2, was temporarily taken out of use and, for 15 years, was used for mining and loading gravel. In Slovakia, according to Government Regulation No. 58/2013, temporarily taken agricultural land must be reclaimed and returned to crop production.

The project aimed to reclaim the degraded area using waste sludge obtained from washing excavated gravel and long-term stored (and therefore biologically minimally active) topsoil treated with compost activated by Bacteriolite. The goal was to return the anthropogenically physically degraded area to a productive state so that, after reclamation, the reclaimed land would achieve a production capacity similar to the arable land on the adjacent control plot, with the potential to apply this reclamation method to other degraded locations if successful.

MATERIALS AND METHODS

The reclamation took place near the village of Horná Seč, in an area of Fluvisols formed on Tertiary sediments (gravel and sand) of the Hron River, unevenly covered with Holocene fluvial flood clays.

The physical part of the reclamation included the excavation and removal of scarp gravel was done from a layer 0–80 cm thick. The resulting depression with compacted original soil parent material was treated by disking. Waste sludge obtained from washing the excavated gravel was used to reconstruct the subsoil, resulting in a total thickness of 0.6 m after soil subsidence. The original topsoil was placed on top, levelled, and loosened in a 0.2 m layer. The biological component of the reclamation involved applying compost made from organic plant matter, activated with the soil aid Bactériolit concentrated (produced by Marcel Mézy Technology, containing an active complex of live composting microorganisms) at a rate of 50 tons per hectare, and adding a legume-cereal mixture. Subsequent appropriate care of the growth included mulching and shallow incorporation into the surface layer of the soil.

Soil sampling and analyses

The first soil sampling was carried out in April 2023, one month after physical reclamation but before the start of biological reclamation. On the reclaimed plot (Anthrozem), a composite soil sample was created by combining seven subsamples. On the neighbouring arable Fluvisol, a composite soil sample was created by combining six subsamples. The control sampling was carried out in April 2024 at the same locations.

Samples were then taken to determine soil structure (0–20 cm) according to the Bakšaev method (Hraško *et al.* 1962), and chemical and biological properties (0–30 cm): soil reaction – potentiometrically in H_2O and 1 M KCl; exchangeable bases (S) and hydrolytic acidity (H) by Kappen's method; carbonate content – volumetrically; total organic carbon (Cox) – oxidimetrically (Hrivňáková *et al.*, 2011); labile carbon (CL) by the method of Loginow *et al.* (1987); and hot water soluble carbon (Chwl) by Körchens and Schulz (1999).

For statistical analysis, one-way ANOVA was used. Differences between Anthrozem and Fluvisol were evaluated using the Tukey test ($P \leq 0.05$).

RESULTS AND DISCUSSION

The residual fine-grained soil material obtained by flushing the excavated stony soil substrate was suitable for subsoil reclamation, as it did not adversely affect plant growth on

Tab. 1: Statistical analysis of soil structure parameters in topsoil of Anthrozem and Fluvisol determined by Tukey's test (significant level $P < 0.05$)

Plot	<0.25 mm dry	MWD_d[1]	K[2]	<0.25 mm wet	MWD_w[3]	K_V[4]	S_W[5]
Anthrozem 2023	10.8^b	1.66^a	3.08^b	15.1^a	0.76^a	2.17^a	1.21^a
Anthrozem 2024	3.67^a	2.43^a	2.49^{ab}	9.00^a	0.83^a	2.92^a	1.30^a
Fluvisol (control)	4.07^a	3.67^b	1.65^a	8.82^a	1.78^b	2.06^a	1.31^a
value P	0.0183	0.0065	0.0250	0.1216	0.0000	0.0454	0.1374
HSD 0,05	± 6.03	± 1.22	± 1.15	± 8.91	± 0.198	± 0.878	± 0.143

Abbreviations: [1]mean weight diameter of structural aggregates dray; [2]Revut structural coefficient; [3]mean weight diameter of water--resistant aggregates wet; [4]vulnerability coefficient of macroaggregates; [5]stability index

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Tab. 2: Statistical analysis of pH, sorption, and organic carbon values in topsoil of Anthrozem (AM) and Fluvisol (FS) determined by Tukey's test (significant level $P < 0.05$).

Plot	pH_{KCl}	H^3	S^4	V^5	CO_3^{2-6}	$C_{OX}{}^7$	$C_L{}^8$	$C_{hwl}{}^9$	L^{10}
		[mmol/kg]		[%]		[g/kg]			
AM[1] 2023	6.66[ab]	7.47[a]	283[a]	97.3[b]	< 0.3	6.88[a]	0.77[a]	0.23[a]	0.12[a]
AM 2024	6.89[b]	6.53[a]	281[a]	97.7[b]	< 0.3	9.39[b]	0.93[a]	0.29[a]	0.11[a]
FS[2] (control)	6.43[a]	11.20[b]	245[a]	95.6[a]	< 0.3	16.30c	2.34[b]	0.57[b]	0.17[b]
value P	*0.0090*	*0.0006*	*0.0968*	*0.0026*		*0.0000*	*0.0000*	*0.0016*	*0.0008*
SD	± 0.21	± 2.12	± 23.63	± 1.01		± 4.00	± 0.72	± 0.16	± 0.03

[1]Anthrozem; [2]Fluvisol; [3]hydrolytic acidity; [4]sum of base cations; [5]base saturation; [6]carbonates, [7]total organic carbon; [8]labile carbon; [9]hot water soluble carbon; [10]lability of organic carbon

the recultivated land. The water resistance of macroaggregates improved slightly one year after reclamation, especially with biological reclamation, but still did not reach the level of the control Fluvisol. Therefore, it is necessary to improve soil structure, particularly by increasing the supply of organic fertilisers, as organic matter is one of the most important factors in the formation and stabilisation of aggregates (Tab. 1).

The Anthrozem topsoil had significantly lower contents of total organic carbon (C_{OX}), labile carbon (C_L), and hot water-soluble carbon (C_{hwl}) compared to the control Fluvisol (Tab. 2). The total carbon content in Anthrozem increased only slightly, though significantly; therefore, it is necessary to continue biological measures aimed at increasing the organic matter content in the soil (Polláková, 2025). pH values and sorption parameters showed that the Fluvisol topsoil had significantly higher acidity than the Anthrozem topsoil.

Maize, sown in the area a year after reclamation, achieved a grain yield of 5.1 t ha^{-1}, which nearly reached the three-year average yield in Slovakia for 2022–2024 (6.5 t ha^{-1}) (Zelená správa, 2025).

Supporting our research, results obtained in real practice, mainly in France, show that microbial inoculation of livestock effluents and field application of microbial preparations improve not only soil properties but also the chemical and energy quality of forage, particularly protein value, digestibility, and nitrogen efficiency, while maintaining or increasing production levels. These improvements contribute directly to greater feed self-sufficiency, reduced dependence on external inputs, improved nitrogen management, higher milk and meat production potential, and increased farm profitability (SOBAC, 2026).

CONCLUSION

Thanks to concentrated Bacteriolite technology, a farmer can activate manure or compost into an active medium capable of fertilizing even waste sludge from excavated gravel, which can then be used as a subsoil layer.

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PART 4

POSTERS

CHEMICAL AND MICROBIAL QUALITY OF ALFALFA AND RED CLOVER MIXTURE SILAGES DEPENDING ON THE SHARE IN THE MIXTURE AND ADDITIVES

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ABSTRACT

This research was conducted to study the influence of ensiled alfalfa and red clover mixtures, mixed at different ratios, as well as the effect of additives – two doses of oak tannin extract and bacterial inoculant on the basic chemical composition and the number of different microorganisms of the silages. With increasing the share of red clover in mixture, there was a significant reduction in proteolysis. Treatments with 50 and 75% red clover had the highest number of lactic acid bacteria and the lowest number of total bacteria. When tannins and LAB inoculants were added, a significant reduction in the level of proteolysis was also observed, which compared to the control treatment was up to 25%.

Keywords: additive, alfalfa, red clover, silage

INTRODUCTION

When alfalfa is ensiled alone, the fermentation outcomes are often suboptimal, because of its high buffering capacity and low sugar and dry matter concentrations (Wang *et al.*, 2019). During the production of alfalfa silage occurs the process of proteolysis in significantly higher extent in comparison with red clover silage (Beauchemin *et al.*, 1997). Jones *et al.* (1995) reported reduction of proteolysis by 70% when mixing extracts of red clover and alfalfa at 1:1 ratio, compared with untreated alfalfa extract in laboratory scale. Many researches connect this fact with presence of polyphenol oxidase in red clover. The positive effect of alfalfa ensiling in a mixture with red clover could also be achieved through richness in vitamins, minerals and other substances, as these two plant species complement each other in terms of these substances (Chiriac *et al.*, 2020). Treatments with commercially available silage additives, such as tannins, could effectively reduce non-protein nitrogen (NPN) formation during ensiling (Tabacco *et al.*, 2006). According to Pys *et al.* (2002), a significant inhibition of protein proteolysis was found in alfalfa-rolled barley silage made with the addition of lactic acid bacteria (LAB) inoculants. This paper discusses the effects of different additives and species share in mixture on the chemical composition and microbial status of alfalfa and red clover mixture silages.

MATERIALS AND METHODS

Green forage was cut in 2024 at the beginning of flowering. The experiment, involving treatments as combinations of six different mixtures and four additives, was performed. The following mixtures were included: alfalfa (A1), alfalfa and red clover at a ratio of 90:10 (A2), 70:30 (A3), 50:50 (A4), 30:70 (A5), and red clover (A6) on a fresh (wilted) matter (FM) basis. The additive factor variants were as follows: control (without additive) (B1), two doses of granulated oak tannin extract (6 g/kg DM (B2) and 12 g/kg DM (B3); 'Essedielle SRL', Italia), and bacterial inoculant 'BioStabil Plus' which contained homofermentative lactic acid bacteria (*Enterococcus faecium* and *Bacillus plantarum*), and heterofermentative lactic acid bacteria (*Bacillus brevis*) with a concentration of 5×10^{10} CFU per gram (B4). The mixtures were ensiled in the polyethylene jars holding 5 dm^3, with three replications. After 56 days the experimental silos were opened and representative samples were taken for chemical analyses.

In the silage following chemical parameters were determined: crude protein (CP), crude fiber (CF), ether extract (EE), crude ash (CA), nitrogen free extract (NFE) and water soluble nitrogen (H_2O-N). NDF and ADF were determined applying the method of Van Soest *et al.* (1991). The following microbial parameters were analyzed: total bacteria count (TB), lactic acid bacteria count (LAB), and yeast and mold count (YM). Statistical analysis of the obtained results was done by analysis of variance procedure with software package STATISTICA, version 8.0 ('STATISTICA 8', Stat. Soft. 2007).

RESULTS AND DISCUSSION

With an increase in the proportion of red clover in the mixture, there was a decrease in the crude protein content and significant reduction of proteolysis under the influence of polyphenol oxidase (Table 1). Also, the addition of tannin and inoculant led to a significant reduction of proteolysis, which was consistent with Tabacco *et al.* (2006). Similar to our investigation, Li *et al.* (2018) stated that the crude protein content in silage differed significantly between the mixtures of alfalfa and red clover, and ranged from 163.1 g/kg DM in pure red clover silage to 183.7 g/kg DM in pure alfalfa. According to Contreras-Govea *et al.* (2013), the ADF content of the control variant of alfalfa silage was 227.7 g/kg DM, while the ADF content of the variant with the inoculant *Lactobacillus plantarum* was 218.5 g/kg DM silage, which supports the results of our experiment. Other variations of the examined chemical parameters can be explained by the relative change in their mutual relationship, due to the loss of volatile ingredients (ammonia, free acetic and butyric acid) when preparing the samples by drying in accordance with AOAC methods, as well as due to the extraction of part of the lactic acid with diethyl ether when determining the fat content (Đorđević *et al.*, 2011).

CONCLUSION

From the results it is evident that addition of red clover to alfalfa when ensiling, as well as the use of oak tannins and inoculants led to a significant reduction of proteolysis, which is considered the most important effect of the tested factors on the quality and nutritional value of silage. The determined trend is particularly significant due to the fact that alfalfa is the most important and cheapest source of protein in rations for dairy cows in Serbia, and the obtained values should be used when balancing the ration in accordance with current normatives. The variation of other parameters of the chemical composition was mostly insignificant, and of no importance for the use of silage. A more detailed examination of the influence of the type and dose of tannins is planned, due to the possibility that, in excessive amounts, they may have a negative effect on protein utilization.

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Tab. 1: Chemical composition of silages (g/kg DM) and number of microorganisms (log cfu/g)

	CP	CF	EE	NFE	CA	NDF	ADF	H2O-N	LAB	TB	YM
Mixture											
A1	195.8^{ab}	295.4	40.6	377.2	90.8	506.6	400.7	387.4^{a}	8.02^{b}	8.66^{a}	2.82^{b}
A2	196.7^{ab}	298.8	37.9	374.9	91.6	505.5	403.1	365.1^{b}	8.10^{ab}	8.62^{ab}	2.61^{c}
A3	199.6^{a}	298.1	38.7	373.3	90.4	508.9	402.3	380.0^{ab}	7.99^{b}	8.60^{bc}	2.64^{bc}
A4	197.2^{ab}	295.2	38.2	378.1	91.3	506.4	403.2	311.3^{c}	8.16^{a}	8.54^{d}	2.81^{b}
A5	197.2^{ab}	295.3	39.0	376.1	92.4	505.7	399.8	315.7^{c}	8.17^{a}	8.57^{cd}	2.80^{bc}
A6	187.2^{b}	296.8	39.5	387.1	89.3	503.3	400.3	300.0^{c}	8.10^{ab}	8.59^{bcd}	3.02^{a}
Additive											
B1	193.4	297.7^{a}	38.0	377.4	93.3	507.2	405.3^{a}	405.3^{a}	8.11	8.60	2.68^{b}
B2	194.4	297.0^{ab}	38.1	380.9	89.5	505.6	402.8^{ab}	337.7^{b}	8.02	8.58	2.83^{ab}
B3	196.5	300.6^{a}	39.4	373.3	90.1	507.1	400.6^{ab}	327.1^{b}	8.11	8.62	2.84^{a}
B4	198.1	291.1^{b}	40.4	379.6	90.8	504.5	397.6^{b}	302.7^{c}	8.12	8.59	2.79^{ab}
ANOVA											
A	*	ns	ns	ns	ns	ns	ns	**	*	**	**
B	ns	*	ns	ns	ns	ns	*	**	ns	ns	*
AXB	ns	ns	ns	ns	ns	ns	ns	**	**	**	**

Values in columns with a different uppercase letters are significantly different based on the LSD test (P<0.05); F-test: * – P<0.05; ** – P<0.01; ns – F-test not significant.

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CITRIC AND FORMIC ACID FOR STABILIZATION OF TOTAL MIXED RATIONS

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ABSTRACT

Total mixed rations (TMR) are exposed to aerobic deterioration after mixing, critically accelerated by elevated temperatures. This study evaluates the efficacy of CitroFor stabilizer (citric and formic acid, 95:5 diluted 1:4 with water) across three TMR types (dry cows, lactating cows, post-partum cows) at two temperatures (20 and 30 degrees C) using continuous temperature monitoring (84 h, n = 3). The dry cow TMR showed the highest benefit: at 30 degrees C, CitroFor at 4 l/t fresh matter (FM) reduced the temperature increase from +12.5 to +7.7 degrees C (38%), with a statistically significant ANOVA result (F = 6.39; p = 0.033) and complete elimination of mould and yeast. For lactating cow TMR, even 4 l/t was insufficient to prevent mould and yeast growth. The 2 l/t dose was counterproductive for post-partum TMR (critical time shortened by 10%). Results highlight a fundamental interaction between TMR type, stabilizer dose and temperature regime with practical implications for seasonal feeding management.

Keywords: aerobic spoilage; dairy cattle; yeast inhibition; temperature monitoring; dose-response interaction

INTRODUCTION

Aerobic spoilage of TMR is a major economic and nutritional challenge in dairy farming. The warming of TMR above ambient temperature signals uncontrolled microbial activity driven primarily by yeasts and moulds that oxidise residual fermentation products. At temperatures above 40 °C, Maillard reactions reduce amino acid bioavailability (Borreani *et al*., 2018). Organic acids – particularly propionic, formic and citric acid – are known to suppress aerobic deterioration in silage and TMR (Gheller *et al*., 2021; Seppälä *et al*., 2013). However, the interaction between TMR nutritional composition, stabilizer dose and ambient temperature has not been systematically addressed. The present study tested CitroFor, a novel combination of citric and formic acid, across contrasting TMR types and temperature regimes.

MATERIAL AND METHODS

Three TMR types prepared on a commercial dairy farm (Netluky, Czech Republic) were tested in November 2025 at the Institute of Animal Science. TMR composition is summarised in Table 1. Treatments were: Control (A) – no additive; CitroFor 2 l/t FM (B); CitroFor 4 l/t FM (C). The stabilizer (95 parts of citric acid diluted 1:4 with water + 5 parts formic acid) was applied by surface spraying. Samples of each treatment were distributed into labelled polyethylene bags (3 replicates × 3 treatments × 3 TMR types × 2 temperatures = 54 bags). Bags were stored at constant 20 °C or 30 °C in climate chambers for 84 hours. Temperature was recorded every hour using DS1922L data loggers. Visual assessment (mould, yeast, heating, sliminess) was performed at 72 h on a 0–3 scale (0 = none, 3 = severe). Statistical analysis used one-way ANOVA (α = 0.05) with the final temperature at 84 h as the dependent variable.

RESULTS AND DISCUSSION

ANOVA results and key temperature data are presented in Table 2. A statistically significant treatment effect was found only for dry cow TMR at 30 °C (F = 6.39; p = 0.033). CitroFor at 4 l/t reduced the final temperature from 44.4 ± 1.2 °C to 37.1 ± 4.1 °C, a reduction of 7.3 °C, keeping TMR safely below the critical 40 °C threshold for Maillard reactions. The temperature increase above ambient was reduced from +12.5 °C (control) to +7.7 °C (38% reduction). Visual assessment confirmed complete suppression of mould and yeast growth at 4 l/t for dry cow TMR even after 72 h at 30 °C (score 0/3; Table 3).

In contrast, lactating cow TMR – characterised by a high proportion of maize silage (33.3% FM) and concentrate (17.6% FM), and high starch content (27.2% DM) – showed mould (score 0.67/3)

Tab. 1: Composition of tested TMR (kg/head/day; % of total fresh matter)

Component	TMR 1 Post-partum		TMR 2 Lactation		TMR 4 Dry cows	
	kg/head/d	%	kg/head/d	%	kg/head/d	%
Alfalfa-grass silage	13.0	24.8	21.0	38.9	20.0	68.3
Maize silage (35% DM)	22.0	41.9	18.0	33.3	6.0	20.5
Wheat straw	—	—	—	—	3.0	10.2
Wet maize grain	4.0	7.6	3.5	6.5	—	—
Liquid feed MGP	2.0	3.8	2.0	3.7	—	—
Concentrate mix	11.5	21.9	9.5	17.6	0.3	1.0
TOTAL	**52.5**	**100.0**	**54.0**	**100.0**	**29.3**	**100.0**
DM (%)	49.3	—	47.5	—	40.6	—
NEL (MJ/kg DM)	7.17	—	6.75	—	4.79	—
NDF (% DM)	15.4	—	16.6	—	33.4	—
Starch (% DM)	28.6	—	27.2	—	—	—

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Tab. 2: ANOVA results and temperature data at 84 h for all TMR × temperature combinations (ns = not significant; *p < 0.05; n.a. = not applicable)

TMR type	Temp. (°C)	F value	p value	Control (°C)	CF 4 l/t (°C)	Reduction
Post-partum	20	1.09	0.394 ns	n.a.	n.a.	n.s.
Post-partum	30	0.98	0.428 ns	n.a.	n.a.	n.s.
Lactation	20	0.24	0.792 ns	n.a.	n.a.	n.s.
Lactation	30	0.39	0.693 ns	n.a.	n.a.	n.s.
Dry cows	20	1.40	0.316 ns	n.a.	n.a.	n.s.
Dry cows	**30**	**6.39**	**0.033***	**44.4 ± 1.2**	**37.1 ± 4.1**	**−7.3 °C (38%)**

Tab. 3: Visual assessment scores at 72 h / 30 °C and temperature increase above ambient (scale 0–3; CF = CitroFor; n.d. = not determined)

TMR type	Mould	Yeast	Heating (°C above ambient)	Note
Dry cows — Control	0/3	0/3	+12.5	Maillard risk
Dry cows — CF 4 l/t	0/3	0/3	+7.7	Safe < 40 °C
Lactation — CF 4 l/t	0.67/3	1.33/3	n.d.	Insufficiently protected
Post-partum — CF 2 l/t	n.d.	n.d.	n.d.	Critical time −10%

and yeast (score 1.33/3) contamination despite 4 l/t application (Table 3). The abundant water-soluble carbohydrates provide an ideal substrate for rapid yeast proliferation that cannot be controlled by the doses tested. For post-partum TMR (highest energy density, 7.17 MJ NEL/kg DM), the 2 l/t dose was counterproductive: the critical time to visible spoilage was shortened by approximately 10%, likely because a sub-inhibitory acid concentration stimulates rather than suppresses yeast metabolism.

The selective effect of CitroFor across TMR types can be explained by differences in buffering capacity and substrate availability. Dry cow TMR contains high structural NDF (33.4% DM) which physically limits oxygen diffusion into the mass and slows microbial activity to a level manageable by the applied acid mixture. The mechanism of action of citric acid – a polyprotic acid (pKa 3.13, 4.76, 6.40) – involves chelation of metal ions essential for microbial growth and direct antimicrobial activity of undissociated molecules penetrating cell membranes (Li *et al*., 2024). Formic acid (pKa 3.75) acts synergistically by rapid pH depression. The combination thus addresses both the metabolic and physical dimensions of spoilage (Rinne *et al*., 2018).

These findings are consistent with Seppälä *et al.* (2013), who reported that TMR stabilizer response depends strongly on silage quality, and with Gheller *et al.* (2021), who found that 4 l/t organic acid blends were optimal for maize silage-based TMR. The novel contribution of this study is the direct comparison across physiologically contrasting TMR types in a single experiment, revealing that dose recommendations cannot be universal.

CONCLUSION

CitroFor at 4 l/t FM significantly improved aerobic stability of dry cow TMR at 30 °C, reducing temperature increase by 38% and eliminating visible mould and yeast growth. For lactating and post-partum TMR, the tested doses were insufficient or counterproductive due to high fermentable substrate content. The 4 l/t dose is essential during high summer temperatures (>30 °C) for dry cow TMR; 2 l/t may be appropriate under moderate barn conditions for less susceptible TMR types. TMR composition must be considered when selecting stabilizer dose. Repeated trials with larger replication and chemical analysis of water-soluble carbohydrate content are needed to refine dose recommendations.

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COMPARISON OF THE EFFECT OF SILAGE ADDITIVES ON THE FERMENTATION PROCESS OF THE LATTE SORGHUM VARIETY IN SINGLE-CUT USE

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ABSTRACT

Sorghum of the LATTE variety was harvested at the milky-wax maturity stage, with an average dry matter of 26% for minisilage production. Using a silage hydraulic press, the mass was pressed into tubes (diameter 160 mm, length 65 cm), weighed, and closed with a rubber lid equipped with a gas outlet valve. Sorghum forage was preserved with various additives: control without additives (C), organic acid-based additives (OA), and acid salt-based additives (AS), with each variant replicated 6 times. After 5 months of fermentation, the minisilos were opened, and silage samples were analyzed. The highest pH was found in OA (4.09) and was higher than in AS (3.86) and C (3.9, $P<0.05$). The acidity of the aqueous extract was lowest in OA (703 mg KOH) compared to C (1301 mg KOH) and AS (1462 mg KOH) ($P<0.05$). Compared to C silages, OA and AS silages had significantly ($P<0.05$) higher contents of all silage acids (85.3, 87.3, and 64 g/kg DM) and especially acetic acid (25.9, 29.1, and 16.6 g/kg DM in OA, AS, and C silages, respectively). In addition, OA silages had significantly higher propionic acid content (2.71 g/kg DM). The higher content of silage acids in silages with additives not only improved their quality but also increased their stability under aerobic conditions.

Keywords: modelsilage, sorghum silage, LATTE variety, silage additives, silage acids

INTRODUCTION

With the progressing climate change and the increasingly frequent problems with a lack of moisture during vegetation and the associated deterioration of yields of the main fodder crops (silage corn, alfalfa), the task arises of ensuring the fodder base with drought-resistant crops. One such crop is sorghum bicolor [*Sorghum bicolor* (L.) Moench.], which, thanks to breeding for a lower content of anti-nutritional substances (tannins, dhurrin), also appears to be a suitable crop for the production of silage for cattle. Despite its lower content of nitrogenous substances and starch, it can be a suitable supplement to feed rations and also a suitable reserve in case of a shortage of corn and alfalfa silage. The NDF content in sorghum forage is also interesting. With a suitable crop rotation, it can be included after winter crop harvests, yielding one additional harvest in a given year. It is also advantageous to use some sorghum varieties in two cuts, as plants harvested earlier have higher crude protein content. Various preservatives are used for ensiling, both chemical and biological, and their selection is often guided by the dry matter content of the preserved forage. When preserving sorghum, especially with a single-cut harvest, it is often a problem to achieve a higher dry matter content of the forage.

MATERIAL AND METHODS

Sorghum forage of the LATTE variety was grown on the lands of the Mendel University in Brno school agricultural enterprise, which are located in South Moravia in the village of Žabčice (Czech Republic). Plants were harvested at the milky-wax maturity stage of the grain. Harvesting took place in September, and due to adverse weather conditions, it was not possible to wither the plants to a higher dry matter content. The average dry matter content of the ensiled forage was 260 g/kg. The sorghum cut was treated with preservatives based on organic acids (OA) and acid salts (AS); the control group was ensiled without additives (C). The fodder was pressed into cylindrical tubes (diameter 160 mm, length 65 cm), using a silage hydraulic press. Tubes were loaded with a 10 kg weight, closed with a rubber lid equipped with a gas outlet valve, and weighed. Each variant was ensiled in 6 replicates. The tubes were left to ferment for 5 months at ambient temperatures of 20–25°C. After the fermentation period, silage samples were taken for analysis. Essential nutrients were analyzed in the laboratory according to methodologies in accordance with COMMISSION REGULATION (EC) No. 152/2009. Furthermore, according to Mertens (2002) and ISO 16472 (2012), acid detergent fiber (ADF) and neutral detergent fiber (NDF) were determined. Acid detergent lignin (ADL) was determined from the ADF fraction after 3-hour maceration in 72% sulfuric acid, and cellulose was determined by subtracting ADL from the ADF fraction (ADF – ADL). Fatty acids were measured in silage extract by capillary isotachophoresis according to Kvasnička (2000). The determination was performed using the IONOSEP 2001 analyzer (RECMAN–laboratory systems, Ostrava, Czech Republic). Statistical evaluation was performed using the STATISTICA 14 program (TIBCO Software Inc.). The significance of differences was evaluated using a one-factor Analysis of Variance using the Tukey HSD test.

RESULTS AND DISCUSSION

The results of the fermentation process evaluation are shown in Table 1. Despite the low dry matter of the ensiled material, the resulting silages were of acceptable quality. Silages treated with OA and AS showed significantly higher total organic acid and acetic acid contents compared to the control silage. The pH value corresponded to the dry matter of the silages. As for the percentage of lactic acid, the treated silages were relatively balanced, but their values were inconclusively lower than those of the control silage. SHAVER (2003) states that the proportion of lactic acid from the total fermentable acids should reach at least 65-70%. The control silages were closer to the higher value, but the differences were not significant. Nutrient values after laboratory analysis are shown in Table 2.

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Tab. 1: Composition of tested TMR (kg/head/day; % of total fresh matter)

Experimental group		Control (C)		Organic acids (OA)		Acid salts (AS)	
n		6		6		6	
Parameter	**Units**	**Mean**	**S.D.**	**Mean**	**S.D.**	**Mean**	**S.D.**
pH		3.90[a]	0.03	4.09[b]	0.08	3.86[a]	0.02
Acidity of the aq. extract	mg KOH	1300[b]	71.26	703[a]	106.65	1462c	66.23
Ammonia (NH_3)	g/kg of silage	0.26	0.07	0.21	0.03	0.28	0.05
Lactic acid	g/kg DM	46.60	12.35	56.40	12.09	57.70	5.00
Acetic acid	g/kg DM	16.61[a]	3.56	25.85[b]	2.81	29.09[b]	1.81
Propionic acid	g/kg DM	0.82[a]	1.39	2.71[b]	0.83	0.45[a]	0.46
Butyric acid	g/kg DM	0.00	0.00	0.32	0.79	0.00	0.00
Sum of acids	g/kg DM	64.03[a]	14.53	85.29[b]	11.73	87.28[b]	5.46
LA/VFA		2.71	0.76	1.98	0.49	1.96	0.21
LA/AA		2.84[b]	0.81	2.22[ab]	0.54	1.99[b]	0.21
% of LA out of total FA	%	71.77	7.02	65.55	7.05	66.09	2.51

n - number of cases; S.D. - standard deviation; LA - lactic acid; VFA - volatile fatty acids; AA - acetic acid; FA - fatty acids. Means in a row marked with different letters are significantly (P<0.05) different.

Tab. 2: Nutritional value of sorghum silages (nutrients in g/kg of dry matter)

Experimental group	Control (C)		Organic acids (OA)		Acid salts (AS)	
n	6		6		6	
Nutrients in g/kg of DM	**Mean**	**S.D.**	**Mean**	**S.D.**	**Mean**	**S.D.**
Dry matter	260	7.61	268	17.10	264	10.43
Crude Ash	74[b]	1.90	68[a]	2.03	72[b]	0.97
Crude Protein	83[b]	2.69	79[a]	2.90	83[b]	1.90
Ether Extract	23	3.69	23	2.89	24	1.39
Crude Fibre	306[b]	7.08	284[a]	8.42	309[b]	8.19
ADF	375	13.24	377	28.58	379	6.78
aNDF	606[b]	15.19	582[a]	15.97	598[ab]	15.43
ADL	44[a]	2.28	53.6[b]	13.41	43.5[ab]	1.08
Cellulose	331	11.53	323	16.68	336	5.79

n - number of cases; S.D. - standard deviation; ADF - Acid detergent fiber; aNDF - Neutral detergent fiber after sample treatment with amylase; ADL - acid detergent lignin; DM - dry matter. Means in a row marked with different letters are significantly (P<0.05) different.

CONCLUSION

Good quality was found for sorghum silages despite the low dry matter content of the ensiled material. The higher content of silage acids in silages with additives not only improved the quality of silages, but also improved their stability after exposure to aerobic conditions. Sorghum silage should be a suitable supplement to cattle feed rations and a feed reserve in times of climate change with a lack of moisture during the vegetation period.

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DYNAMIC CHANGES IN STRUCTURAL CARBOHYDRATE COMPONENTS DURING THE ENSILING OF SWEET SORGHUM

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ABSTRACT

Sweet sorghum can be used to produce a substantial quantity of biofuel due to its high biological yield and high carbohydrate content. In this study, we investigated the dynamic changes in fermentation characteristics, carbohydrate components, and the bacterial community during the ensiling of wilted and unwilted sweet sorghum. The results revealed a rapid fermentation pattern and high-quality fermentation quality in wilted and unwilted sweet sorghum, wherein lactic acid, and acetic acid accumulated and stabilized during the initial 9 days of ensiling, with the pH values less than 4.2, until 60 days of ensiling. We found that the ensiling of sweet sorghum involved the degradation (5%~10%) of neutral detergent fiber (NDF) and hemicellulose and that the degradation of NDF fit a first-order exponential decay model. A shift in dominance from *Lactococcus* to *Lactobacillus* occurred before the first 9 days of ensiling, and the abundance of *Lactobacillus* (r=-0.68, P<0.001) was negatively correlated with the NDF content. The relative abundances of *Lactobacillus* in wilted and unwilted sweet sorghum after ensiling for 60 days were 76.30% and 93.49%, respectively, and relatively high fermentation quality was obtained. In summary, ensiling is proposed as a biological pretreatment for sweet sorghum for subsequent biofuel production, and unlike other materials, sweet sorghum quickly achieves good fermentation quality and has great potential for bioresource production.

Keywords: sweet sorghum, carbohydrate components, ensiling

INTRODUCTION

The production of bioresources is under great pressure due to global climate change and the continuous emergence of issues such as urbanization and water scarcity. In general, plants that have more carbohydrates and less lignin generally have the benefit of being able to produce significant amounts of biofuel (Mathur *et al*., 2017). Sweet sorghum (*Sorghum bicolor* L. Moench) is a C4 herbaceous annual grass that has high photosynthetic efficiency. Moreover, sweet sorghum has comparatively high energy and carbohydrate contents. After the required processing, the accessible holocellulose in the biomass may be readily used to produce biofuel (Velmurugan *et al*., 2020). However, owing to its short harvest periods and seasonality, sweet sorghum requires appropriate preservation after widespread harvest to guarantee a steady supply throughout the year for subsequent biofuel production. Thus, in this study, we investigated the dynamic changes in carbohydrate components of wilted and unwilted sweet sorghum during ensiling pretreatment, as well as the impact of ensiling processes on the fiber degradation kinetics of sweet sorghum.

MATERIALS AND METHODS

Sweet sorghum plants were harvested at the heading stage in Wuzhong, Ningxia Hui Autonomous Region, China (N 37°43′, E 106°04′, elevation 1240 m). A portion of the test materials was collected immediately after harvest, and additional materials were obtained after 48 h of wilting in the field. The wilted and unwilted materials were shredded into approximately 2 cm pieces using a small plant chopper. The 42 bags (2 treatments × 7 days × 3 replicates) were vacuum-sealed and kept in a room at approximately 20°C. Three bags per treatment were opened after the initial (3d, 6d, 9d), intermediate (14d, 21d, 30d) and late (60d) fermentations. Then the carbohydrate components of silage samples was examined. The contents of neutral detergent fiber (NDF) and acid detergent fiber (ADF) were analyzed using an automatic fiber analyzer (Ankom 2000i full; Ankom Tech Co., Macedon, NY, USA) in accordance with the procedures outlined by Van Soest *et al.* (1991). The hemicellulose (HC) and cellulose (CL) contents were calculated according to the NDF, ADF and acid detergent lignin contents. The NDF, ADF, HC, and CL data were fitted to the following exponential decay model (Eq. (1)) to characterize the fiber degradation properties (Zhao, *et al*., 2018):

$$y = y_0 + a * \exp(-bt) \quad (1)$$

Among these are: y, the total amount of undegraded material at time t; y0, the undegraded portion fraction after 60 days of ensiling; a, the degradable fraction; b, the percentage disappearance rate (day^{-1}) of a; t, the number of ensiling days; and e, 2.7183.

RESULTS AND DISCUSSION

The dynamic changes in the (a) NDF, (b) ADF, (c) HC and (d) CL contents in wilted and unwilted sweet sorghum during the ensiling are shown in Fig. 1(a-d). With increasing ensiling time, the NDF and HC contents in the wilted and unwilted sweet sorghum silages decreased to varying degrees, the ADF and CL

Tab. 1: Degradation kinetic parameters of fiber during the ensiling of sweet sorghum and fitted to a first-order exponential decay model (y = y0 + a * exp (-bt))

Item1	y_0	a	b	R^2
NDF	94.81	5.26	0.25	0.957
ADF	95.55	4.54	0.04	0.821
HC	91.57	8.43	0.55	0.882
CL	96.37	3.67	0.25	0.868

1 NDF, neutral detergent fiber; ADF, acid detergent fiber; HC, hemicellulose; CL cellulose.

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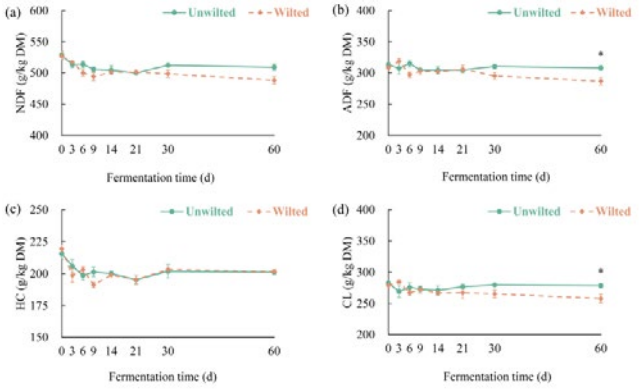


Fig. 1: The dynamic changes in (a) neutral detergent fiber (NDF), (b) acid detergent fiber (ADF), (c) hemicellulose (HC) and (d) cellulose (CL) contents in wilted and unwilted sweet sorghum after 3, 6, 9, 14, 21, 30 and 60 days of ensiling. DM, dry matter. Asterisks indicate significant differences between different treatments on the same days of ensiling (*, significant at P<0.05)

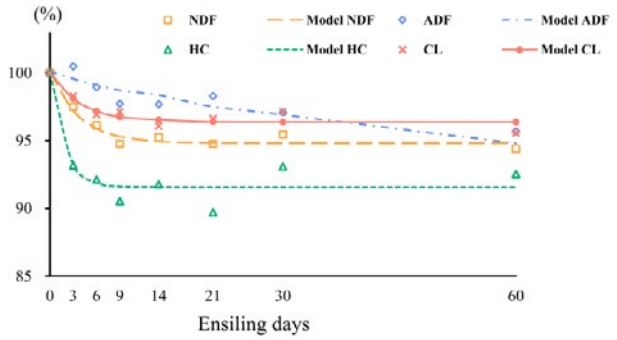


Fig. 2: Degradation kinetic parameters of fiber during the ensiling of sweet sorghum and fitted to a first-order exponential decay model (y = y0 + a * exp (-bt)).

contents in the unwilted sweet sorghum silage remained basically unchanged, and the ADF and CL contents in the wilted sweet sorghum silage tended to decrease during the later stage of ensiling. The degradation mechanism of structural carbohydrates observed in the study could be explained by enzymatic action, acidolysis and microbial activity (Dewar *et al.*, 1963). Unlike other materials, sweet sorghum has a higher content of WSCs and a lower buffering capacity. After the formation of an anaerobic environment, the microbial fermentation process intensifies, and the acid production ability of the microbial mixture becomes stronger, which may lead to a certain degree of degradation of structural carbohydrates.

The measured structural carbohydrate contents were fitted using a first-order exponential decay model, and the kinetic parameters and related fit curves are shown in Table 1 and Fig. 2, respectively. We found that the correlation coefficient (R^2 value) of the NDF degradation curve was 0.957, which indicates that the degree of NDF degradation in sweet sorghum silage can be well predicted using a first-order exponential decay model. Degradation of NDF occurred mainly during the initial 14 days of ensiling, suggesting that structural carbohydrates are more readily degraded during the early phases of ensiling. Similar results were also found in a previous study, in which the combination of *Lactobacillus plantarum* and cellulase increased the breakdown of structural carbohydrates in forage silage. ADF showed slight degradation, and the degradation rate was 0.04, which could be attributed to continued acidolysis. The large reduction in NDF was attributed to the high breakdown of HC, which is consistent with the finding that HC is readily hydrolyzed (McDonald *et al.*, 1991).

CONCLUSION

The ensiling of sweet sorghum involved the degradation (5%~10%) of NDF, and the degradation of NDF fit a first-order exponential decay model. However, in our study, there was no change in the ADL content in wilted or unwilted sweet sorghum silage with increasing ensiling time.

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EFFECT OF PHYSICAL STRUCTURE AND ADDITIVE APPLICATION ON MYCOTOXIN CONTAMINATION OF MAIZE SILAGE

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ABSTRACT

The aim of this study was to assess whether chop length and silage additives affect mycotoxin contamination in maize silage. Maize harvested at the milk wax stage was ensiled with urea, a lactic acid bacteria inoculant, or their combination, particle size was evaluated using the Penn State Particle Separator, and six mycotoxins were quantified by HPLC-MS/MS (88% dry matter basis). Deoxynivalenol and fumonisins were the predominant mycotoxins, HT-2 toxin occurred only in the untreated control, and urea alone or with inoculant reduced deoxynivalenol and prevented HT-2 toxin while chop length had no statistically significant effect on mycotoxin contamination.

Keywords: silage, additeves, urea, biological additive, corn silage

INTRODUCTION

Mycotoxins represent a group of toxic secondary metabolites produced by fungi, primarily belonging to the genera *Fusarium*, *Aspergillus*, and *Penicillium*. Major representatives of these toxins include deoxynivalenol (DON), fumonisins (FUM), HT-2 toxin (HT-2), zearalenone (ZEA), aflatoxins (AFL), and ochratoxins (OTA), which are frequently detected in both concentrate and forage feeds used in ruminant nutrition (Chhaya *et al*., 2024). The aim of this study was to evaluate the occurrence and concentration of selected mycotoxins (DON, FUM, HT-2 toxin, ZEA, OTA, and AFL) in maize silages and to determine the effect of different silage additives (urea, biological additive, and their combination) and chop length on mycotoxin formation.

MATERIALS AND METHODS

In cooperation with PD Kozárovce (conventional farm), maize (FAO 480, dent grain type) was ensiled at the milk–wax stage of grain maturity. The biomass was harvested manually (several plants) and chopped using a shredder (H: 6284 XL). Variant C (control, without additives) was ensiled first, followed by variant U with the addition of urea (at a dose of 0.3%). + The physical structure of variants C and U was determined according to Heinrichs (2025) using the Penn State Particle Separator (PSPS), where the proportion of particles retained on the first sieve with 19 mm openings was 18.0% (longer chop length). Biomass from the same plot was subsequently harvested using a self-propelled harvester (KRONE BIG X), and a biological additive was applied simultaneously (containing *L. plantarum*, *L. rhamnosus*, and *L. buchneri*, min. 3.0×10^{11} CFU/g; applied at a dose of 1 g per 1 t of fresh matter). This biomass was ensiled as variant B (biological additive), after which urea was added (0.3%) and variant B+U was ensiled. The physical structure of variants B and B+U was assessed using the Penn State Particle Separator (PSPS), where the proportion of particles retained on the first sieve with 19 mm openings was 4.0% (shorter chop length). The biomass was compacted using a vacuum sealer (MSW, Motor Technics) and stored in plastic bags (n = 3). After six weeks, samples were collected, dried, and mechanically processed. In cooperation with Romer Labs (Tulln, Austria), the content of six mycotoxins (DON, FUM, HT-2 toxin, ZEA, OTA, AFL) was analysed using multi-mycotoxin analysis (HPLC–MS/MS). Mycotoxin concentrations are reported on an 88% dry matter basis.

RESULTS AND DISCUSSION

Of the six monitored mycotoxins, only DON and FUM were detected in maize silages, and HT-2 toxin was found only in the control. DON was the predominant mycotoxin detected in maize silage, followed by FUM as the mycotoxins with the second highest concentration. Guidance level for DON in cereals and cereal products is 8,000 µg/kg and for maize by-products is 12,000 µg/kg in the EU (Romer Labs, 2024), and according to FDA (2019) for ruminating dairy cattle older than 4 months is advisory level for total ratio 5,000 µg/kg. For FUM is guidance level for maize and maize products 60,000 µg/kg and for compound feed for adult ruminants, 50,000 µg/kg in the EU (Romer Labs, 2024). For lactating dairy cattle is guidance level for FUM 30,000 µg/kg (no more than 50% of diet- dry weight) according to FDA (2019). None of the analysed samples of maize silage exceeded the specified guidance levels for DON and FUM. There is no EFSA and FDA guidance concerning T-2/HT-2, but potentially harmful levels for cattle are 700-1,500 µg/kg in total ration dry matter (Dairyland, 2016). The HT-2 toxin was only present in the C silage without additives, at a concentration below potentially harmful level. The application of additives (urea, biological additive, and their combination) inhibited the formation of HT-2 toxin in maize silages. Kalúzová *et al.* (2022) confirmed the significant effect of the addition of urea and biological additive on reducing the content of T-2 toxin in maize silages. The addition of urea and the combination of biological additive with urea significantly ($P < 0.05$) reduced the DON concentration, unlike the application of the biological additive alone. This agrees with the results of Kalúzová *et al.* (2022). In maize silages treated with the biological additive, a significantly higher ($P < 0.05$) content of DON and FUM was observed. Gallo *et al.* (2021) tested several inoculants containing lactic acid bacteria and found out a positive effect ($P < 0.05$) on reducing AFB1 concentration, but a different effect on DON concentration in maize silage (control: 2215.0 µg/kg vs. with biological additives 2629.0–4373.0 µg/kg, $P > 0.05$). Skládanka *et al.* (2013) found out that grass silage treated with a biological–enzymatic inoculant had a comparable DON content as control ($P < 0.05$) and significantly higher concentration ($P < 0.05$) in silage with chemical additive. In the present study, the addition of urea and the combination of the biological additive with urea did not significantly affect the FUM concentration. Maize silage with biological additive had statistically significant ($P < 0.05$) higher concentration of FUM. Skládanka *et al.* (2013) detected the presence of FUM only in grass silages treated with a biological–enzymatic inoculant, in contrast to the control and silages treated with a chemical additive. Overall, the shorter length of cut showed lower total mycotoxin average content compared with the longer length of cut (446.2 vs. 522.5 µg/kg) however, the differences were not statistically significant. Similarly, Toledo *et al.* (2020) found out that particle size did not affect the presence of mycotoxins in maize silage.

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Tab. 1: Influence of physical structure (stratified with the Penn State Particle Separator) and additive use on mycotoxin contamination of maize silage (in µg/kg)

Chop length	Variant	Parameter	DON	FUM	ZEA	HT-2	AFB1	OTA
Longer	C	x̄	2131.2^{abc}	138.2^{a}	ND	235.8	ND	ND
		SD	206.9	21.8	/	86.0	/	/
	U	x̄	612.4^{a}	136.8	ND	ND	ND	ND
		SD	45.1	10.8	/	/	/	/
Shorter	B	x̄	1198.6^{b}	185.9^{a}	ND	ND	ND	ND
		SD	23.6	56.2	/	/	/	/
	B+U	x̄	754.6^{c}	176.0	ND	ND	ND	ND
		SD	56.7	55.6	/	/	/	/
All Variants		x̄	1174.2	160.2	ND	235.8*	ND	ND
		SD	626.8	45.2	/	86.0*	/	/
Longer chop length		x̄	522.5 (total mycotoxins)					
Shorter chop length		x̄	446.2 (total mycotoxins)					

C: control, U: urea, B: biological additive, ND: not detected, *only in C variant, values with the same index in the column are statistically significant at P<0.05

CONCLUSIONS

This study evaluated the occurrence of selected mycotoxins in maize silages and assessed the effects of silage additives and chop length on their formation. Among the monitored mycotoxins, maize silages from a Slovak conventional farm were predominantly contaminated with DON, followed by FUM, whereas ZEA, AFLB1, and OTA were not detected. The HT-2 toxin was detected only in the untreated control silage. Importantly, none of the detected mycotoxin concentrations exceeded the current EU or FDA guidance levels for ruminants. The application of silage additives affected mycotoxin formation. The use of urea alone and in combination with a biological additive significantly reduced DON concentrations, while the biological additive applied alone was associated with higher DON and FUM concentrations. The formation of HT-2 toxin was inhibited by all additive treatments, confirming their protective effect against this mycotoxin. In contrast, FUM concentrations were not significantly affected by urea or the combined treatment. Chop length had no statistically significant effect on mycotoxin concentrations; however, maize silages with a shorter length of cut showed a lower average total mycotoxin content compared with those with a longer length of cut. These findings suggest that the concentrations of mycotoxins in silage are influenced more significantly by the addition of additives than by physical structure.

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ENDOGENOUS *LACTOCOCCUS LACTIS* AND *ENTEROCOCCUS FAECIUM* IMPROVE THE SILAGE QUALITY AND AEROBIC STABILITY OF *CYPERUS ESCULENTUS* STEM AND LEAF

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ABSTRACT

This study aimed to investigate the effect of dominant endogenous lactic acid bacteria isolated from natural *Cyperus esculentus* stems and leaves silage on the quality of the silage and to provide a basis for improving the quality of *Cyperus esculentus* stems and leaves silage in South Xinjiang. Five treatment groups were established in this experiment: CK (control group), PA (adding 5×10^7 CFU/g FM *Enterococcus faecium*), EF (adding 5×10^7 CFU/g FM *Pediococcus lactis*), LP (adding 5×10^7 CFU/g FM *Lactiplantibacillus plantarum Lactobacillus plantarumas*), and PE (adding 5×10^7 CFU/g FM *Pediococcus lactis+Enterococcus faecium*,1:1). Each treatment was performed in three replicates. After 60 days of fermentation, sensory evaluation was performed to detect nutritional components, fermentation indicators, microbial content, and aerobic stability time. At 3d, 7d, 15d, 30d and 60d of fermentation, the water-soluble carbohydrate (WSC) content in PE group was significantly higher than that in CK group. The pH value and ammonia nitrogen content (NH_3-N) of all treatment groups were significantly lower than those of the CK group at 3d, 7d, 15d, 30d and 60d of fermentation. The number of lactic acid bacteria (LAB) in the PE, PA, and EF groups was significantly higher than that in the CK group ($P < 0.05$). The number of molds in all treatment groups was significantly lower than that in the CK group ($P < 0.05$). The number of aerobic bacteria (AB) in the PA, EF, and PE groups was significantly lower than that in the LP group ($P < 0.05$). The 72-hour in vitro gas production of *Cyperus esculentus* silage is higher than that of *Cyperus esculentus* stems and leaves. In summary, adding different lactic acid bacteria, especially endogenous ones, can effectively improve the quality of pea stem and leaf silage and improve the feeding value.

Keywords: *Cyperus esculentus* L., Silage, Lactobacillus, Fermentation quality, Subordinate function

INTRODUCTION

Currently, the primary approach used by researchers both domestically and internationally to improve silage quality is the addition of exogenous lactic acid bacteria (LAB) inoculants (Queirozo *et al*., 2013; Zhang *et al*., 2009). *Cyperus esculentus* stems and leaves represent high-quality silage raw materials: they are rich in soluble sugars, crude protein, and other nutrients that can meet the fermentation requirements of LAB, thereby ensuring silage quality. At the same time, their utilization enables the recycling of agricultural waste, providing low-cost feed for the livestock industry and alleviating environmental pressure. In practical applications, the specific method and dosage of inoculant addition must be comprehensively determined based on factors such as differences in silage raw material types and storage environmental conditions, and further adjusted and optimized according to actual silage outcomes to ensure that the final silage quality meets the expected goals (Li *et al*., 2020). Commonly used LAB inoculants at present are mainly composed of homofermentative LAB, often combined with sugar sources or cellulase to ensure the fermentation quality of *C. esculentus* stems and leaves (Jin *et al.,* 2020; He *et al*., 2022). However, research on the addition of excellent LAB naturally attached to the surface of *C. esculentus* stems and leaves remains relatively scarce, and systematic comparisons of the fermentation effects of endogenous bacteria are lacking. From a theoretical perspective, fermentation raw materials themselves carry a certain number of native LAB strains, which are more adapted to the plant's growth environment and thus more likely to survive stably therein (Amer *et al*., 2024). During the silage fermentation process, these naturally occurring LAB strains participate in the construction and dynamic evolution of the microbial community structure, a process that may have positive effects on the quality and functional characteristics of the final fermentation products (Wang *et al*., 2018). In contrast, exogenous LAB inoculants refer to microbial preparations obtained by artificially screening and culturing target LAB strains before adding them to the fermentation raw material. Although such exogenous inoculants can also exert the physiological functions of LAB during fermentation, their adaptability to the plant's own living environment is often somewhat inferior compared with LAB strains naturally attached to the raw material surface. In addition, the natural antimicrobial substances contained in *C. esculentus* itself can inhibit the growth and reproduction of some LAB strains, thereby making it difficult for the silage system to decrease to a suitable pH level and affecting the silage outcome (Chou *et al*., 2023).

Therefore, in the present study, two types of lactic acid bacteria tolerant to the antimicrobial substances of *C. esculentus*, namely Pediococcus acidilactici and Enterococcus faecium, were first isolated and screened from the natural silage system of *C. esculentus* stems and leaves. These two strains were then used as a specialized lactic acid bacteria inoculant and applied to the silage material of *C. esculentus* stems and leaves. Subsequently, by systematically analyzing the nutritional quality indices, fermentation characteristic parameters, and aerobic stability of the resulting silage products, the specific mechanisms through which these two lactic acid bacteria affect the silage performance of *C. esculentus* stems and leaves were further investigated, thereby providing a theoretical basis for improving the silage quality of this raw material.

MATERIALS AND METHODS

Experimental Materials

The *Cyperus esculentus* used in this study were sourced from the No. 6 planting area of the 54th Regiment, 3rd Division of the Xinjiang Production and Construction Corps, located at 38°60' N, 77°09' E, with an altitude of 1 231 m. The growing period in this region was from May 27 to October 7, 2024. On October 4, the stems and leaves of *C. esculentus* in this planting area were mown at a height of 3–4 cm above the ground, and then chopped to a length of 2–3 cm using a grinder with a kneading function. The processed stems and leaves were subsequently packed into transparent polyethylene bags measuring 30 cm × 40 cm × 0.24 mm, with each bag containing

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500 g ± 10 g of material. After sealing with a vacuum sealer, 10 silage bags were prepared for each treatment. All silage samples were fermented at 18–23 °C for 60 days, after which the bags were opened for aerobic stability analysis and sensory evaluation. The detailed nutritional composition of the silage raw material used in this experiment is shown in Tab. 1.

Two types of biological additives were used in this study. The first type consisted of two bacterial strains with good tolerance to the antimicrobial substances of *Cyperus esculentus*, which were isolated, identified, and screened from natural silage samples of *C. esculentus* stems and leaves, namely *Pediococcus acidilactici* (GenBank Accession No. OQ630001) and *Enterococcus faecium* (GenBank Accession No. OQ629997). The second type was a commercially available *Lactiplantibacillus* plantarum preparation purchased from Xinjiang Tiankang Biotechnology Co., Ltd., with a viable count of 3×10^9 colony-forming units (CFU)/g.

Experimental Design

A total of five treatment groups were established in this experiment: a control group (CK, sprayed with an equal volume of sterile water), EF (*Enterococcus faecium*, 5×10^7 CFU/g fresh matter, FM), PA (*Pediococcus acidilactici*, 5×10^7 CFU/g FM), LP (*Lactiplantibacillus plantarum*, 5×10^7 CFU/g FM), and PE (*P. acidilactici* + *E. faecium*, 1 : 1, 5×10^7 CFU/g FM), with five replicates per treatment. L. plantarum and E. faecium were cultured in MRS liquid medium, while *P. acidilactici* was cultured in M17 liquid medium. The viable cell count of each bacterial suspension was determined using the plate count method (Gao *et al*., 2022), and the required volume of each suspension was calculated based on the application rate per unit fresh weight for subsequent silage inoculation.For inoculation, the respective bacterial suspensions were uniformly sprayed onto the chopped C. esculentus stem and leaf material using a sterile sprayer. After thorough mixing for each treatment, 500 g of the inoculated material was weighed and packed into polyethylene bags (40 cm × 30 cm × 0.24 mm) equipped with a one-way exhaust valve, followed by vacuum sealing. All silage bags were then incubated at room temperature (20 ± 3 °C) for natural fermentation until the end of the fermentation period (Yang *et al*., 2024). Sampling was conducted on days 3, 7, 15, 30, and 60 of ensiling. At each sampling time point, three silage bags were randomly selected from each treatment group. The contents of each bag were thoroughly mixed, and analytical samples were collected to determine nutrient composition, fermentation quality parameters, and microbial community counts (Wang, 2024).

The experimental animals used in the in vitro gas production test were three healthy fistulated sheep from the Teaching and Experimental Station of the College of Animal Science and Technology, Shihezi University. This experiment was approved by the Animal Welfare Committee of Shihezi University, with the ethical approval number A2023-226. The sheep were fed three times daily at 08:00, 14:00, and 20:00, and had free access to water.

Within 2 hours after the morning feeding (08:00) of the experimental sheep, rumen fluid samples were collected from each of the three sheep via rumen fistula. After collection, all samples were thoroughly mixed and immediately transferred to a thermally insulated container for transport back to the laboratory. In the laboratory, the mixed rumen fluid was preprocessed by filtration through four layers of gauze. During the entire filtration process, CO_2 gas was continuously bubbled through the system to maintain a strictly anaerobic environment. To minimize the impact of handling on the samples, all the above procedures were performed rapidly in a constant-temperature water bath maintained at 39 °C.

Exactly 200 mg of *Cyperus esculentus* stem and leaf raw material and 200 mg of the same material after 60 d of ensiling were weighed separately using a precision balance. The two types of samples were then loaded into in vitro fermentation syringes, with six replicate syringes prepared for each sample. During each batch of incubation, six blank samples (containing culture medium only) were also set up in parallel. A small amount of medical-grade petroleum jelly was applied to the front one-third of the plunger of each syringe. The samples were then gently loaded into the syringes while the rubber tubing of each syringe was clamped to prevent sample overflow from the tip. Finally, the loaded syringes were placed in a constant-temperature water bath at 39 °C and preheated for 30 min (Liu *et al*., 2025).

Determination Indicators and Methods

To ensure the standardization and reliability of sensory evaluation, the scoring method proposed by the German Agricultural Society was adopted in this study. After opening the silage bags, the quality grade of the silage was comprehensively evaluated based on three dimensions of the *Cyperus esculentus* stem and leaf silage: odor characteristics, physical structure, and appearance color.

For the determination of nutritional components, classic and widely recognized methods were used for each index: dry matter (DM) content was measured by oven drying at 105 °C; crude protein (CP) content was determined using the Kjeldahl method; water-soluble carbohydrate (WSC) content was analyzed by the anthrone colorimetric method; neutral detergent fiber (NDF) and acid detergent fiber (ADF) contents were measured using the Van Soest method; and ash content was determined by ignition at 550 °C (GB/T 20806-2022).

The key fermentation parameters were determined using the following methods: the pH of the silage material was measured directly with a pH meter (Model pHs-3c, Leici); the contents of organic acids produced during fermentation, including lactic acid (LA), acetic acid (AA), propionic acid (PA), and butyric acid (BA), were quantitatively analyzed by high-performance liquid chromatography (HPLC); and the change in ammonia nitrogen (NH_3-N) content in the silage system was determined by the phenol-hypochlorite colorimetric method (DB15/T 1458-2018).

The microbial community counts in the *Cyperus esculentus* stem and leaf silage were determined using the plate count method. For different types of microorganisms, specific media and culture conditions were used: aerobic bacteria (AB) were cultured on nutrient agar at 30 °C under aerobic conditions; lactic acid bacteria (LAB) were cultured on MRS medium at 37 °C under anaerobic conditions; yeasts were cultured on malt extract agar at 27 °C under aerobic conditions; and

Tab. 1: Nutrient composition of *cyperus esculentus* 1. silage raw material (DM/%)

Items	pH	DM/%	CP/% DM	EE/% DM	Ash/% DM	WSC/% DM	NDF/% DM	ADF/% DM
Content	6.75	37.16	11.98	3.13	13.27	17.96	49.37	27.61

molds were cultured on high-salt Czapek–Dox agar at 27 °C under aerobic conditions (General Administration of Quality Supervision, 2002).

During counting, intact single colonies were used as the identification standard, and plates with colony numbers in the range of 10–100 were selected for statistical analysis to ensure the accuracy of the results. The number of target

microorganisms per gram of fresh silage (FM), expressed as colony-forming units (CFU), was calculated using the following formula (DENG *et al*., 2023):

(1)

$$\text{Microbial count}(\text{CFU/g FM}) = \\ = \frac{\text{number of colonies} \times \text{dilution factor} \times 1000(\mu\text{L})}{\text{inoculation volume}(\mu\text{L})}$$

To accurately evaluate the aerobic stability of the silage, the relevant measurements were conducted on the 60th day of fermentation. First, the opening of each silage bag was carefully cleaned to reduce the risk of microbial and contaminant introduction and to minimize rapid moisture loss from the silage material (Wang *et al*., 2019). A multi-point temperature recorder was used to monitor temperature changes in the samples: multiple temperature probes were placed at the center of the material within each silage bag, while three additional probes were placed in the surrounding environment to track ambient temperature in real time. All probes were programmed to record temperatures at 10-minute intervals, and three sample probes were assigned to each treatment group. The aerobic stability time was defined as the time required for the temperature of the silage sample to rise 2 °C above the ambient temperature, which indicates the onset of spoilage and deterioration (Muck, 2013).

In Vitro Gas Production

The in vitro gas production method described by Menke *et al.* (Menke *et al.*, 1997) was used in this experiment to simulate the rumen fermentation environment. At different time points after the start of incubation, the gas production of each sample was recorded based on the readings from the glass syringes.

Data Analysis

The data were initially processed using Excel 2024. One-way analysis of variance (ANOVA) was performed using SPSS 26.0, and Duncan's multiple range test was applied for mean comparisons. Graphs were generated using Origin 2024.

For the comprehensive evaluation of silage quality and aerobic stability, the membership function method was used for quantitative analysis in this study. Among the indicators, those positively correlated with silage quality (including dry matter, crude protein, water-soluble carbohydrates, lactic acid, acetic acid, and aerobic stability time) were calculated using formula (2). In contrast, indicators negatively correlated with silage quality (including pH, ash, neutral detergent fiber, acid detergent fiber, and ammonia nitrogen) were calculated using formula (3) (Sun *et al*., 2021). After obtaining the membership function values for all indicators, the weighted average was calculated based on the corresponding weight of each indicator. The silage performance of different treatment groups was then ranked according to the magnitude of the weighted average (a higher average indicates better silage quality, and vice versa).

$$\mathrm{F_{ij}}^{+} = \frac{(\mathrm{X_{ij}} - \mathrm{X_{min}})}{(\mathrm{X_{max}} - \mathrm{X_{min}})} \quad \textbf{(2)}$$

$$\mathrm{F_{ij}}^{-} = 1 - \frac{(\mathrm{X_{ij}} - \mathrm{X_{min}})}{(\mathrm{X_{max}} - \mathrm{X_{min}})} \quad \textbf{(3)}$$

In the above formulas, F_{ij} represents the membership value of the *j*-th index for the *i*-th treatment; X_{ij} is the measured value of the *j*-th index for the *i*-th treatment; and X_{max} and X_{min} denote the maximum and minimum values, respectively, of the *j*-th index across all experimental treatment groups.

RESULTS AND ANALYSIS

Sensory Evaluation of *Cyperus esculentus* Stem and Leaf Silage During Fermentation Under Different Treatments

Tab. 2 presents the sensory evaluation results of the silage from each treatment group after 60 days of fermentation. The control group was predominantly yellowish-green with only a small amount of brown coloration, and its odor was a mixture of a weak sour smell and a faint grassy aroma. The material texture was relatively loose and not sticky to the touch. The LP group exhibited a yellowish-brown color, with a slightly stronger sour odor than the CK group, and the texture was soft and also non-sticky. The PA group maintained a yellowish-green appearance, with an odor combining a faint aroma and a distinct sourness, and the texture was similarly soft and non-sticky. The EF group showed a yellowish-brown color mixed with some green, and its sour aroma was less intense than that of the PA group; however, the material remained soft, only slightly moist, and not sticky. The PE group was yellowish-green with a clear sour aroma, and the texture was moderately soft and moist, also without stickiness. No mold was observed in any of the treatment groups during the sensory evaluation, indicating that all treatments met the basic storage requirements for silage.

Tab. 2: Sensory evaluation of *cyperus esculentus* l. silage

Treatment	Color	Texture	Scent	Aggregate score	Grades
CK	1	2	10	13	Second-class good
LP	2	2	11	15	First-classexcellent
PA	2	2	12	16	First-class good
EF	2	2	11	15	First-class good
PE	2	3	13	18	First-class good

Tab. 3: Changes of nutritional quality of *cyperus esculentus* silage with different additives

Items	Time	Treatments					SEM	*P*-value
		CK	PA	EF	LP	PE		
DM/%	3 d	32.18 ± 0.20^{b}	32.39 ± 0.27^{ab}	32.51 ± 0.18^{ab}	32.20 ± 0.19^{b}	32.69 ± 0.25^{a}	0.051	0.047
	7 d	30.66 ± 0.09^{c}	31.57 ± 0.18^{a}	31.55 ± 0.07^{a}	31.25 ± 0.06^{b}	31.67 ± 0.11^{a}	0.029	0.001
	15 d	30.67 ± 0.11^{c}	31.29 ± 0.16^{ab}	31.41 ± 0.11^{ab}	31.10 ± 0.16^{b}	31.56 ± 0.08^{a}	0.059	0.007
	30 d	29.83 ± 0.32^{c}	31.74 ± 0.16^{ab}	31.94 ± 0.09^{a}	31.34 ± 0.05^{b}	32.02 ± 0.22^{a}	0.060	0.000
	60 d	28.95 ± 0.15^{c}	31.07 ± 0.15^{b}	31.13 ± 0.06^{b}	30.70 ± 0.11^{b}	31.72 ± 0.15^{a}	0.058	0.000
CP/% DM	3 d	11.47 ± 0.06^{b}	11.67 ± 0.05^{a}	11.70 ± 0.03^{a}	11.61 ± 0.04^{ab}	11.74 ± 0.02^{a}	0.020	0.014
	7 d	11.25 ± 0.03^{c}	11.65 ± 0.04^{a}	11.67 ± 0.01^{a}	11.53 ± 0.02^{b}	11.69 ± 0.01^{a}	0..015	0.000
	15 d	10.94 ± 0.02^{c}	11.06 ± 0.04^{ab}	10.99 ± 0.02^{bc}	10.98 ± 0.04^{bc}	11.14 ± 0.04^{a}	0.016	0.022
	30 d	9.68 ± 0.02^{d}	9.82 ± 0.04^{c}	10.19 ± 0.05^{a}	9.96 ± 0.04^{b}	10.25 ± 0.03^{a}	0.018	0.000
	60 d	8.29 ± 0.02^{c}	8.62 ± 0.08^{b}	8.75 ± 0.04^{ab}	8.44 ± 0.01^{c}	8.87 ± 0.04^{a}	0.023	0.001
NDF/% DM	3 d	48.97 ± 0.04^{a}	48.73 ± 0.07^{a}	48.65 ± 0.06^{a}	48.88 ± 0.04^{a}	48.85 ± 0.06^{a}	0.026	0.215
	7 d	48.47 ± 0.19^{a}	47.42 ± 0.29^{a}	47.70 ± 0.15^{ab}	46.97 ± 0.15^{bc}	46.68 ± 0.18^{c}	0.089	0.008
	15 d	47.82 ± 0.24^{a}	46.38 ± 0.23^{abc}	46.30 ± 0.21^{bc}	46.71 ± 0.28^{ab}	45.72 ± 0.20^{c}	0.106	0.020
	30 d	46.80 ± 0.24^{a}	45.73 ± 0.26^{abc}	45.44 ± 0.31^{bc}	46.18 ± 0.15^{ab}	45.10 ± 0.34^{c}	0.123	0.032
	60 d	45.48 ± 0.29^{a}	43.86 ± 0.31^{b}	43.83 ± 0.11^{b}	44.01 ± 0.13^{b}	42.96 ± 0.15^{c}	0.098	0.003
ADF/% DM	3 d	27.51 ± 0.30^{a}	26.44 ± 0.19^{ab}	26.29 ± 0.17^{ab}	26.70 ± 0.47^{ab}	26.87 ± 0.15^{b}	0.131	0.152
	7 d	26.64 ± 0.19	26.54 ± 0.27	26.47 ± 0.22	26.52 ± 0.20	26.44 ± 0.28	0.114	0.586
	15 d	26.50 ± 0.27	26.48 ± 0.18	26.41 ± 0.21	26.48 ± 0.21	26.39 ± 0.14	0.094	0.912
	30 d	26.20 ± 0.29	26.21 ± 0.15	26.04 ± 0.19	26.06 ± 0.22	26.02 ± 0.16	0.095	0.476
	60 d	26.16 ± 0.21	26.05 ± 0.28	26.29 ± 0.33	26.02 ± 0.34	25.97 ± 0.32	0.155	0.961
WSC/% DM	3 d	12.28 ± 0.11^{c}	12.55 ± 0.08^{b}	12.62 ± 0.06^{ab}	12.40 ± 0.07^{bc}	12.85 ± 0.05^{a}	0.036	0.006
	7 d	9.32 ± 0.18^{a}	8.56 ± 0.09^{bc}	8.55 ± 0.14^{bc}	8.83 ± 0.14^{b}	8.31 ± 0.04^{c}	0.059	0.003
	15 d	8.92 ± 0.10^{a}	8.11 ± 0.09^{bc}	7.96 ± 0.07^{c}	8.50 ± 0.25^{ab}	7.51 ± 0.08^{d}	0.063	0.000
	30 d	5.97 ± 0.09^{c}	6.77 ± 0.33^{ab}	6.65 ± 0.28^{bc}	6.21 ± 0.10^{bc}	7.39 ± 0.03^{a}	0.094	0.006
	60 d	4.09 ± 0.05^{d}	5.84 ± 0.04^{b}	5.82 ± 0.08^{b}	5.19 ± 0.08^{c}	6.46 ± 0.15^{a}	0.042	0.000
Ash/% DM	3 d	13.09 ± 0.08^{a}	12.96 ± 0.04^{a}	12.95 ± 0.06^{a}	13.00 ± 0.06^{a}	12.62 ± 0.16^{b}	0.042	0.047
	7 d	13.03 ± 0.06^{a}	12.68 ± 0.11^{ab}	12.80 ± 0.14^{ab}	12.92 ± 0.08^{ab}	12.52 ± 0.16^{b}	0.054	0.095
	15 d	12.87 ± 0.06^{a}	12.59 ± 0.06^{b}	12.60 ± 0.06^{b}	12.78 ± 0.07^{ab}	12.37 ± 0.06^{c}	0.029	0.003
	30 d	12.59 ± 0.08	12.54 ± 0.05	12.51 ± 0.08	12.52 ± 0.12	12.32 ± 0.02	0.036	0.243
	60 d	12.29 ± 0.02	12.27 ± 0.02	12.30 ± 0.02	12.29 ± 0.01	12.24 ± 0.03	0.011	0.497

Note: Different lowercase letters as superscripts in the same row of data indicate significant differences (P<0.05), while the same lowercase letters or no letters indicate no significant differences (P>0.05). Same as below.

Changes in Nutritional Quality of *Cyperus Esculentus* Silage During Fermentation Under Different Treatments

Tab. 3 shows the changes in dry matter, crude protein, neutral detergent fiber, acid detergent fiber, water-soluble carbohydrates, and ash contents in each treatment group during fermentation. After 60 days of fermentation, the DM content in all treatment groups decreased compared with the initial stage of ensiling.Regarding the differences among groups at different fermentation stages: on days 3 and 7 of ensiling, the DM content in the PE group was significantly higher than that in the control group and the LPgroup ($P < 0.05$), while no significant differences were observed among the other treatment groups ($P > 0.05$). At day 30 of fermentation, the DM contents in the EF group and the PE group showed significant advantages and were significantly higher than that in the LP group ($P < 0.05$). By day 60, the DM content in the PE group was significantly higher than that in all other treatment groups ($P < 0.05$), indicating that this mixed-culture treatment was the most effective in reducing DM loss during ensiling of *Cyperus esculentus* stems and leaves.

Throughout the entire ensiling period, the CP content showed an overall trend of gradual decline, and this downward trend began to level off after 30 days of fermentation. On day 7 of ensiling, the CP content in the LP group was significantly lower than that in the PE group, the EF group, and the PA group ($P < 0.05$), while no significant differences were observed among the other groups ($P > 0.05$). At day 15 of fermentation, the CP content in the PE group showed a significant advantage and was significantly higher than that in the EF and LP groups ($P < 0.05$). By day 60, the CP content in the PE group remained significantly higher than that in the PA group ($P < 0.05$).

During the ensiling process, the contents of NDF and ADF showed a consistent trend, both decreasing continuously from the start to the end of fermentation. On day 3 of fermentation, there were no significant differences in NDF content among the treatment groups ($P > 0.05$). For ADF content, however, the PE group was significantly lower than the control group ($P < 0.05$), while the other treatment groups (EF, PA, LP) showed no significant differences compared with either PE or CK ($P > 0.05$).At days 15 and 30 of fermentation, the NDF content in the PE group exhibited a significant advantage and was significantly lower than that in the CK and LP groups ($P < 0.05$). By day 60, the NDF content in the PE group remained significantly lower than that in the CK group and all other single-strain treatment groups

($P < 0.05$).After the entire fermentation period, the PE group had the lowest NDF and ADF contents among all treatments respectively. These results indicate that the mixed-culture treatment was the most effective in degrading the fibrous components of *Cyperus esculentus* stems and leaves during ensiling.

Throughout the entire fermentation period, the WSC content showed a continuous downward trend. By day 7 of ensiling, the WSC content in all lactic acid bacteria treatment groups had decreased significantly compared with day 3, and all treatment groups had significantly lower WSC contents than the control group ($P < 0.05$).At day 15 of fermentation, the WSC content in the PE group was significantly lower than that in the CK group and all other single-strain treatment groups ($P < 0.05$), indicating that the PE group had the highest WSC consumption efficiency at this stage.By day 30, the trend in WSC content had shifted: the PE group exhibited a significantly higher WSC content than the PA, EF, and CK groups ($P < 0.05$).At day 60, the WSC content in the CK group was significantly lower than that in all LAB-treated groups ($P < 0.05$). Among the LAB treatments, the PE group had a significantly higher WSC content than the CK group and all other single-strain groups ($P < 0.05$), while the LP group had a significantly lower WSC content than the PA and EF groups ($P < 0.05$). These results suggest that different LAB strains exhibit significant differences in their efficiency of WSC utilization during ensiling.

Throughout the entire fermentation period, the ash content showed only minor fluctuations. On day 3 of ensiling, the ash content in the PE group was significantly lower than that in the other treatment groups ($P < 0.05$). On day 7, the ash content in the control group was significantly higher than that in the PE group, while no significant differences were observed between the remaining treatment groups and either CK or PE ($P > 0.05$).At day 15 of fermentation, the ash contents in the LP and CK groups were significantly higher than that in the PE group ($P < 0.05$), and the PE group maintained a significantly lower ash content compared with the other treatments ($P < 0.05$).By days 30 and 60 of fermentation, there were no significant differences in ash content between any of the lactic acid bacteria-treated groups and the CK group ($P > 0.05$).

Changes in Fermentation Quality of Cyperus Esculentus Stem and Leaf Silage During Ensiling Under Different Treatments

The changes in pH and NH_3-N content after the addition of different lactic acid bacteria treatments are shown in Tab. 4. With respect to pH, the values in all lactic acid bacteria-treated groups and the control group gradually decreased with increasing fermentation time. Throughout the entire ensiling period, the pH in the LAB-inoculated groups remained consistently lower than that in the CK group.By day 60 of fermentation, the pH of the PE group had dropped to the lowest level among all treatments (4.87), whereas the CK group exhibited the highest pH (6.45).

In terms of NH_3-N content, the PE group showed a significant advantage: throughout the entire fermentation process, the NH_3-N content in the PE group remained significantly lower than that in all other treatment groups ($P < 0.05$). At day 60 of

Tab. 4: Changes changes in fermentation quality of *cyperus esculentus* stem and leaf silage during fermentation under different treatments

Items	Time	Treatments					SEM	*P*-vale
		CK	PA	EF	LP	PE		
pH	3 d	6.70 ± 0.01^{a}	6.59 ± 0.01^{c}	6.58 ± 0.01^{c}	6.63 ± 0.01^{b}	6.49 ± 0.01^{d}	0.002	0.001
	7 d	6.69 ± 0.01^{a}	6.41 ± 0.01^{c}	6.40 ± 0.01^{c}	6.57 ± 0.02^{b}	6.17 ± 0.01^{d}	0.002	0.000
	15 d	6.63 ± 0.01^{a}	6.21 ± 0.01^{c}	6.19 ± 0.01^{c}	6.31 ± 0.01^{b}	6.07 ± 0.01^{d}	0.002	0.000
	30 d	6.53 ± 0.01^{a}	6.10 ± 0.02^{c}	6.11 ± 0.01^{c}	6.22 ± 0.01^{b}	5.53 ± 0.01^{d}	0.002	0.000
	60 d	6.45 ± 0.01^{a}	5.47 ± 0.01^{c}	5.62 ± 0.01^{c}	6.01 ± 0.01^{b}	4.87 ± 0.01^{d}	0.001	0.000
LA/g/kg	3 d	0.31 ± 0.01^{d}	0.41 ± 0.01^{b}	0.41 ± 0.01^{b}	0.37 ± 0.01^{c}	0.48 ± 0.01^{a}	0.004	0.000
	7 d	0.45 ± 0.02^{bc}	0.53 ± 0.02^{ab}	0.53 ± 0.01^{ab}	0.43 ± 0.02^{c}	0.58 ± 0.03^{a}	0.012	0.012
	15 d	0.47 ± 0.01^{d}	0.64 ± 0.01^{b}	0.65 ± 0.02^{b}	0.52 ± 0.02^{c}	0.72 ± 0.02^{a}	0.008	0.000
	30 d	0.50 ± 0.01^{d}	0.86 ± 0.02^{b}	0.86 ± 0.03^{b}	0.63 ± 0.01^{c}	0.98 ± 0.02^{a}	0.015	0.000
	60 d	0.53 ± 0.01^{d}	0.97 ± 0.02^{b}	1.04 ± 0.05^{b}	0.72 ± 0.02^{c}	1.28 ± 0.03^{a}	0.003	0.000
AA/g/kg	3 d	0.17 ± 0.01^{a}	0.14 ± 0.01^{b}	0.07 ± 0.01^{d}	0.12 ± 0.01^{bc}	0.11 ± 0.01^{bc}	0.001	0.008
	7 d	0.22 ± 0.01^{a}	0.23 ± 0.01^{a}	0.17 ± 0.01^{b}	0.21 ± 0.01^{a}	0.23 ± 0.02^{a}	0.005	0.022
	15 d	0.27 ± 0.01^{b}	0.32 ± 0.02^{ab}	0.31 ± 0.01^{ab}	0.26 ± 0.01^{b}	0.34 ± 0.02^{a}	0.007	0.022
	30 d	0.25 ± 0.02^{d}	0.37 ± 0.01^{b}	0.37 ± 0.01^{b}	0.31 ± 0.01^{c}	0.45 ± 0.02^{a}	0.006	0.000
	60 d	0.26 ± 0.01^{d}	0.46 ± 0.01^{b}	0.49 ± 0.01^{ab}	0.38 ± 0.01^{c}	0.52 ± 0.01^{a}	0.006	0.000
NH3-N/g/kg	3 d	0.07 ± 0.01^{a}	0.03 ± 0.00^{b}	0.03 ± 0.01^{b}	0.03 ± 0.01^{b}	0.03 ± 0.00^{b}	0.003	0.006
	7 d	0.11 ± 0.01^{a}	0.07 ± 0.00^{bc}	0.07 ± 0.01^{bc}	0.09 ± 0.01^{ab}	0.06 ± 0.00^{c}	0.004	0.009
	15 d	0.12 ± 0.01^{a}	0.11 ± 0.01^{a}	0.10 ± 0.01^{a}	0.11 ± 0.01^{a}	0.07 ± 0.01^{b}	0.003	0.008
	30 d	0.17 ± 0.01^{a}	0.11 ± 0.01^{b}	0.12 ± 0.01^{b}	0.12 ± 0.01^{b}	0.08 ± 0.01^{c}	0.002	0.000
	60 d	0.23 ± 0.01^{a}	0.18 ± 0.01^{b}	0.18 ± 0.01^{b}	0.19 ± 0.01^{ab}	0.17 ± 0.00^{b}	0.004	0.012

Tab. 6: Changes in the number of microorganisms during silage fermentation of *cyperus esculentus* stems and leaves

Items	Time	Treatments					SEM	P-value
		CK	PA	EF	LP	PE		
LAB (log CFU/g FM)	3 d	8.25^{d}	8.63^{b}	8.65^{b}	8.49^{c}	8.78^{a}	0.014	< 0.001
	7 d	8.28^{d}	8.92^{ab}	8.87^{ab}	8.63^{c}	8.98^{a}	0.010	< 0.001
	15 d	8.42^{b}	9.49^{a}	9.50^{a}	9.47^{a}	9.51^{a}	0.029	< 0.001
	30 d	8.17^{d}	9.11^{bc}	9.20^{ab}	8.97^{c}	9.23^{ab}	0.021	< 0.001
	60 d	7.74^{c}	8.71^{b}	8.77^{b}	8.69^{b}	8.88^{a}	0.012	< 0.001
Yeast (log CFU/g FM)	3 d	5.03^{a}	4.55^{c}	4.78^{b}	4.89^{ab}	4.41^{c}	0.030	< 0.001
	7 d	5.27^{a}	4.93^{bc}	5.07^{ab}	5.22^{a}	4.76^{c}	0.033	0.001
	15 d	4.84^{b}	4.70^{c}	4.58^{d}	4.80^{bc}	7.74^{a}	0.016	0.001
	30 d	4.54^{a}	4.31^{c}	4.27^{c}	4.41^{b}	4.33^{bc}	0.011	< 0.0001
	60 d	3.80^{ab}	3.62^{cd}	3.54^{d}	3.72^{bc}	3.65^{cd}	0.009	0.001
Mold (log CFU/g FM)	3 d	3.80^{c}	3.86^{abc}	3.89^{abc}	3.92^{ab}	3.85^{bc}	0.025	0.062
	7 d	3.83^{a}	3.29^{c}	3.54^{b}	3.30^{c}	3.57^{b}	0.013	< 0.001
	15 d	4.14^{a}	3.53^{c}	3.60^{c}	3.87^{b}	3.51^{d}	0.026	< 0.001
	30 d	3.77^{a}	< 2.00	< 2.00	3.62^{ab}	< 2.00	0.007	0.076
	60 d	4.15^{a}	< 2.00	< 2.00	3.71^{b}	< 2.00	0.053	0.022
AB (log CFU/g FM)	3 d	9.09^{d}	9.21^{c}	9.19^{c}	9.45^{a}	9.33^{b}	0.005	< 0.001
	7 d	8.81^{a}	8.39^{d}	8.39^{d}	8.56^{b}	8.46^{c}	0.003	< 0.001
	15 d	8.38^{b}	8.45^{b}	8.66^{a}	8.30^{b}	8.32^{b}	0.016	< 0.001
	30 d	8.36^{ab}	8.11^{c}	8.13^{c}	8.36^{ab}	8.26^{b}	0.014	< 0.001
	60 d	7.86^{a}	7.53^{bc}	7.40^{c}	7.57^{b}	7.41^{c}	0.016	< 0.001

Tab. 5: Subordinate function analysis of different treatments on silage quality of cyperus esculentus

Index	Treatment				
	CK	PA	EF	LP	PE
pH	0.01	0.81	0.78	0.62	0.98
LA	0.25	0.91	0.93	0.89	0.96
AA	0.04	0.86	0.88	0.77	0.89
DM	0.18	0.85	0.85	0.76	0.92
CP	0.22	0.89	0.89	0.65	0.95
Ash	0.94	0.54	0.56	0.56	0.55
NH_3-N	0.22	0.90	0.91	0.88	0.97
WSC	0.02	0.86	0.84	0.53	0.96
NDF	0.43	0.70	0.73	0.64	0.89
ADF	0.64	0.70	0.43	0.73	0.89
Aerobic stability	0.03	0.86	0.81	0.56	0.97
Average	0.25	0.82	0.81	0.70	0.89

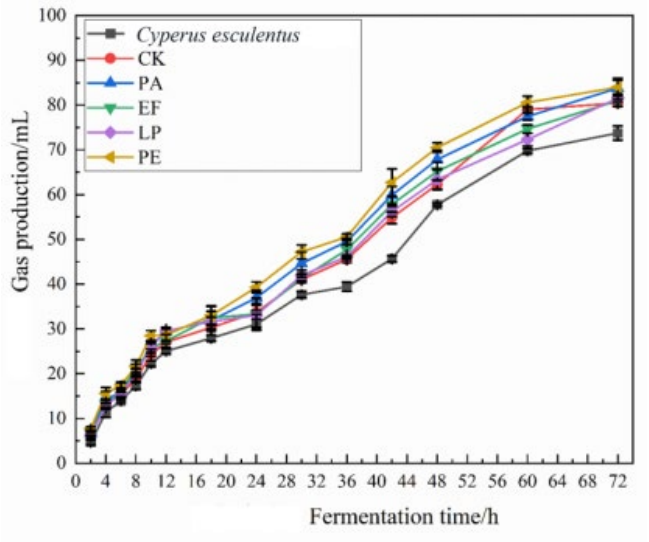


Fig. 1: In vitro cumulative gas production curves of *cyperus esculentus* and its silage

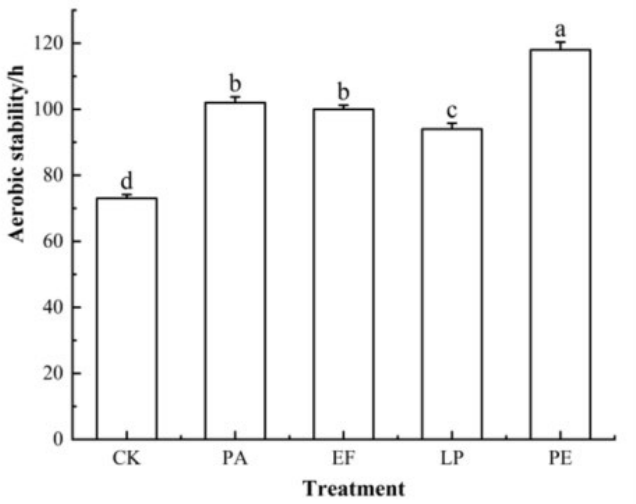


Fig. 2: Aerobic stability of *cyperus esculentus* silage

fermentation, the NH_3-N content in the control group was significantly higher than that in the PA, EF, and PE groups ($P < 0.05$).

With respect to lactic acid content, all treatment groups showed a gradual increase over the course of fermentation. Among these, the PE group maintained a significantly higher lactic acid content than all other treatments throughout the entire ensiling period ($P < 0.05$). The lactic acid contents in the PA and EF groups were significantly higher than those in the LP group and the control group ($P < 0.05$).By day 60 of fermentation, the lactic acid contents of the treatment groups were ranked from highest to lowest as follows: PE > EF > PA > LP > CK. These results further indicate that the PE treatment was the most effective in promoting lactic acid accumulation during ensiling.

In terms of acetic acid content, all treatment groups also exhibited a gradual increase throughout the entire ensiling period. At day 60 of fermentation, the acetic acid content in the PE group showed a significant advantage and was significantly higher than that in all other treatment groups except the EF group, as well as the control group ($P < 0.05$). However, there was no significant difference in acetic acid content between the EF and PE groups ($P > 0.05$), suggesting that the mixed culture and Enterococcus faecium have similar effects in promoting acetic acid synthesis during ensiling.

Changes in Microbial Counts During Fermentation Under Different Treatments

As shown in Tab. 5, the lactic acid bacteria (LAB) counts in all treatment groups increased gradually during the first 30 days of fermentation, but began to decline after day 30. From day 7 to day 60 of ensiling, the LAB counts in all LAB-inoculated groups were significantly higher than those in the control group (CK) ($P < 0.05$).On day 7 of ensiling, the LAB counts in the PE group,PA group,and EF group were significantly higher than those in the LP group ($P < 0.05$). On day 15, there were no significant differences in LAB counts among the treatment groups ($P > 0.05$). By day 60, the PE group had the highest LAB count among all treatments ($P < 0.05$).

The yeast and aerobic bacteria (AB) counts showed a continuous decreasing trend throughout the entire ensiling period. On day 15 of fermentation, except for the PA group, the yeast count in the EF group was significantly lower than that in the control group and the other treatments ($P < 0.05$). On day 30, the yeast counts in all lactic acid bacteria-treated groups were significantly lower than that in the CK group ($P < 0.05$), but differences existed among the LAB groups; the yeast count in the LP group was significantly higher than those in the PA and EF groups ($P < 0.05$). By day 60, the CK group had the highest yeast count among all treatments.

By day 7 of fermentation, the differences among groups had changed: the aerobic bacteria (AB) counts in the PA, EF, and PE groups were significantly lower than that in the LP group ($P < 0.05$), while no significant differences were observed among the PA, EF, and PE groups themselves ($P > 0.05$).At day 15 of ensiling, the AB count in the EF group was significantly higher than those in the other treatment groups ($P < 0.05$).By day 30 of fermentation, the AB counts in the PA and EF groups had become significantly lower than those in the remaining treatment groups ($P < 0.05$).

In vitro Cumulative Gas Production of *Cyperus esculentus* and Its Silage

As shown in Fig. 1, the in vitro gas production of both *Cyperus esculentus* and its silage gradually increased with the extension of fermentation time, reaching the maximum value at 72 h, after which the gas production tended to stabilize. Throughout the entire fermentation process, the gas production of the silage was consistently higher than that of the original *Cyperus esculentus*, and the PE treatment group exhibited the highest gas production.

Effects of Different Treatments on the Aerobic Stability of *Cyperus esculentus* Stem and Leaf Silage

In terms of differences among groups, there was no significant difference in aerobic stability time between the EF and PA groups ($P > 0.05$), whereas significant differences were observed among all other treatment groups($P < 0.05$). Notably, the aerobic stability time of all lactic acid bacteria (LAB)-inoculated groups was extended by 29–46 h compared with the control group. Among these, the PE group, which received both *Pediococcus acidilactici* and *Enterococcus faecium*, exhibited a significantly longer aerobic stability time than all other treatment groups ($P < 0.05$), indicating that this mixed-culture treatment was the most effective in improving the aerobic stability of *Cyperus esculentus* stem and leaf silage.

Comprehensive Evaluation of *Cyperus Esculentus* Stem and Leaf Silage Quality Under Different Treatments

A membership function-based comprehensive analysis method was employed to construct a comprehensive evaluation system using 11 key indicators. The specific evaluation results are presented in Tab. 6. In this method, the comprehensive evaluation score serves as a quantitative criterion for assessing silage quality, with higher scores indicating superior silage quality and lower scores indicating poorer quality.

After 60 days of fermentation, the comprehensive ranking of silage quality for *Cyperus esculentus* stem and leaf silage across the different treatment groups was as follows: PE group > PA group > EF group > LP group > CK group.

DISCUSSION

Effects of Lactic Acid Bacteria on the Nutritional Quality of *Cyperus esculentus* Stem and Leaf Silage

The core objective of adding lactic acid bacteria inoculants during the silage of *Cyperus esculentus* stems and leaves is to facilitate rapid and efficient fermentation. It accelerates lactic acid accumulation and pH decline in the early silage stage, inhibits the proliferation of undesirable microorganisms, thereby extending the effective storage period of silage, maintaining its quality stability, and preventing rapid deterioration (Sharp *et al*., 1994). *Pediococcus acidilactici* and *Enterococcus faecium* used in this study are both homofermentative LAB, capable of normal growth under low pH conditions. Their inoculation can promote the proliferation of beneficial LAB in the silage system, inhibit the activity of harmful microorganisms such as Clostridium and aerobic bacteria, reduce gas production and dry matter loss during silage, and improve the silage quality of *Cyperus esculentus* stems and leaves (Zhao *et al*., 2017). When LAB multiply to a certain population size, they inhibit the metabolism of undesirable bacteria through nutrient competition and acidic environment construction, reducing the inefficient consumption of WSC (Li *et al*., 2022). This explains the significant slowdown in the WSC degradation rate of all treatment groups after 30 days of silage observed in this study. Homofermentative LAB can reduce protein hydrolysis in silage (Zhang *et al*., 2023), which is consistent with the results of this study: the CP content in all LAB-inoculated groups was significantly higher than that in the control group, indicating that inoculation effectively reduces protein degradation loss.NH_3-N is a key indicator of silage quality; higher levels denote more extensive amino acid and protein decomposition, indicating poor fermentation (Zhao *et al*., 2017). This degradation during silage is mainly driven by undesirable microbial metabolism (Niu *et al*., 2018). Thus, regulating the silage microbial community and inhibiting harmful microbes are critical to reducing NH_3-N production. Additionally, standardized silage preparation and controlled storage conditions can further mitigate protein and amino acid degradation, enhancing overall silage efficacy (Jiang *et al*., 2020).

Based on the results obtained in this study, inoculation with LAB was able to reduce the loss of CP in *Cyperus esculentus* stem and leaf silage to a certain extent. From a mechanistic perspective, LAB can convert part of the protein in the silage into lactic acid and other organic acids through their fermentation metabolism (Wang *et al*., 2021). This transformation effectively reduces the production of NH_3-N in the system. Therefore, the relatively lower proportion of NH_3-N in the LAB-inoculated silage groups directly indicates less extensive protein degradation and superior fermentation quality. In addition, the results of the present study also showed that all LAB inoculation treatments decreased the NDF and ADF contents of *Cyperus esculentus* silage. In the feed nutritional evaluation system, NDF and ADF contents are key indicators affecting feed intake and digestibility in livestock (Liu *et al*., 2020). Higher NDF and ADF contents indicate a greater proportion of fibrous material, which may limit feed intake and reduce digestibility. Moreover, factors such as chop length, limitations in the fibrolytic enzyme activity of the strains used, and insufficient fermentation duration can also influence NDF and ADF contents in the final silage. Feed with high fiber content is difficult for the animal digestive system to fully break down and utilize. Particularly for ruminants, the digestion of such feed requires a longer retention time in the rumen and involves more extensive microbial metabolism (Suwignyo *et al*., 2021). In contrast, when NDF and ADF contents are lower, the proportion of fibrous material is reduced, making the feed easier for animals to consume and improving its digestibility and absorption efficiency. This, in turn, can promote animal growth, development, and overall production performance (Hu *et al*., 2020; Xian *et al*., 2022).

Effects of Lactic Acid Bacteria on the Fermentation Quality and Microbial Counts of *Cyperus esculentus* Stem and Leaf Silage

The results of this study showed that after 60 days of fermentation, the pH of the PE group had dropped to the lowest level among all treatments (4.87), although this value still did not meet the pH standard for high-quality silage (Sun *et al.*, 2023). It is worth noting that the rate of pH decline during *Cyperus esculentus* ensiling was slower than that observed with other conventional silage materials, and the overall pH remained relatively high. This observation is consistent with the findings of previous studies (Li *et al.*, 2020). Inoculation with the composite LAB additive significantly reduced the pH of *Cyperus esculentus* silage, which laid a favorable foundation for further improving its silage quality in subsequent research. Based on these results, future experiments could attempt to combine molasses with composite LAB additives(Li *et al*., 2022) to investigate the potential synergistic effects of this combination on the fermentation quality of *Cyperus esculentus* silage.

From the perspective of the effects of different LAB inoculation types, both the single addition of endogenous dominant LAB and the application of composite LAB preparations significantly reduced the pH of *Cyperus esculentus* stem and leaf silage. However, it should be noted that the pH in the treatment group supplemented with commercial LP remained relatively higher compared with the other LAB-treated groups. This result suggests that the secondary metabolites naturally abundant in *Cyperus esculentus*, such as phenolic compounds, flavonoids, and saponins—all of which possess broad-spectrum antimicrobial activity—may have inhibited the normal growth and metabolism of the commercial *L. plantarum* strain to some extent through multiple mechanisms, including membrane disruption and the inhibition of key enzyme activities (Yang *et al.*, 2022). Therefore, to mitigate the inhibitory effects of these endogenous substances on LAB activity and further optimize the silage quality of *Cyperus esculentus* stems and leaves, future studies could consider adopting a combined approach involving both commercial LAB additives and endogenous dominant LAB strains.

The leaves of *Cyperus esculentus* are rich in various antioxidant substances, including flavonoids, carotenoids, coumarins, phenolics, alkaloids, terpenoids, and anthraquinones (Yan *et al*., 2010). Among these, flavonoids and cardiac

glycosides have well-documented effects against certain diseases. Previous studies have confirmed that flavonoids possess strong antimicrobial activity, exerting significant inhibitory effects on most bacteria and molds, with bacteria generally being more susceptible to flavonoid inhibition than molds (Yan *et al*., 2010;.Jing *et al*., 2016). In the present study, two endogenous LAB strains isolated from the natural ensiling system of *Cyperus esculentus* stems and leaves, together with one commercial *Lactiplantibacillus plantarum* strain, were used as silage fermentation additives. The results showed that the endogenous dominant LAB and the mixed LAB additive were better able to adapt to the internal environment of *Cyperus esculentus* stem and leaf silage: compared with the commercial *L. plantarum* treatment and the control group, these two treatment groups exhibited significantly lower pH values and significantly higher LAB counts. Notably, the silage quality of *Cyperus esculentus* stems and leaves treated with commercial *L. plantarum* was significantly inferior to that of the groups treated with endogenous LAB. This difference is presumably related to the structural characteristics and active substances of *Cyperus esculentus* itself—the externally added commercial *L. plantarum* may have been affected by the antimicrobial substances present in the stems and leaves, leading to inhibited growth and reproduction of the strain, which in turn resulted in a slower decline in pH during silage fermentation.

Although the pH values of all treatment groups in this study remained higher than the standard for high-quality silage, no spoilage was observed throughout the fermentation process, and no mold or decay was detected at the end of fermentation. This phenomenon may be closely related to the inherent antimicrobial components of *Cyperus esculentus* itself. Previous studies have found that ethanol extracts of *Cyperus esculentus* leaves exhibit significant inhibitory effects against *Shigella*, *Salmonella*, *Staphylococcus aureus*, and *Escherichia coli* (Jing *et al*., 2016).

In vitro Cumulative Gas Production of Cyperus esculentus and Its Silage

The gas produced during in vitro fermentation mainly consists of carbon dioxide and methane. From a source perspective, gas production is closely related to the carbohydrates and CP present in the forage, as these are the primary substrates for microbial fermentation. The cumulative gas production can reflect the fermentability of the forage, and this indicator is mainly influenced by two factors: the actual content of fermentable organic matter and the activity level of rumen microorganisms. Under relatively consistent conditions, higher-quality forage generally produces a greater volume of gas in the rumen environment. In the present study, the in vitro gas production of *Cyperus esculentus* silage at 72 h was higher than that of the original *Cyperus esculentus* material, indicating that the digestibility of *Cyperus esculentus* was improved after ensiling.

CONCLUSION

The three LAB strains used in this study all improved the quality of *Cyperus esculentus* stem and leaf silage and extended its aerobic stability to varying degrees. Among these, the endogenous LAB strains isolated from the natural ensiling system of *Cyperus esculentus* stems and leaves exhibited better performance than the commercial LAB strain. In terms of strain combinations, the mixed inoculation of *Pediococcus acidilactici* and *Enterococcus faecium* was more effective than singlestrain inoculation. The comprehensive ranking of silage quality after inoculation with different strains was as follows: PE > PA > EF > LP > CK. This further confirms the advantage of using endogenous mixedculture LAB to optimize the ensiling effect of *Cyperus esculentus* stem and leaf silage.

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FORAGE PRODUCTION FROM REWETTED PEAT GRASSLAND

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ABSTRACT

The rewetting of agriculturally used peat grasslands is expected to reduce or prevent greenhouse gas emissions. However, its effects on forage quality and yield remain unclear. This study aimed to evaluate the potential nutritive quality of vegetation from the rewetted part of one plot (plot of interest). In addition, vegetation from an adjacent non-rewetted plot was sampled as a control. Vegetation samples were collected from up to three fixed sampling areas shortly before each planned cut. In recent years, both plots were managed extensively, with up to two cuts per year on the rewetted plot and up to three cuts per year on the control plot. The collected material was dried at 40 °C in a drying box to hay dry matter content and subsequently subjected to extended Weender analysis.

Results indicate that vegetation from the rewetted part of the plot of interest provided acceptable nutritive quality for the intended animal categories, with values comparable to those of the control plot. However, determining an appropriate cutting date is already challenging on grassland on mineral soils and becomes even more difficult on rewetted peatlands. Moreover, even when mowing is possible, harvesting the cut biomass is not always feasible.

Keywords: potential forage quality, rewetted peat grassland

INTRODUCTION

The use of drained peatlands for food or feed production is one of the sources of greenhouse gas emissions in agriculture (Poyda *et al.*, 2016). In southern Germany as well, drained peatlands are still used agriculturally, for example as grassland for intensive fodder production. Rewetting can reduce or prevent greenhouse gas emissions from drained peat grasslands (Bockermann *et al.*, 2024). Efforts have therefore been made in recent years to rewet peat grasslands where possible. Rewetting should be carried out in such a way that peat grasslands can remain in agricultural use after rewetting, for example as extensive grassland for fodder production or pasture. Nevertheless, it remains unclear how forage quality and productivity will change under such conditions.

The aim of this study was to evaluate the potential forage quality of vegetation from a rewetted part of one plot during the first harvest season after structural measures for controlled rewetting, aimed at maintaining the water table level at up to 10 cm below the soil surface. This plot had not been actively drained for several years prior to the measures. Under wet weather conditions, the water table had previously risen even above the soil surface in some areas, resulting in their temporary inundation. The structural measures were designed to prevent such conditions while also retaining water within the plot during dry periods. Moreover, the potential nutritive quality of vegetation from an adjacent non-rewetted plot was evaluated as a control.

MATERIAL AND METHODS

Forage sampling was conducted on two plots. The first plot (plot of interest; total 6.0 ha) included a 3.5 ha rewetted area, where structural measures were implemented in September 2023. These measures aimed to enable controlled rewetting of the plot—retaining groundwater and precipitation to maintain an average water table level of approximately between 10 and 20 cm below the soil surface—while also regulating the water table level and lowering it at harvest. The second plot (control plot; 4.2 ha), bordering the plot of interest, was not influenced by structural measures and remained non-rewetted, although it was no longer actively drained. Both plots had been managed extensively in recent years, with up to two cuts per year on the rewetted part of the plot of interest and up to three cuts per year on the control plot. Additionally, at the two cuts (first and third), the non-rewetted part of the plot of interest was sampled at one spot.

Vegetation samples were collected during the year 2024 shortly before planned cuts from up to three previously selected spots at a cutting height of ~8 cm. Samples were dried in a drying box at 40 °C and analysed using classical wet-chemical methods at the Department of Quality Assurance and Analytical Services Laboratory of the Bavarian State Research Centre for Agriculture in Grub. An extended Weender analysis was performed, determining crude ash, crude protein, crude fiber, sugars, aNDFom (ash-free neutral detergent fiber), ADFom (ash-free acid detergent fiber), enzymatically soluble organic matter, utilizable protein, metabolizable energy for ruminants, metabolizable energy for horses, and water-soluble carbohydrates.

Statistical analyses were conducted in SAS 9.4 using PROC MEANS and PROC MIXED, with plot as a fixed factor and planned harvest date nested within plot.

RESULTS AND DISCUSSION

In the year 2024, weather conditions were generally wet. As a result, not only the rewetted part of the plot of interest but also its non-rewetted areas, as well as parts of the control plot, were partially waterlogged. Moreover, it was not possible to lower the water table level before harvest on the rewetted part of the plot of interest as planned. Consequently, neither of the two plots could be cut uniformly at one time, and the first cutting date was delayed compared to the usual harvest dates (around July 1 for the plot of interest and June 15 for the control plot). The earliest and latest dates of the first cut were July 4 and September 4, respectively. Overall, five cutting dates were required for the first cut considering both plots (three for the rewetted part and four for the non-rewetted part of the plot of interest, and three for the control plot). Only the non-rewetted parts of the plot of interest were cut twice, whereas the control plot (including a small non-rewetted part of the plot of interest) was cut three times. As already mentioned, under normal conditions, the plot of interest is cut at least twice per year, and the control plot at least three times per year. Usually, a strip of old grass of approsimately 0.5 ha is left uncut each year on the rewetted part of the plot of interest. However, in the year 2024, a larger area of the rewetted part could not be cut even once, resulting in a strip of old grass of around 1 ha. This area could not be harvested for forage production in the following year either (due to insufficient vegetation quality), indicating considerably higher instability in forage yield than what can already occur under normal conditions.

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Potential nutritive values of vegetation from both plots are shown in Table 1. For the rewetted part of the plot of interest, metabolizable energy (ME) for ruminants across the three dates of the first cut varied only slightly, between 9.61 ± 0.25 and 9.71 ± 0.11 MJ/kg DM. In addition, one sample was collected from the non-rewetted part of the plot of interest in the first and third cut, yielding comparable ME values of 9.66 and 10.62 MJ/kg DM, respectively. On the control plot, sampling during the first cut was only possible at the second cutting date, when the largest area was harvested, resulting in a mean ME of 9.52 ± 0.07 MJ/kg DM. In the second and third cut, mean ME values were 10.47 ± 0.29 and 10.96 ± 0.15 MJ/kg DM, respectively. Mean crude protein values were low in samples from the rewetted part of the plot of interest. However, due to acceptable ME content, the utilizable crude protein was notably higher than crude protein content and comparable to values from the first cut of the control plot (114.67 ± 1.54 g/kg DM) and the non-rewetted part of the plot of interest (119 g/kg DM; one sampling point). Similarly, aNDFom and ADFom contents in material from the rewetted part of the plot of interest were comparable to those from the first cut of the control plot (618.00 ± 8.19 and 364.00 ± 17.06 g/kg DM for aNDFom and ADFom, respectively) and the non-rewetted part of the plot of interest (620.00 and 365.00 g/kg DM for aNDFom and ADFom, respectively). These values were expected due to the late first cutting date of the first cut (Andueza *et al.*, 2021). ELOS, an indicator of readily digestible organic matter, remained within recommended ranges for grassland forage, confirming sufficient potential feed quality of vegetation from the rewetted part of the plot of interest.

The low variability in nutritive quality of collected material from the rewetted part of the plot of interest across the planned first cut dates, despite the prolonged harvest period, is likely due to the simultaneous presence of senescent and newly emerging biomass.

Tab. 1: Potential nutritive values (mean ± SD) of forage from rewetted and non-rewetted peat grassland plots

Item	Plot of interest		Control plot (non-rewetted) (n = 3)
	Rewetted part (n = 3)	Non-rewetted part (n = 2)	
Crude protein (g/kg DM)	79.42 ± 5.88	125.00 ± 52.33	126.11 ± 49.48
Utilisable protein (g/kg DM)	117.89 ± 0.54	130.00 ± 15.56	132.00 ± 15.82
aNDFom (g/kg DM)	616.94 ± 4.70	530.50 ± 126.57	499.00 ± 129.95
ADFom (g/kg DM)	362.67 ± 11.86	299.00 ± 93.34	282.89 ± 79.01
ELOS (g/kg DM)	468.00 ± 32.05	604.00 ± 104.65	629.33 ± 104.04
WSC (g/kg DM)	104.53 ± 27.82	143.90 ±58.83	154.08 ± 38.03
Metabolisable energy – ruminants (MJ/kg DM)	9.67 ± 0.06	10.14 ± 0.68	10.32 ± 0.73
Metabolisable energy – horses (MJ/kg DM)	7.71 ± 0.08	8.35 ± 1.20	8.58 ± 1.26

Means did not differ significantly (P > 0.05).

n = number of cut dates for the rewetted part and number of cuts for the non-rewetted part of the plot of interest and the control plot. DM = dry matter; aNDFom = ash-free neutral detergent fiber; ADFom = ash-free acid detergent fiber; ELOS = enzymatically soluble organic matter; WSC = water-soluble carbohydrates.

CONCLUSION

In the first year after structural measures for controled rewetting, the potentional nutritive quality remained acceptable for feeding of fattening cattle and horses. However, it was not always possible to cut the whole area at the planned date or to harvest all cut biomass. Therefore, even the nutritive quality should stay acceptable, the harvest is not always possible and losses of whole cuts or parts of areas by some cuts has to be considered.

Acknowledgement

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INHIBITION OF CLOSTRIDIA GROWTH BY *LACTIPLANTIBACILLUS PLANTARUM* AND *LACTOCOCCUS LACTIS*

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ABSTRACT

Clostridia are undesirable microorganisms in silage, particularly in low dry matter forage, where their growth can impair silage quality and animal performance. This study screened more than 200 lactic acid bacteria for their ability to inhibit clostridia. Two newly isolated strains, *Lactiplantibacillus plantarum* DSM 34271 and Lactococcus lactis DSM 34262, showed clear inhibitory effects against *Clostridium tyrobutyricum* in agar diffusion assays. Based on these results, both strains were evaluated as silage inoculants in three slightly wilted grass materials. Compared with untreated controls, inoculated silages exhibited significantly lower pH values, increased lactic acid concentrations, reduced butyric acid formation and lower weight losses after 90 days of storage. These findings indicate improved fermentation quality and suggest effective suppression of clostridial activity by the selected strains.

Keywords: *Lactiplantibacillus plantarum*, *Lactococcus lactis*, clostridia inhibition, silage

INTRODUCTION

Clostridia are spore-forming, anaerobic gram-positive bacteria that ferment carbohydrates, lactic acid, as well as amino acids and proteins. When clostridia drive the fermentation process, they lead to a reduction in feeding value. For feeding purposes it is very important to keep counts of clostridia spores at a low level to avoid health problems and impaired animal performance.

In a screening project, a broad range of lactic acid bacteria were evaluated for their potential to inhibit clostridial growth. Two newly isolated strains, *Lactiplantibacillus plantarum* (LP) DSM 34271 and *Lactococcus lactis* (LC) DSM 34262, demonstrated strong inhibitory activity against clostridia in agar diffusion assays. Based on these results, three ensiling trials with slightly wilted grass were conducted in which the two isolated lactic acid bacteria were compared with an untreated control regarding their influence on various quality parameters.

MATERIALS AND METHODS

The inhibition of clostridia was examined using an agar diffusion test. *Clostridium tyrobutyricum* DSM 2637 spores have been diluted to reach semi-confluent growth and enclosed into RCM agar plates. Supernatants of more than 200 strain cultures (80 µl) were put into wells and incubated anaerobically (AN) at 37 °C for 24 h. A clear zone (halo) around the well shows the inhibition of clostridia (Schoster *et al.*, 2013).

For the silage trials, grass material from three different sources with dry matter concentrations ranging from 27 to 36%, was slightly wilted. Each chopped grass material was ensiled either without treatment (control) or with *Lactiplantibacillus plantarum* DSM 34271 or *Lactococcus lactis* DSM 34262. The lactic acid bacteria were sprayed onto the grass as a liquid bacterial solution with a concentration of 2.5×10^5 cfu/g FM. A quantity of 3 kg forage was ensiled in plastic buckets to observe fermentation processes and losses after 90 days of storage at 20 °C. Forage samples were analyzed to determine forage quality (DM, pH-value, fermentation pattern and ammonia-N). Fermentation weight losses were determined based on the mass difference of the mini silos before and after ensiling.

RESULTS AND DISCUSSION

The two selected lactic acid bacteria showed a clear inhibition zone in the agar diffusion test, shown in Table 1 and Figure 1. *Lactococcus lactis* DSM 34262 had a stronger effect against the test strain *Clostridium tyrobutyricum* compared to *Lactiplantibacillus plantarum* DSM 34271.

The mean pH-value in the untreated control was 4.72. Inoculated silages reached significantly lower pH values of 3.98 and 4.25, respectively. Consistent with the pH pattern, lactic acid concentrations were significantly higher in the treated silages (7.65 and 5.98 g per 100 g DM) compared with the control silage (3.11 g per 100 g DM). These results indicate improved preservation in the treated grass. The lower concentrations of butyric acid and ammonia-N in the treated materials are consistent with the observed pH and lactic acid pattern. Additionally, silages with higher lactic acid concentrations exhibited lower weight losses, presumably due to the inhibition of spoilage microorganism growth (control: 2.88 g/100 g FM; treatment: 1.36 and 1.84 g/100 g FM). Values are shown in Table 2.

Tab. 1: Inhibition zones of investigated lactic acid bacteria

Strain	Incubation [C°]	Halo [mm]
L. plantarum DSM 34271	37°C / AN	1.5
L. lactis DSM 34262	37°C / AN	5.0

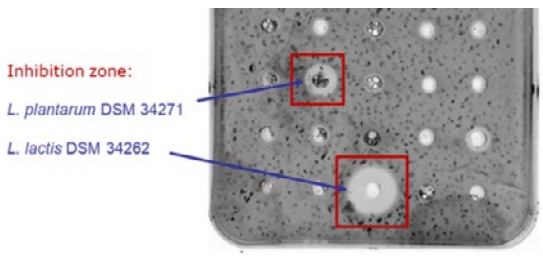


Fig. 1: Inhibition zones of investigated lactic acid bacteria

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Tab. 2: Comparison of the parameters of mean values from three silage trials using slightly wilted grass material after 90 days of storage

		Mean values of three silage trials / 90 d				
Parameter	**Unit**	**Control**	**LP (DSM 34271)**	**p-value**	**LC (DSM 34262)**	**p-value**
DM	g/100 g FM	31.0	32.1	0.159	31.5	0.451
pH – value		4.72	3.98*	<0.001	4.25*	0.013
Ammonia-N	g/100 g DM	0.30	0.15	0.064	0.22	0.192
Weight losses	g/100 g FM	2.88	1.36*	0.004	1.84*	0.019
Lactic acid	g/100 g DM	3.11	7.65*	<0.001	5.98*	<0.001
Acetic acid	g/100 g DM	1.33	0.68	0.083	0.93	0.268
Butyric acid	g/100 g DM	0.56	0.05*	0.027	0.20	0.130

*Indicates mean values within a line are significantly different at $p < 0.05$ (Mann-Whitney-Test) in comparison to the control group.

CONCLUSION

The present study demonstrates that the newly isolated strains *Lactiplantibacillus plantarum* DSM 34271 and *Lactococcus lactis* DSM 34262 effectively inhibit the growth of clostridia and improve silage fermentation quality. Both strains showed clear antagonistic activity against *Clostridium tyrobutyricum* in agar diffusion assays, with *L. lactis* DSM 34262 exhibiting the strongest inhibition. When applied as silage inoculants to slightly wilted grass with elevated clostridia risk, both strains significantly enhanced fermentation performance. The main effects included a pronounced reduction in pH, increased lactic acid production, lower weight losses and reduced formation of butyric acid, indicating suppressed clostridial activity. These results confirm the suitability of both strains as effective biological silage additives and highlight their practical potential to improve silage preservation and feeding quality under challenging ensiling conditions.

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INVOLVEMENT OF 4-PENTENOIC ACID IN CAUSING QUALITY DETERIORATION OF NETTLE SILAGE: STUDY OF ANTIBACTERIAL MECHANISM

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Keywords: *Pediococcus pentosaceus*, nettle silage, 4-pentenoic acid, ammonia

INTRODUCTION

Ensiling legumes such as alfalfa presents challenges because of their high buffer capacity and protein content, as well as their low levels of water-soluble carbohydrates (WSCs), especially for nettle (Zhang *et al*., 2010). Recently, researchers showed that inoculation with *Pediococcus sp*. can improve the quality of legume silage by increasing lactic acid (LA) production and reducing pH levels (Kuppusamy *et al*., 2020). The relative abundance of spherical LAB, such as *Pediococcus spp*., remains stable in nettle silage (Huang *et al*., 2023). However, various substances, including condensed tannins and flavonoids, can inhibit *Pediococcus sp.* activity during ensiling or under specific conditions (Bae *et al*., 2023). We investigated whether *Pediococcus sp.* can influence fermentation characteristics or overcome these challenges in nettle silage.

MATERIAL AND METHODS

Pediococcus sp. strains were isolated from nettle silage under natural fermentation conditions. The nucleotide sequence data of the bacteria were deposited in the National Center for Biotechnology Information under accession number "OL455604" (accessed on November 19, 2021). Nettle (*Urtica cannabina*) was harvested on September 20, 2022, from the wild in Shawan County in the central mountains of Tianshan, Xinjiang, China (E 84°58′–86°24′; N 43°26′-45°20′). *P. pentosaceus* was evenly sprayed on each bag of the silage surface and completely mixed for storage. There were four fermentation period treatments (7, 15, 30, and 60 h), two inoculation treatments, and five replicates for each treatment, resulting in 40 bags of samples. Fresh silage samples (20 g) were used to analyze fermentation characteristics. The LA, acetic acid (AA), propionic acid (PA), pH, and ammonia (AN) levels were measured as described previously (Huang *et al*., 2023). Samples were selected after 7, 30, and 60 d of ensiling. Total DNA was extracted from each silage sample using a commercial DNA Kit (FastDNA® Spin Kit for Soil, MP Biomedicals, Irvine, CA, USA). The amplicons were purified and analyzed as previously described (Huang *et al*., 2023). For metabolite analysis, 50 g of fresh sample was collected on each natural fermentation day (after 7, 30, and 60 d of ensiling) of nettle silage, with five replicates for each silage. The minimum inhibitory concentrations of the substances against *P. pentosaceus* were determined using the broth dilution method. The characteristic data of the two silage groups were analyzed using Student's t-test. Data were analyzed using SPSS 22 Statistics (SPSS, Inc., Chicago, IL, USA). Differences between treatments were considered significant at $P < 0.05$. Bioinformatic analysis of the silage microbiota and metabolites was performed using the Majorbio Cloud platform (https://cloud.majorbio.com).

RESULTS AND DISCUSSION

As shown in Table 1, during fermentation, The LA content was highest but the AA content was lowest in PP-treated plants compared with that in control plants after 7 d of ensiling ($p < 0.05$). The AN content was lower in the PP group than in the control group after 30 and 60 d of ensiling ($p < 0.05$). A ratio of LA-to-AA < 3 indicates heterolactic fermentation during ensiling, in which LAB such as *Lactobacillus buchneri* can convert WSC into LA, AA, and CO_2, resulting in a decrease in the DM. In the present study, inoculation with *P. pentosaceus* did not change the fermentation type or lead to a LA-to-AA ratio <3, but the content of AA decreased by 21.62% after 7 d of ensiling, indicating that *P. pentosaceus* can reduce DM loss during the early stage of ensiling fermentation. In this study, inoculation with *P. pentosaceus* decreased AN production during nettle ensiling. AN is correlated with undesirable bacteria (such as *Clostridium* and *Enterobacter*) and plant proteolytic enzyme activity, both of which are inhibited at a pH below (Bae *et al*., 2023; Ren *et al*., 2020; Ozogul *et al*., 2016). However, we showed that the pH was > 7 after inoculation, indicating that another factor was responsible for the reduction of AN during ensiling. Some species of *Pediococcus* can significantly decrease AN production (87.48% decline) in *Escherichia coli* (Ozogul *et al*., 2016). Thus, our results suggest that *P. pentosaceus* can inhibit undesirable bacterial activity during nettle ensiling.

Pediococcus pentosaceus was isolated from naturally fermented nettle silage, the correlation between *Pediococcus* and organic acids such as 4-pentenoic acid ($R^2 = -0.6323$, $p < 0.001$) and gluconic acid ($R^2 = -0.2512$, $p < 0.001$) was extremely negatively significant during nettle ensiling. The minimum inhibitory concentrations of 4-pentenoic acid and gluconic acid against *P. pentosaceus* were 16 and 32 mg/mL, respectively. We first observed that 4-pentenoic acid was present during the natural fermentation of nettle silage and that its content was almost stabilized during ensiling (decreased by 4.48% after 90 d of fermentation), inhibiting *P. pentosaceus* in vitro.

CONCLUSION

Inoculation with *P. pentosaceus* decreased ammonia production (from 9.00% to 6.10% of total nitrogen) in nettle silage. However, the quality of nettle silage cannot be improved because of interference by 4-pentenoic acid, which exerts antibacterial effects.

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Tab. 1: The fermentation characteristics of nettle silage % dry matter

Items	Ensiling days	Treatment		SEM	P value
		CK	PP		
pH	7	7.92	7.64	0.132	< 0.001
	15	7.77	7.87	0.083	0.263
	30	8.35	8.37	0.058	0.059
	60	8.46	8.01	0.132	< 0.001
LA	7	2.60	2.80	0.227	0.18
	15	3.27	3.58	0.459	0.273
	30	ND	ND	–	–
	60	ND	ND	–	–
AA	7	1.85	1.45	0.218	0.027
	15	1.92	2.05	0.360	0.238
	30	ND	ND	–	–
	60	ND	ND	–	–
PA	7	0.06	0.05	0.015	0.414
	15	0.09	0.09	0.032	0.097
	30	ND	ND	–	–
	60	ND	ND	–	–
AN (%TN)	7	1.06	1.48	0.25	0.167
	15	3.59	4.12	0.33	0.254
	30	6.71	5.05	0.55	0.039
	60	9.00	6.14	0.11	< 0.001

CK: control; PP: inoculation with *P. pentosaceus*; LA: lactic acid; AA: acetic acid; PA: propionic acid; AN: ammonia nitrogen; TN: total nitrogen.

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IT IS POSSIBLE TO ENSILE AMARANTH (*A. HYPOCHONDRIACUS*) PLANTS GROWN IN THE CONDITIONS OF THE CZECH REPUBLIC?

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ABSTRACT

The objective of this study was to evaluate the effect of growth phase of *Amaranthus hypochondriacus* on chemical composition and digestibility of green plants. Plants were collected during the growing season according to the days since sowing. Amaranth plants show decent dry matter degradation after 24 hours in the rumen (*in sacco* method), from 70 to 84.0%. The NDF content in the plant increased during vegetation; the nitrogenous substance content ranged from 6.1 to 8.5% and starch content from 2.9 to 9.4% in dry matter. + Amaranth plants show decent dry matter degradation after 24 hours in the rumen (in sacco method), from 70 to 84.0%.

Keywords*:* amaranth, yield, chemical composition, digestibility

INTRODUCTION

Amaranth, in Czech called laskavec (*Amaranthus* spp.), is a pseudocereal with high nutritional value, great economic potential, and a large number of usable species and varieties (Ulbricht *et al*., 2009). The great potential of amaranth lies in its use as feed for ruminants, which are capable of consuming and utilizing even weedy species of amaranth; interest in amaranth as feed has currently increased due to its high content of nutrients and fatty acids (Peiretti *et al*., 2018). Ensiling is an effective way to reduce the oxalic acid content in amaranth fodder (Blaney *et al*., 1972) and to increase the digestibility and reduce the content of antinutritional substances (Cervantes, 1990).

The objective of this study was to evaluate the effect of growth phase of *Amaranthus hypochondriacus* on chemical composition and digestibility of green plants.

MATERIAL AND METHODS

The sowing of amaranth seeds (var. *A. hypochondriacus*) on the IAS plots took place on April 15, 2023, with maize as the preceding crop. The plants were not treated chemically during the entire growing season, nor were they fertilized. The plant itself has a robust root system and makes good use of the nutrient reserves in the soil. Plants were collected during the growing season according to the days since sowing, and the condition of the stand and the plants was always described; collections were carried out at three locations on the plot (collection site size 50 × 100 cm). The height of the plants and their weight were measured; the plants were then cut and dried at 55 °C. After drying, the plant samples were ground to particle sizes of 2 and 1 mm for determining nutrient content in the laboratory (1 mm samples) and for subsequent degradation of the samples in the rumen (2 mm). In green samples, nutrient degradation in the rumen of cannulated Holstein cows was detected by *in sacco* method (incubation for 24 hours in bags with pore size 51 µm). Plant samples were analysed for ash, crude protein (CP), ether extract (EE), crude fibre (CF), neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL).

RESULTS AND DISCUSSION

Table 1 shows the yield potential of amaranth plants for green fodder, in different stages of maturity.

For comparison, according to the approximate yields of field crops at different farming intensities (Mendel University in Brno), the yield of maize for silage is 40 t/ha with a whole-plant dry matter content of 32% and the yield of alfalfa is 50 t/ha in green mass with standard cultivation methods. With lower inputs, however, the yield of maize for silage is 25 t/ha and alfalfa 40 t/ha. Compared to these crops, unfertilized amaranth achieves a green mass yield of 32–81 t/ha. Amaranth, due to its growth potential even in unsuitable soil and climatic conditions, can be used for the production of quality forage (Abbasi *et al*., 2012); its nutritional value depends on the stage of growth at which it is harvested (Pospíšil *et al*., 2009). Peiretti *et al.* (2018) monitored the nutritional value of the forage of the species *A. caudatus* depending on the growth stage in order to determine how the growth stage affects the chemical composition, in vitro digestibility, and fatty acid profile, and thus recommend the potential of the forage for feeding ruminants.

Rezaei *et al.* (2009) monitored the nutritional values of fresh and ensiled forage of the species A. hypochondriacus, treated with molasses in amounts of 0, 5, and 10% in the fresh state. Based on chemical composition, mineral content, in vitro digestibility, and favorable protein quantity values, these authors recommend the forage as suitable for feeding ruminants and also as ensilable if conditions for drying it are not available. Pond and Lehmann (1989) claim that including the species A. cruentus in amounts of up to 50% can successfully replace traditionally fed bulky feeds, such as alfalfa, thanks to its low fiber content, high protein content, and absence of toxins in the vegetative parts of the plants. They also demonstrate that the added amaranth served as an energy source for lamb growth and had no negative impact on gains or nutrient conversion.

Tab. 1: Yield potential of amaranth plants for green fodder, in different stages of maturity

Days after sowing	Plant height (cm)	Yield of green mass (t/ha)	Average yield of green mass (t/ha)	Average dry matter of the crop (%)
80th day	67–100	28.7–36.7	32.0	16.3
100th day	108–134	49.5 – 64.4	56.8	16.4
110th day	143–189	77.3–84.7	82.2	19.5
120th day	135–178	56.4–75.4	67.2	19.2
140th day	157–180	69.2–96.1	81.0	20.2

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Tab. 2: How the growth stage affects the chemical composition

Days after sowing	NDF	ADF	ADL	CP	starch	Dry matter losses (%) after 24 hours in sacco
80th day	33.8	24.9	3.5	8.5	2.9	84.0
100th day	38.7	29.9	3.6	6.6	3.2	77.9
110th day	40.2	30.9	3.3	6.1	9.4	72.0
120th day	42.1	32.4	3.5	7.8	6.1	73.9
140th day	43.6	32.6	3.8	6.9	5.0	69.9

CP – crude protein; NDF – neutral detergent fibre; ADF – acid detergent fibre; ADL – acid detergent lignin

CONCLUSION

When comparing the dry matter of the crop throughout the entire growing season, it is surprising that the dry matter of the crop hardly changes. The plants grow continuously throughout the entire vegetation period, are green, and the leaves hardly fall off. The NDF content in the plant increased during vegetation from 33.8 to 43.6% in dry matter. What is interesting is the almost unchanged ADL content in the plant (3.3 to 3.8%) in the plant dry matter throughout the entire vegetation period, so there is no lignification of the plants. Moreover, the plants themselves are robust and strong. A relatively high ash content was determined in the plants (15.1–21.3% in dry matter). The nitrogenous substance content ranged from 6.1 to 8.5%; starch content from 2.9 to 9.4% in dry matter. Amaranth plants show decent dry matter degradation after 24 hours in the rumen (in sacco method), from 70 to 84.0%. In comparison with amaranth, corn silage shows a dry matter digestibility of 58–74% after 24 hours in the rumen, and grass silage 64–85%. With the aging of the pasture, there is a slight decrease in the digestibility of the forage.

Acknowledgement

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PREPARATION AND FERMENTATION OF MIXED SILAGE OF COTTON STALK AND CORN STOVER

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ABSTRACT

Cotton is a globally economic crop with an enormous yield of cotton stalk, which are usually difficult to prepare high-quality silage alone. To explore the high-value utilization approach of cotton stalk, this experiment designed corn stover silage mixed with cotton stalk at ratios of 0%, 10%, 25%, 50% and 100%. The inoculant *Lactiplantibacillus plantarum* and cellulase were added for silage preparation. The results showed that corn stover silage was good fermentation quality with pH below 4.0 and lactic acid content higher than 3% of fresh matter, while the cotton stalk silage was poor quality. With the increase in the mixing ratio of cotton stalk, the pH of the silage increased and the lactic acid content decreased. The fermentation quality was lower significantly ($P < 0.05$) when the mixing ratio of cotton stalk exceeded 25%. The combined addition of lactic acid bacteria and cellulase shows a bacteria-enzyme synergistic effect, improving the fermentation quality of mixed silage.

Keywords: cotton stalk, corn stover, mixed silage, fermentation quality, feed utilization technology

INTRODUCTION

Cotton and corn, as important global economic and food crops, generate a large amount of straw resources through large-scale cultivation. Corn stover can be converted into a core roughage source for livestock through high-quality silage fermentation. However, cotton stalk has low nutritional value due to high lignification and anti-nutritional factors such as free gossypol, and it is poorly palatable when fed directly, which is a key bottleneck limiting its use as feed (Wang *et al.*, 2024). For a long time, a large amount of cotton stalk has been discarded or burned, resulting in the waste of biomass resources and increasing environmental burden.

This study focuses on mixed silage of cotton stalk and corn stover, systematically exploring how mixing ratios, microbial inoculants, and other key factors affect fermentation characteristics. The goal is to provide theoretical support and practical references for the high-value utilization of cotton stalk, cost reduction and efficiency improvement in livestock farming, and the development of a green circular agricultural economy.

MATERIALS AND METHODS

Cotton (*Gossypium hirsutum*) stalks were obtained from the Experimental Base of Institute of Cotton Research of Chinese Academy of Agricultural Sciences (Anyang, China), and corn (*Zea mays*) stovers from the Experimental farm of Zhengzhou University (Xingyang, China). Silage additives included L. plantarum FG1 (Zhengzhou University, Zhengzhou, China) and cellulase (CEL, Nanning, China). The mixing ratios of cotton stalk to corn stover were 0, 10, 25, 50, and 100%. Treatments were designed as control, CEL, FG1, and CEL FG1. Silage was prepared with polyethylene plastic bags, vacuum-sealed, and stored at room temperature (20–25°C). After 60 days of fermentation, samples were taken and analyzed for fermentation quality.

RESULTS AND DISCUSSION

Table 1 shows the chemical compositions of the two straw types. Cotton stalk had higher pH, dry matter (DM) and crude protein (CP) but lower neutral detergent fiber (NDF) and acid detergent fiber (ADF) than corn stover.

Fig. 1 illustrates the fermentation quality. Corn stover had 60%–70% moisture (Fig. 1-a) and exhibited favorable fermentation when ensiled alone: pH < 4.0 (Fig. 1-b), lactic acid > 3% in FM (Fig. 1-c), and ammonia nitrogen (NH_3-N) < 0.5% (Fig. 1-d), meeting high-quality silage criteria. In contrast, pure cotton stalk silage had higher pH and lower lactic acid, indicating poor fermentation. Fermentation improved at a 10%–25% cotton stalk mixing ratio, but when the ratio exceeded 25%, pH rose significantly and lactic acid dropped markedly, resulting in inferior quality. Thus, the mixing proportion of cotton stalk should not exceed 25% in silage or fermented total mixed ration (TMR).

Adding lactic acid bacteria and cellulase did not significantly improve fermentation, and no obvious synergistic effect was observed. A significant increase in ammonia nitrogen occurred only in the 10% cotton stalk mixed silage group, and the mechanism remains unclear. Future research will focus on the effects of cotton stalk chemical composition on silage fermentation and the associated protein metabolic pathways and regulatory mechanisms.

Tab. 1: Chemical composition of cotton stalk and corn stover

Item	Cotton stalk	Corn stover
pH	7.12	6.18
Dry matter (DM, %)	79.10	27.78
CP (% DM)	14.65	9.17
NDF (% DM)	40.80	42.77
ADF (% DM)	32.77	32.23

DM, dry matter; CP, crude protein; NDF, neutral detergent fiber;ADF, acid detergent fiber.

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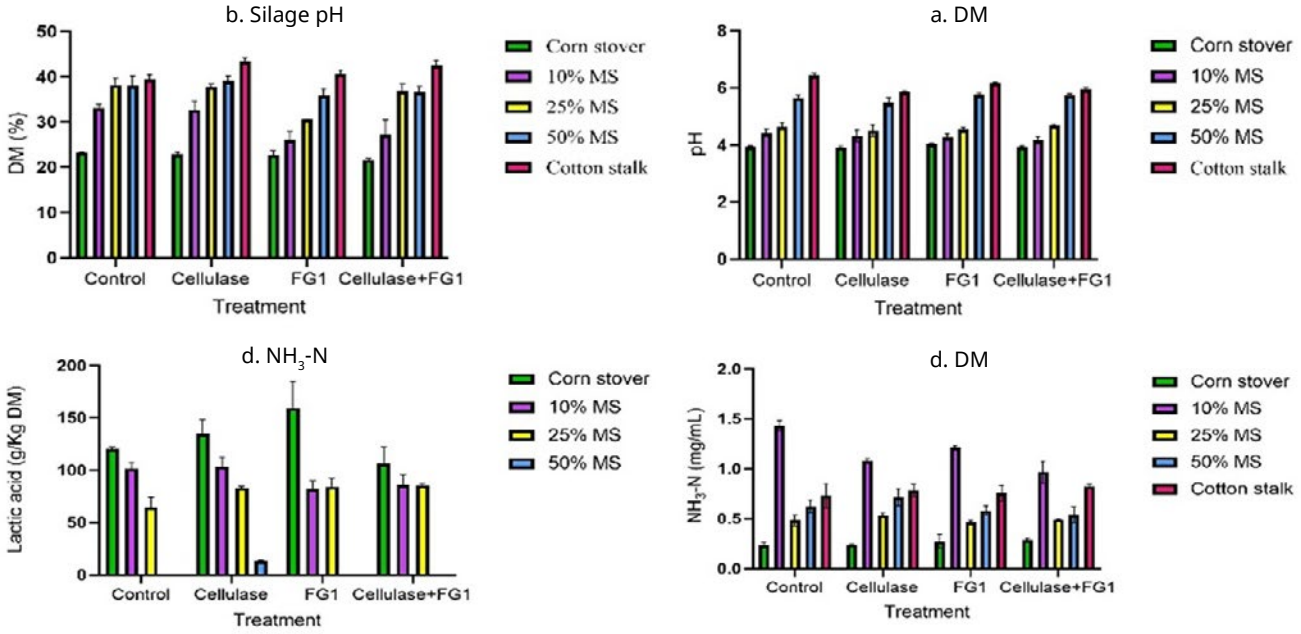


Fig. 1: Fermentation quality of mixed silage prepared with cotton stalk and corn stover

CONCLUSION

This study confirms that ensiling cotton stalks alone often leads to poor fermentation quality, whereas mixing them with corn stalks can effectively improve fermentation performance. When the proportion of cotton stalks exceeds 25%, the fermentation quality of the mixed silage significantly decreases. Therefore, when preparing silage or fermented total mixed rations, the mixing ratio of cotton stalks should not exceed 25%. This study can provide baseline data support for the high-value utilization of cotton stalks.

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REDUCING DRY MATTER LOSSES AND EMISSIONS IN MAIZE SILAGE AND BIOGAS SYSTEMS USING A SILAGE ADDITIVE CONTAINING *L. DIOLIVORANS*

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ABSTRACT

Silage additives are widely used to improve maize silage quality, yet their effects on the carbon footprint (CF) of silage production and biogas systems remain rarely studied. This study evaluated a biological silage additive containing homo- and heterofermentative lactic acid bacteria and assessed its impact on silage quality, dry matter (DM) losses during anaerobic and aerobic storage, biogas and methane yield, and the CF of maize-to-electricity generation. Maize silage prepared with (TM) and without silage additive (UM) was stored for 112 days and monitored during nine weeks of aerobic exposure. TM showed improved fermentation, higher acetic acid concentrations, strong suppression of yeasts and molds, markedly enhanced aerobic stability (72 h → 223 h), and substantially lower DM losses (21.5% → 5.21%). Biogas and methane yields increased by 5.9% and 6.8%, respectively. Using Global Warming Potential over a 100 year time horizon (GWP100), TM reduced the CF by 13.3% at the ensiling stage and by 6.9% at electricity generation stage compared to UM. Overall, the additive improved preservation efficiency and methane recovery, lowering the climate impact of maize-based biogas systems primarily by reducing losses rather than altering emission processes, although biogenic CO_2 emissions were not considered. exposure and reduced yeast and mold counts ($p < 0.01$).

Keywords: carbon footprint, silage additive, biogas, maize silage

INTRODUCTION

Maize silage is a key substrate for biogas production, with biogas yield strongly depending on silage quality (FNR 2016; Vervaeren *et al.* 2010). Silage additives have been shown to improve fermentation and reduce dry matter (DM) losses and spoilage, thereby affecting biogas yield and environmental impact (Borreani *et al.* 2018; Wróbel *et al.* 2023). However, the influence of silage additives on the carbon footprint (CF) of maize-based biogas systems remains rarely studied. This study therefore investigates the effects of a biological silage additive on silage quality, biogas yield, and CF, with the aim of assessing whether improved fermentation not only stabilizes maize silage but also reduces the CF of biogas-to-electricity generation.

MATERIAL AND METHODS

Maize (36.2% DM) was harvested from a farm in northern Germany in September 2023, weighed before ensiling, and divided into two silos: an untreated control (UM) and a treated silage (TM). TM was inoculated with a mixture of homo- and heterofermentative lactic acid bacteria (LAB) (*L. diolivorans*, *L. buchneri*, *L. rhamnosus*) at 250,000 CFU/g FM. Silos were sealed 112 days (anaerobic phase) and then opened (aerobic phase); silage quality was monitored over nine weeks of aerobic exposure. Analyses included pH, lactic and acetic acids, yeast and mold counts, aerobic stability (Honig, 1990), and biogas production assessed by batch tests according to VDI 4630. Fresh matter losses during anaerobic ensiling phase and aerobic fed-out phase were calculated from the difference between harvested and fed-out material. Treatment effects between UM and TM were analyzed using one-way ANOVA ($p < 0.05$). CF assessment followed ISO 14067 using a cradle-to-grid life cycle assessment and were performed to quantify and compare the CF associated with a) ensiling (cultivation & ensiling) and b) electricity generation (entire chain - cultivation, ensiling, digestion, and electricity generation in a combined heat and power unit). Functional units were 1 kg FM for ensiling and 1 kWh_{el} for electricity generation. The CF evaluation was applied using the IPCC (AR6) Global Warming Potential over a 100 year time horizon (GWP100) characterization factors (IPCC, 2021), which include fossil CO_2 and CH_4, and N_2O emissions, while assigning biogenic CO_2 a global warming potential of zero and thus excluding it from the climate impact assessment.

RESULTS AND DISCUSSION

The silage additive significantly improved silage quality and preservation, as reflected by higher acetic acid concentrations (TM: 3.58% DM; UM: 1.40% DM) and pronounced suppression of spoilage microorganisms. Yeast counts decreased significantly from 27,200 CFU/g FM in UM to 180 CFU/g FM in TM, indicating effective inhibition of aerobic spoilage (Table 1).

Tab. 1: Average values of the monitoring parameters for silage quality of the control (UM) and with a biological silage additive treated maize silage (TM) for three months opening period

Parameter	Unit	Maize silage		
		UM	TM	p-value
DM	g/kg FM	331.77 ± 26.90	348.01 ± 22.09	0.1047
oDM	g/kg DM	980.74 ± 43.90	981.58 ± 37.00	0.5989
Lactic acid	% DM	5.79 ± 1.97	4.31 ± 1.01	0.0236
Acetic acid	% DM	1.40 ± 0.33	3.58 ± 0.48	< 0.0001
pH	–	3.66 ± 0.11	3.83 ± 0.08	< 0.0001
ASTA	h	72.40 ± 16.99	223.20 ± 16.35	< 0.0001

DM= Dry matter. FM = Fresh matter. ASTA = Aerobic stability time assessment. CFU = Colony forming unit

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Accordingly, aerobic stability increased significantly from 72 h to 223 h, substantially reducing the risk of deterioration upon air exposure. As a result, DM losses declined from 21.5% in UM to 5.21% in TM. Although heterofermentative LAB such as *L. buchneri* may cause additional DM losses through CO_2 formation during anaerobic fermentation, the present results demonstrate that under practical conditions the benefits of improved aerobic stability and reduced yeast activity clearly outweighed potential fermentation-related losses (Wróbel et al. 2023). Overall, the additive shifted fermentation toward reduced spoilage and DM losses, thereby increasing the amount of usable substrate for downstream utilization.

Biogas production

Biogas and methane yields of the silages were evaluated over the full observation period. TM achieved a higher biogas yield (241.67 ± 17.08 Nl/kg FM) than UM (228.26 ± 12.45 Nl/kg FM), corresponding to an increase of 5.9%, while methane yield increased by 6.8% from 122.12 ± 12.45 Nl/kg FM for TM to 130.38 ± 9.16 Nl/kg FM for UM.

Carbon footprint

At the ensiling stage, CF under GWP100 amounted to 37.24 g CO_2-eq kg/FM for TM and 42.93 g CO_2-eq kg/FM for UM, corresponding to a 13.3% reduction. At the electricity generation stage, TM showed a 6.9% lower CF than UM, mainly driven by loss-induced upstream replacement (i.e., cultivation and input requirements rise when DM losses increase). However, GWP100 does not reflect that biogenic CO_2 released through DM losses represents foregone methane potential and thus lost energy generation. Accounting for biogenic CO_2 would therefore further accentuate CF differences between treatments.

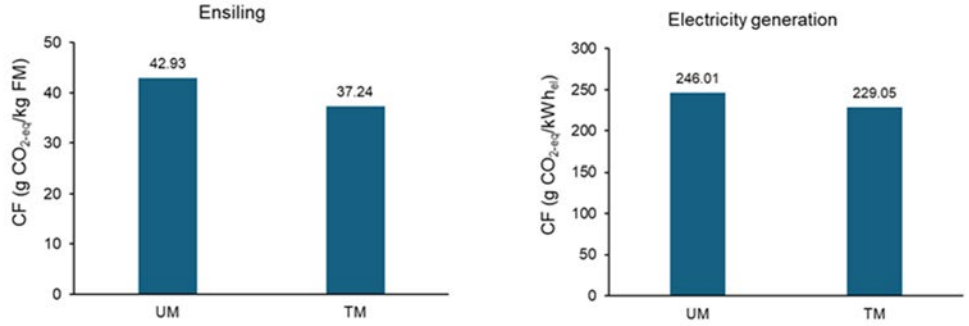


Fig. 1: Carbon footprint of untreated (UM) and treated maize silage (TM) under Global Warming Potential (GWP 100) for the ensiling process (a) and the full pathway up to electricity generation (b) from biogas

CONCLUSION

Even without accounting for biogenic CO_2 emissions, this study demonstrates that the silage additive containing *L. diolivorans* reduces the climate impact of maize silage production and maize-based biogas systems by minimizing dry matter losses and improving substrate efficiency. Improved fermentation quality and aerobic stability preserve more biomass for methane production, slightly increase methane yield, and reduce land and input requirements, thereby lowering emissions per unit of electricity produced. The results highlight silage additives as an effective method to mitigate climate impact when combined with good management.

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SUMMER CATCH CROPS FOR FORAGE CONSERVATION

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ABSTRACT

A range of dicot and monocot summer catch crops were grown on a small scale with the aim to test their ensilability. They were harvested in autumn and ensiled directly without wilting, in part with straw. After three months of anaerobic storage their fermentation quality and aerobic stability were determined. The average fermentation coefficient was reasonably high, while the aerobic stability without straw was rather low. The dry matter content < 200 g/kg of most dicots requires an absorbent to prevent silage effluent in practice.

Keywords: Brassicaceae, Asteraceae, Gramineae, ensilability, fermentation coefficient

INTRODUCTION

Catch crops are grown in the period between two main crops to build up biomass and humus, to diversify crop rotation, cover the soil, protect from soil erosion, improve soil life and fertility, increase yield of main crops, inter alia. Moreover, some have the potential to be used as feed, especially in times of feed scarcity. The aim of the study was to evaluate this potential with a broad range of commercially available summer catch crops including their ensilability.

MATERIAL AND METHODS

A total of 12 different catch crops belonging to the families of Gramineae, Brassicaceae and Asteraceae were sown at the end of July 2024, after the harvest of barley, a minimum of 1 ha each, in the area of Northern Saxony. It is a region of arid months in spring and summer with a total rainfall of 460 mm in 2024. Plant height, stocking density and phenological stage were determined every two weeks starting from the beginning or mid of September respectively to monitor plant growth and development. Forages were sampled regularly to determine fermentability (Schmidt *et al*., 1971). From these species, 6 were finally ensiled on laboratory scale as Rostock Model Silages (ROMOS): *Sinapis alba* (mustard), *Eragrostis tef* (teff), *Avena strigosa* (lopsided oat), *Helianthus annuus* (sunflower), *Brassica oleracea* (wild cabbage), *Guizotia abyssinica* (ramtil). All forages were ensiled being chopped without wilting. The following treatments applied: untreated control (CON) and a mix of *E. faecium*, *P. pentosaceus* and *L. plantarum* (LAB). Furthermore, dicotyledons were either mixed with straw of winter barley or not to achieve a target dry matter (DM) of >30 % applying the aforementioned treatments. Laboratory scale silages were opened after three months of storage at ambient temperature. Feed value, fermentation quality (DLG, 2006) and aerobic stability for 7 d (Honig, 1990; Martens *et al*., 2022) were assessed.

RESULTS AND DISCUSSION

The DM concentrations of the grasses were >200 g/kg while the dicotyledons remained mostly between 110-200 g/kg throughout the autumn (Table 1) making necessary an adsorbing ensiling partner such as straw. Straw though limited fiber digestibility, which was highest in wild cabbage and teff (700 g/kg DM). The fermentability coefficient (FC) predicted that all forages were moderately difficult to easy to ensile at all times (FC>35) except for ramtil, which presented a high BC with a low DM resulting in a borderline mean FC (Table 1). All silages were very well fermented as butyric acid was not detectable, and acetic acid remained in an acceptable range (Table 2, DLG 2006). Ammonia formation was limited with the highest values of lopsided oat and sunflower/straw with 90 g/kg total N (Tab. 2). However, fermentation losses were high for the grasses, sunflower and wild cabbage (CON) between 150-180 g/kg (Tab.2). Sunflower silages were aerobically least stable, increasing in temperature only after 2 d, probably because of their high sugar contents (Table1) followed by cabbage during the fourth day (data not shown). In the aerobic phase, yeast growth was highest in the grasses and sunflower silages coupled with high condensation from oxidation (Fig.1).

Tab. 1: Theoretical mean ensilability of catch crops across time, cut between September – November 2024

Forage	Scientific name	n	DM	WSC	BC	WSC/BC	FC	NO_3
Abyssinian mustard	*Brassica carinata*	12	168^{fg}	252^{bc}	67.6^{b}	3.76^{de}	46.9^{de}	0.02
Wild cabbage	*Brassica oleracea*	17	148^{g}	278^{ab}	63.5^{bc}	4.40^{bcd}	50.0^{de}	0.10
Turnip rape	*Brassica rapa*	15	190^{ef}	228^{c}	41.4^{e}	5.54^{ab}	63.3^{ab}	0.02
Summer forage rape	*Brassica napus*	11	177^{f}	240^{bc}	54.5^{d}	4.45^{bcd}	53.3^{bcde}	0.03
Yellow mustard	*Sinapis alba*	3	241^{bc}	162^{d}	68.6^{ab}	2.38^{f}	43.1^{ef}	0.32
Sunflower	*Helianthus annuus*	23	171^{f}	318^{a}	56.2^{cd}	5.71^{a}	62.8^{abc}	0.85
Ramtil	*Guizotia abyssinica*	15	125^{h}	217^{c}	76.6^{a}	2.88^{ef}	35.5^{f}	0.57
Spring oat	*Avena sativa*	17	250^{b}	162^{d}	36.7^{ef}	4.43^{bcd}	60.5^{abc}	0.63
Lopsided oat	*Avena strigosa*	16	226^{cd}	162^{d}	32.4^{f}	5.14^{abc}	63.6^{a}	0.39
Teff	*Eragrostis tef*	18	275^{a}	120^{d}	34.1^{ef}	3.52^{def}	55.7^{abcd}	0.42
Sudan grass	*Sorghum sudanense*	19	209^{de}	163^{d}	41.3^{e}	3.96^{cde}	52.6^{cde}	0.34
Ryegrass/clover	*Lolium multiflorum, Trifolium incarnatum, T. pratense*	9	170^{fg}	223^{c}	58.4^{cd}	3.85^{de}	47.8^{de}	0.16
s.e.			1.351	3.012	0.512	0.076	0.628	0.06

DM (g/kg); WSC water soluble carbohydrates (g/kg DM); BC buffering capacity; FC fermentability coefficient; NO_3 (g/kg DM); different superscript letters within a column mean significant differences between forages ($p < 0.05$)

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Tab. 2: Fermentation parameters (g/kg DM) of catch crops ensiled without or with barley straw after 90 d storage

Forage species	Treatment	Ensiled on	DM (g/kg)	pH	Lactic acid	Acetic acid	Ethanol	NH3-N (g/kg N)	Fermentation losses
Wild cabbage	CON	28. 10. 2024	143	3.89	145	27.3	11.7	68.9	160
Wild cabbage	LAB	28. 10. 2024	146	3.83	155	23.6	9.23	57.7	75.7
W.cabbage/straw	CON	28. 10. 2024	361	4.21	63.6	15.6	4.65	83.7	76.5
W.cabbage/straw	LAB	28. 10. 2024	362	4.07	81.7	12.1	3.05	77.1	37.2
Yellow mustard	CON	04. 09. 2024	233	4.31	104	25.3	5.50	70.7	56.3
Yellow mustard	LAB	04. 09. 2024	230	4.14	116	14.8	1.32	52.1	49.1
Y. mustard/straw	CON	04. 09. 2024	377	4.33	69.7	19.9	3.84	61.2	-16.7
Y. mustard/straw	LAB	04. 09. 2024	383	4.25	77.3	15.0	1.04	64.8	89.0
Sunflower	CON	28. 10. 2024	160	4.04	93.7	16.5	27.9	60.1	176
Sunflower	LAB	28. 10. 2024	159	3.81	127	15.7	20.7	61.4	180
Sunflower/straw	CON	28. 10. 2024	351	4.21	65.0	13.6	7.47	89.5	87.0
Sunflower/straw	LAB	28. 10. 2024	364	4.04	69.0	10.0	4.67	54.9	33.6
Ramtil	CON	01. 11. 2024	136	4.29	108	32.9	2.66	63.7	77.2
Ramtil	LAB	01. 11. 2024	136	4.07	148	24.3	3.22	50.5	103
Ramtil/straw	CON	01. 11. 2024	299	4.32	67.6	13.3	2.80	64.4	45.4
Ramtil/straw	LAB	01. 11. 2024	300	4.24	79.1	15.6	2.31	61.4	98.3
Lopsided oat	CON	19. 09. 2024	187	3.91	119	21.9	2.48	89.1	146
Lopsided oat	LAB	19. 09. 2024	183	3.85	125	12.2	0.90	56.1	161
Teff	CON	19. 09. 2024	257	4.03	73.6	15.9	2.92	53.6	181
Teff	LAB	19. 09. 2024	256	3.92	86.5	11.0	2.50	47.7	182
s.e.			0.69	0.00	0.71	0.17	0.29	0.81	2.31
P									
Forage			<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Inoculant			0.204	<0.001	<0.001	<0.001	<0.001	<0.001	0.661
Forage*Inoculant			0.258	<0.001	<0.001	<0.001	0.178	<0.001	<0.001

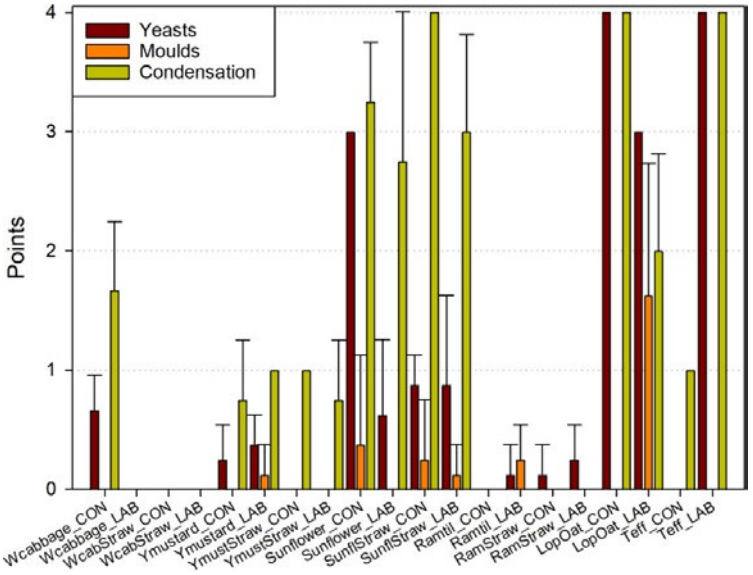


Fig. 1: Yeasts, moulds and condensation scored by visual assessment after 7 d of aerobic stability test (0-4 points); CON Control, LAB Inoculated

CONCLUSION

Ensiling a mixture of catch crops with straw in small units such as bales is recommended to minimize losses from effluent and aerobic deterioration, at the expense of digestibility. A technological on farm solution is required.

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THE EFFECT OF DIFFERENT DRY MATTER CONTENT ON THE FERMENTATION PROCESS AND AEROBIC STABILITY OF ALFALFA SILAGE

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ABSTRACT

The aim of this study was to determine the effect of different dry matter contents of ensiled alfalfa on fermentation quality and the resulting aerobic stability. Alfalfa was ensiled in PE bags and treated with an additive containing a lactic acid bacteria inoculant and an aromatic salt. The quality of the fermentation process was assessed by determining the fermentation products and nutritive value according to Commission Regulation (EU) 771/2024. Aerobic stability was assessed by continuous temperature measurement in a measuring device. The critical temperature for the onset of aerobic instability was defined as the point at which the silage temperature increased by more than 3 °C. It was found that increasing dry matter content, the production of all fermentable acids, including lactic acid, decreased, and protein degradation (%degree of proteolysis) decreased. The aerobic stability of the tested operational alfalfa silages lasted 125-135 hours from air exposure. It has been confirmed that high-quality primary fermentation (Quality class II) is only one of the factors influencing the resulting aerobic stability, which adds to the complexity of this issue.

Keywords: alfalfa silage, dry matter content, fermentation quality, aerobic stability

INTRODUCTION

The dry matter content of alfalfa (*Medicago sativa*), or rather the dry matter composition of this silaged forage, has a fundamental influence on the resulting quality of the fermentation process, as well as the intake of the finished silage. The lack of fermentable carbohydrates in silaged alfalfa is technologically solved by increasing the dry matter content through intensive silage to a value of 40–45%, although the largest volume of alfalfa silages in our country is produced in the dry matter range of 35–40%. It is known that increasing the dry matter content improves the conditions for fermentation in favour of lactic fermentation. It is known that silage above 50% dry matter does not have a positive effect on feed intake, but in addition, technological problems arise with trampling of the silaged material, microbial heating and mould. When silage is silaged for more than 48 hours, nutrient losses of over 10% occur. Quality changes are also related to the proportion of leaves and stems in the material. The proportion of leaves also varies with the order of mowing, is related to the density of the stand and also decreases with the age of the stand. Šimko et al. (2006) report that the proportion of leaves in the early phase of the first mowing is in the range of 42–48 %, while in the second mowing it drops to a lower value (28–45 %).

MATERIAL AND METHODS

Alfalfa stands were harvested at the end of budding, bundled and subsequently harvested and ensiled using the technology of pressing into PE bags with the addition of a combined microbial-chemical silage additive at a dose of 1l/ton of matter. The additive contained five strains of homofermentative lactic acid bacteria and potassium salt of sorbic acid. Alfalfa was harvested on a farm in the Veľké Uherce area in the Trenčín Region in the Partizánske District. Veľké Uherce is located at an altitude of approximately 220 m above sea level with an annual precipitation of approximately 600–700 mm. The operational experiment was aimed at evaluating the qualitative and nutritional characteristics of alfalfa haylage depending on the dry matter content. At the same time, silage samples were assessed for aerobic stability according to the Honig methodology (1990) based on the course of changes in silage temperature after opening. The obtained data were statistically processed and evaluated using the Tibco Statistica CZ program. One-factor variation according to ANOVA was used in the data evaluation, and the statistical significance of the differences was tested using the Scheffe test at a significant level of $p < 0.05$.

RESULTS AND DISCUSSION

Selected indicators of the fementation process of the evaluated alfalfa silages are listed in Table 1.

The average dry matter content of the operational alfalfa silages was 422.3 ± 116.4 g.kg^{-1}, which corresponds to the optimal range for alfalfa silages, in which high-quality fermentation occurs simultaneously with minimal losses. This is also confirmed by the study of Li *et al.* (2018), who achieved similar results. However, there was a relatively high variability in dry matter content between individual samples (from 330.2 to 639.9 g/kg). The positive effect of treatment with a microbial-chemical additive on the quality of the fermentation process is also confirmed by Ding *et al.* (2011) and Peng *et al.* (2024). The average pH value (4.61 0.09) was within the optimal range for alfalfa silages, from which it can be concluded that the silage mass was sufficiently acidified, with

Tab. 1: Average fermentation characteristics (n = 6)

DM	pH	TA	LA	AA	LA/VFA	∑ acids	DP	Ethanol
%		mg KOH/100 g	%					
422.3±116.4	4.61±0.09	1167.67±391	2.18±0.49	0.66±0.17	1.31±0.02	7.40±2.80	6.30±0.65	0.04±0.02

DM... Dry matter, TA... Titrate acidity, LA... Lactic acid, AA... Acetic acid, DP... Degree of proteolysis

Tab. 2: Average nutritional value of lucerne silage (in DM)

CP	RDP	CF	ADF	NDF	DCF	NFC	DMD	NEL
g.kg^{-1}	%	g.kg^{-1}			%	g.kg^{-1}	%	MJ
198.9 ±8.7	74.73±1.78	282.5±17.4	328.6±13.2	358.7±10.4	21.61±4.07	282.6±15.6	65.24±1.53	4.30±0.04

CP... Crude protein; RDP...Rumen degradability of protein; CF... Crude fiber; ADF...Acid detergent fiber; NDF...Neutral detergent fiber; DCF... Rumen degradability of CF; NFC... Non-fiber carbohydrates; DMD... Digestibility of dry matter; NEL... Netto energy of lactation

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the exception of the silage with the highest dry matter content (633.9 g/kg), which showed insufficient acid content and high pH values. Hashemzadeh-Cigari *et al.* (2014) also state that a high-quality fermentation process takes place at a pH of 4.5 or less. The average lactic acid content was 2.18 ± 0.49%, which corresponds to the results of Gua *et al.* (2019), who found 2-3% of this acid in the analysed samples. The lowest LA content was in sample 10 (1.23%), which was due to the extremely high dry matter content. The same results were achieved by Loučka *et al.* (2017), who found a lactic acid content of 1.13% in alfalfa silage with a dry matter content of 56.3%. Similarly, Wang *et al.* (2019) described that LAB activity is significantly weakened in silages with a high dry matter content. The average acetic acid content (0.66 ± 0.17%) corresponds to the homofermentative type of fermentation and the optimal range, which is in line with the findings of Ding *et al.* (2011). According to Kung *et al.* (2018), the propionic acid content in quality silages should be < 0.1%. The tested operational silages reached only trace amounts (0.02 ± 0.01%), which is in line with the findings of Gua *et al.* (2019) and Penga *et al.* (2024). Butyric and valeric acids were not detected in any of the samples. Ding *et al.* (2011) also achieved the same results. The sum of acids in the dry matter was on average 7.40 ± 2.80%. The high value in samples 5, 8 and 9 indicates quality fermentation, while silage 10 again affected the overall average with a low value (2.48%). The consequence of the high dry matter content in this sample was a strongly reduced fermentation. The degree of proteolysis (6.3%) indicates a low degree of CP degradability. A positive correlation was confirmed between the degree of proteolysis and CP degradability (r = 0.901), with the degree of proteolysis increasing with increasing CP degradability.

The nutritional value of the operational alfalfa silages is documented in Table 2. The results shown in the table show that the average crude protein content was within the acceptable range for alfalfa silages (198.9 ± 8.7 g/kg DM). Kung *et al.* (2018) report that alfalfa silage harvested at the budding stage has around 20% crude protein. Similar crude protein results were also achieved by Wang *et al.* (2020) and Ding *et al.* (2011). The average protein degradability is at a medium level (74.73±1.78%), as 75-90% of alfalfa CP is microbially degraded in the rumen. The content of crude fibre (282.5 ± 17.4%) and ADF fractions (328.6 ± 13.2 g/kg DM) and NDF (358.7 ± 10.4 g/kg DM) correspond more to the values of the harvest at the beginning of flowering. Similar results are also reported by Guo *et al.* (2019). The degradability of crude fibre of alfalfa silages in the rumen is at the level of 21.61 ± 4.07%. A higher level is usually analysed in the first cuts due to a lower degree of lignification of the tissues. The average dry matter digestibility (65.24 ± 1.53%) corresponds to the relatively low content of fibre fractions. In the set of evaluated silages, it was confirmed that silages with a lower ADF content had higher dry matter digestibility. The calculated negative correlation (r = −0.886) between ADF content and digestibility shows that each 1% decrease in ADF content increases dry matter digestibility by approximately 0.5–1%. The average energy concentration of NEL (4.30 ± 0.04 MJ/kg DM) corresponds to the energy content commonly achieved in protein silages. The energy content is always related to the level of digestibility of organic matter and the crude fibre content, or the harvest stage of the forage.

The results showed certain differences in resistance to aerobic spoilage, both in the time of onset of degradation itself and in the maximum temperature reached. From the course of the curves (Fig. 1), it can be stated that all tested silage samples did not show aerobic instability, as they exceeded the temperature limit of 23 °C between the 125th and 135th hour of aeration. The heating of the silage was apparently caused by mould activity and occurred in all samples more or less simultaneously, with the maximum between the 140th and 150th hour. The highest increase in pH was recorded in the silage with the highest dry matter content (639.9 g/kg). Indicating a more pronounced destabilisation of the silage after opening. This change in pH may be due to the intensive activity of lactate-assimilating yeasts and bacteria that metabolise the formed lactic acid, leading to deacidification and loss of stability (Pahlow *et al.*, 2003). A stable or decreasing pH value indicates fermentation equilibrium, while an increase in pH indicates an undesirable microbial process.

Despite the low ethanol finding (0.05%), according to Muck *et al.* (2018), latent yeast activity cannot be completely interpreted. The results of testing the aerobic stability of the evaluated silages indicate that the application of the combined silage preparation used led not only to an improvement in the fermentation process but also to an increase in the aerobic stability of the silages. This trend is in line with the results of the studies by Saarisalo *et al.* (2006) and Jaakkola *et al.* (2010), who confirmed that the combination of Lactobacillus plantarum with sodium benzoate was more effective in enhancing aerobic stability than the microbial combination of *Lactobacillus plantarum* and *Lactobacillus buchneri* alone.

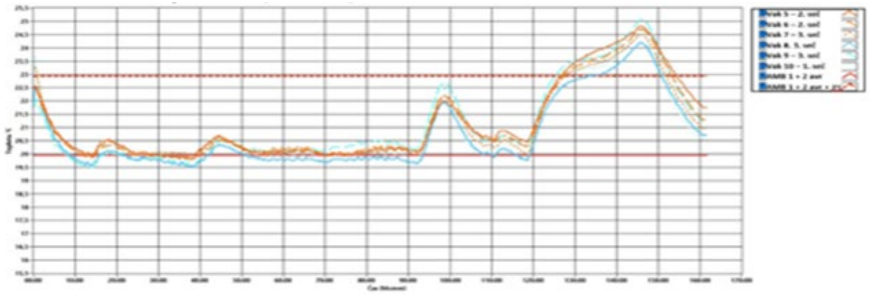


Fig. 1: Aerobic stability of packed lucerne silages

CONCLUSION

This study evaluated the effect of different dry matter contents during ensiling lucerne using PE bag technology on the final quality and aerobic stability. It is important to note that the dry matter content in some bags showed great variability. A microbial chemical preservative was used during preservation in all studies. With increasing dry matter content, a decrease in the production of fermentable acids, including LA, was found. No butyric acid production was, and the degree of proteolysis was low. DOM was lower than 66%, and the NEL concentration was also low (4.30 MJ/kg DM). Operational alfalfa silages did not show increased susceptibility to aerobic spoilage with high dry matter content. The findings indicate that the addition of the silage additive used limited the development of yeast and fungi and prevented unwanted heating.

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FOAM FOR COVERING SILAGE: OPPORTUNITIES AND LIMITATIONS

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ABSTRACT

Conventional covering of bunker silos relies on plastic films to maintain anaerobic conditions during ensiling. This method is labour-intensive and generates substantial plastic waste. A foam-based sealing method developed at TFZ research centrum in Germany has been proposed as an environmentally friendly alternative. The foam is biodegradable, feed-safe, and potentially applicable both immediately after silo filling and during feed-out. This study evaluated the foam's functional performance under laboratory condition, focusing on sealing ability, required thickness, fermentation outcomes and aerobic stability. Laboratory glass silos and simulated feed-out tubes were used. Results show that foam thickness strongly affects mould exclusion, which reduced fermentation efficiency and resulted in higher losses compared with air-tight controls. During feed-out simulation trail, foam reduced aerobic spoilage and weight losses. Study suffered from practical limitations in form of missing application device. Proper application appears critical, especially regarding uniform thickness. Further technological development is required before practical implementation.

Keywords: silage sealing, foam covering, aerobic deterioration, fermentation quality

INTRODUCTION

A successful ensiling process is associated with low losses, as lactic acid bacteria efficiently convert substrates into fermentation products, while other undesirable microorganisms are less efficient and therefore cause greater fermentation losses (McDonald *et al*., 1991). Studies have shown that silage losses in bunker silos can be high, reaching up to 26% of dry matter (Spörndly and Nylund, 2016). These high losses are largely due to handling during filling and feed-out (Muck and Holmes, 2000). Today, plastic films are used to cover silage and thereby maintain anaerobic conditions. The conventional method of covering the silo with plastic films is labor-intensive and involves potential safety risks during covering and uncovering. In addition, recycling and disposal of the film after use is laborious and problematic from an environmental perspective. In Germany, the Technologie- und Förderzentrum institute (TFZ) has developed a spray-on foam composed of natural oils, fibres, alginate, and natural rubber. Once applied, the foam hardens into an elastic surface that adheres to the silage mass and can be removed mixed with the feed. The concept appears promising, but its effectiveness, required thickness, and suitability under Nordic conditions remain unclear. This study aimed to evaluate the potential and limitations of the foam method for bunker-silo sealing and feed-out protection.

MATERIAL AND METHODS

The foam consists of two main fractions that are mixed together immediately upon application. Each component consists of several raw materials that are mixed separately. The foam consisted of rapeseed oil, sodium alginate, cellulose fiber, calcium sulfite in one fraction, and natural rubber, water, sodium benzoate, sorbic acid, glycerol in second fraction. The foam is intended to be spray-applied to the surface of the silo. Unfortunately, a lack of the necessary equipment meant that the foam in this experiment was applied using a pouring method. Grass–legume herbage (third cut) was wilted to 35% DM and chopped to 3 cm length was used. Two types of experiments were designed, both without silage additive application. In the storage trial three foam thicknesses (9, 17, 21 mm) were compared with a control sealed with airlock only. For this purpose, 1.7 L laboratory silos were used, and forage was packet at density of 167 kg DM/m^3. In the feed-out simulation trial, two PVC tubes (11 cm × 1.8 m) were filled with wilted forage at density of 168 kg DM/m^3. All silos were stored at 3–21 °C for 180 days. After the storage, silages were analyses for chemical and microbial composition. Tube silos were cut by 15 cm weekly to simulate slow feed out at 20 °C. One column was foam covered after each removal: the other remained uncovered. Weight losses, temperature, mold, yeast and chemical composition were monitored in removed silage fractions.

RESULTS AND DISCUSSION

Results from storage trail are presented in Table 1. Overall fermentation quality of silages was poor across all treatments, mainly due to low water-soluble carbohydrate content and high buffer capacity of the forage. All silages showed high pH and low lactic acid production, indicating dominance of heterofermentative lactic acid bacteria. Mould growth declined with increasing foam thickness, and at ~17 mm mould was fully excluded. This requirement differs from TFZ recommendations (5–8 mm), potentially due to poorer application quality from manual pouring. However, yeast populations remained high in all foam treatments. Silage losses were greater in foam-covered silos compared with the control. Some losses were likely caused by evaporation of water from the foam, which contains ~20% moisture.

Results from feed-out simulation trial howed that foam covering during feed-out reduced aerobic spoilage substantially (Table 2). The foam-covered tube exhibited lower temperature increases, lower aerobic microbial growth and markedly reduced weight losses compared with the uncovered control. Differences in temperature were exibited from third week of exposure. These results show promise for foam as a feed-out barrier, especially during prolonged removal intervals.

CONCLUSION

Foam as a silage cover shows potential, particularly for protecting exposed surfaces during feed-out where it reduced aerobic losses. However, without a proper equipment foam did not achieve fermentation quality comparable to air-tight control. Proper application appears critical, especially regarding uniform thickness. Before foam can serve as a practical or economic alternative to plastic film, improved application technology, cost reduction and further optimisation are required.

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Tab. 1: Chemical and microbial composition of silage in the foam-thickness test (n=3). Control = lid with fermentation lock; Foam 1 = 0.9 cm; Foam 2 = 1.7 cm; Foam 3 = 2.1 cm

Analyses	Unit	Treatment				LSD*	P-value
		Control	Foam 1	Foam 2	Foam 3		
DM	%	34.7	34.9	35.1	34.6		
pH		5.2	5.3	5.2	5.2	0.18	0.2
NH3-N	% total N	8.0c	8.4[b]	8.9[a]	9.1[a]	0.32	0.001
Lacic acid	% DM	1.9[b]	2.2[b]	2.4[b]	3.8[a]	1.07	0.02
Acetic acid	% DM	0.4[a]	0.2[b]	0.3[b]	0.3[b]	0.06	0.004
Propion. acid	% DM	0.6[b]	0.5[a]	0.6[b]	0.7[b]	0.10	0.02
Butyric acid	% DM	<0.05	<0.05	<0.05	<0.05	0.11	0.3
Ethanol	% DM	0.8[a]	0.4c	0.5[b]	0.6[b]	0.08	0.001
2.3-[b]utandiol	% DM	0.6	0.6	0.5	0.6	0.16	0.3
Yeast	log cfu/g	1.7[a]	4.2c	4.2c	3.9[b]	0.22	0.001
Moulds	log cfu/g	1.7[b]	4.3[a]	2.7[b]	1.7[b]	1.36	0.01
Weight losses	% DM	4.7[b]	16.4[a]	15.4[a]	15.3[a]	6.87	0.001

Means in the same column with different uppercase letters differed (P <0.05). *Least signifiant difference.

Tab. 2: Silage quality during feed-out simulation test (n=1)

Treatment	Exposure time	DM (%)	pH	Weight losses	Moulds	Yeasts
				% DM	log cfu/g	
Foam	1st week	34.5	4.9	0.3	<1.7	5.8
Control		35.8	5.0	2.1	<1.7	5.8
Foam	2nd week	34.6	5.0	0.9	<1.7	6.3
Control		36.2	5.1	4.0	<1.7	6.2
Foam	3rd week	34.4	5.0	1.2	3.5	6.2
Control		35.1	5.2	6.2	5.4	7.0
Foam	4th week	34.5	5.0	1.5	4.2	5.9
Control		32.5	8.1	9.1	8.0	8.0
Foam	5th week	34.5	5.0	1.7	5.2	7.0
Control		34.4	5.6	11.4	6.0	7.2

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TRADE-OFF BETWEEN FERMENTATION QUALITY AND AEROBIC STABILITY OF ALFALFA SILAGE

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ABSTRACT

Alfalfa (*Medicago sativa* L.) is difficult to ensile due to high crude protein content (192 g/kg DM) and associated buffering capacity. A 4x2 factorial experiment (51-day continuous temperature monitoring) tested four preservation treatments (untreated control, enzymatic inoculant with hydrolytic enzymes, control inoculant without enzymes, formic acid) at 20 degrees C and 30 degrees C in three replicates. The enzymatic inoculant produced the most pronounced thermal response (+0.76 degrees C at 30 degrees C; Cohen's d = 0.240) and the optimal fermentation profile (LA:VFA ratio 3.46 at 30 degrees C; VFA 2.50% DM), but exhibited the lowest aerobic stability at elevated temperature (31 h vs. 93 h for formic acid). Formic acid demonstrated minimal and temperature-independent dry matter losses (0.32-0.54%) with excellent aerobic stability across both temperature regimes. Significant treatment x temperature interactions were identified for VFA ($F = 794.32$; $p < 0.001$) and DM losses ($F = 50.57$; $p < 0.001$). The study reveals a fundamental trade-off between fermentation quality and aerobic stability with practical implications for seasonal optimization of alfalfa silage conservation.

Keywords: alfalfa, silage, enzymatic inoculant, formic acid

INTRODUCTION

Ensiling of alfalfa (*Medicago sativa* L.) represents an important forage preservation technology, yet the combination of high buffering capacity (high crude protein) and low water-soluble carbohydrates makes it one of the most challenging forages to ensile. Biological additives containing lactic acid bacteria (LAB) and hydrolytic enzymes are widely used to optimize fermentation, but their temperature-dependent performance has not been systematically studied. Continuous temperature monitoring provides real-time information on silage metabolic activity and offers a novel methodological approach for comparative additive evaluation. The objective of this study was to evaluate the effects of biological and chemical preservation strategies on fermentation quality, DM losses, and aerobic stability of alfalfa silage at two contrasting storage temperatures.

MATERIALS AND METHODS

Chopped alfalfa (*M. sativa* L.) harvested at the vegetative stage (DM 359.5 g/kg FM; CP 191.9; ADF 317.2; NDF 399; ash 113 g/kg DM) was ensiled in vacuum LDPE bags (~500 g FM). Four treatments were applied: untreated control (CON) with distilled water; enzymatic inoculant (ENZ) containing *Pediococcus pentosaceus* + *Lactiplantibacillus plantarum* with beta-glucanase and xylanase (1 g/t); control inoculant (INO) with *L. plantarum* + *Levilactobacillus brevis* without enzymes (1 g/t); and formic acid (FOR, 85%, 4 L/t FM). A completely randomized 4×2 factorial design was used with two storage temperatures (20 +/– 1.5 degrees C and 30 +/– 2.1 degrees C for 4 weeks, then reduced to 20 degrees C) in three replicates (n = 24 units). Temperature was recorded by DS1922L sensors every 3 h for 51 days (13 May – 2 July 2025). After fermentation, aerobic stability was assessed over 7 days at 1-hour intervals following Honig's method (time to +2 degrees C above ambient). Fermentation quality (lactic acid, VFA) was determined by isotachophoresis (IONOSEP 2001). Data were analyzed by two-way ANOVA with Tukey HSD post-hoc test (Statistica v.13; alpha = 0.05).

RESULTS AND DISCUSSION

Two-way ANOVA (Table 1) revealed significant main effects and interactions for all fermentation parameters ($p < 0.05$). The treatment x temperature interaction was particularly strong for VFA ($F = 794.32$) and DM losses ($F = 50.57$), confirming that additive performance is highly temperature-dependent.

Fermentation profile, aerobic stability and DM losses are summarised in Table 2. All treatments produced well-fermented silage with pH 4.15–4.52 and no detectable butyric acid, confirming effective clostridial inhibition across all preservation methods and temperatures. Lactic acid content was not significantly different between treatments ($F = 26.29$; interaction $p = 0.17$), indicating that all strategies supported adequate lactic fermentation.

The enzymatic inoculant achieved the highest LA:VFA ratio (3.46) at 30 degrees C, indicating optimal homofermentative dominance with minimum undesirable by-products in the liquid phase. Paradoxically, ENZ 30 degrees C also showed the worst aerobic stability (31 h) and the highest DM losses (1.68%), compared with 93 h and 0.54% for formic acid at 30 degrees C. This fundamental trade-off arises because the high lactic acid concentration produced by efficient homofermentation provides an ideal substrate for lactate-assimilating yeasts upon aerobic exposure; aerobic stability was negatively correlated with lactic acid content ($r = -0.67$; $p < 0.01$).

The enzymatic inoculant produced the most pronounced thermal signature (+0.76 degrees C above CON at 30 degrees C; Cohen's d = 0.240), reflecting hydrolytic enzyme activity liberating fermentable substrates from cell walls, which fuels increased microbial metabolic heat. At 20 degrees C the thermal effect was smaller (+0.18 degrees C; Cohen's d = 0.136), demonstrating the temperature-dependence of enzymatic kinetics. Formic acid showed minimal temperature deviation from control (non-significant at 30 degrees C; Cohen's d = 0.032), consistent with its non-biological acidification mechanism.

Tab. 1: Two-way ANOVA: F-values for key fermentation parameters

Source of variation	df	pH	VFA	DM losses
Treatment (T)	3	1245.4*	2285.6*	109.7*
Temperature (°C)	1	58.33*	81.00*	38.45*
T × °C interaction	3	102.6*	794.3*	50.57*

* $p < 0.05$; NS = not significant; VFA = volatile fatty acids; DM = dry matter.

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Tab. 2: Fermentation profile, DM losses and aerobic stability of alfalfa silage

Treatment	pH	LA (% DM)	VFA (% DM)	LA/VFA	DM losses (%)	Aerobic stability (h)
CON 20 °C	4.150^{a}	9.40^{a}	3.42^{c}	2.75^{b}	0.43^{c}	57
CON 30 °C	4.160^{a}	9.10^{a}	3.33^{b}	2.73^{b}	1.62^{d}	76
ENZ 20 °C	4.190^{b}	8.83^{a}	2.94^{e}	3.00^{bc}	0.61^{c}	>168
ENZ 30 °C	4.245^{c}	8.66^{a}	2.50^{g}	3.46^{a}	1.68^{d}	31
INO 20 °C	4.375^{d}	7.61^{a}	3.00^{f}	2.54^{b}	0.20^{a}	>168
INO 30 °C	4.310^{e}	7.86^{a}	3.53^{a}	2.22^{c}	0.88^{c}	40
FOR 20g°C	4.515^{f}	6.95^{a}	2.56^{h}	2.71^{b}	0.32^{ab}	93
FOR 30 °C	4.400^{d}	7.12^{a}	2.82^{d}	2.52^{bc}	0.54^{bc}	76
Pooled SEM	*0.003*	*0.024*	*0.010*	*0.002*	*0.090*	—

Means with the same superscript letter within columns do not differ ($p > 0.05$, Tukey HSD). Shaded rows highlight the key trade-off. CON = control; ENZ = enzymatic inoculant; INO = inoculant without enzymes; FOR = formic acid; LA = lactic acid; VFA = volatile fatty acids (acetic + propionic); SEM = standard error of mean; aerobic stability = time to +2 degrees C above ambient

Formic acid demonstrated the smallest temperature sensitivity among all treatments: DM losses increased by only 71% from 20 to 30 degrees C (0.32–0.54%), compared with 337% for INO and 174% for ENZ. Aerobic stability for formic acid was 76–93 h (classified as excellent by Honig's system) at both temperatures, outperforming all biological treatments and supporting its reconsideration as a reliable preservative for warm climates or slow feed-out systems.

The control inoculant (INO) achieved the lowest DM losses at 20 degrees C (0.20%) with perfect aerobic stability (>168 h), demonstrating that heterofermentative LAB without enzymatic supplementation can be highly efficient in temperate storage conditions. At 30 degrees C, INO showed the highest total VFA (3.53% DM) and acetic acid (3.21% DM), likely reflecting greater heterofermentative activity under warmer conditions.

CONCLUSIONS

Continuous temperature monitoring identified characteristic thermal signatures of individual preservation strategies, with the enzymatic inoculant showing the highest metabolic response (+0.76 degrees C at 30 degrees C). A fundamental trade-off was identified between fermentation quality and aerobic stability: enzymatic inoculant achieved the best fermentation profile (LA:VFA 3.46) but the poorest aerobic stability (31 h) at 30 degrees C, while formic acid provided excellent stability (76–93 h) and minimal, temperature-independent DM losses (0.32–0.54%). For immediate consumption in moderate climates (below 25 degrees C), enzymatic inoculants with rapid feed-out are recommended.

For long-term storage or warm climates (above 25 degrees C), formic acid offers the most economically reliable performance. Preservation strategy must be selected in combination with anticipated storage temperature and feed-out management.

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TRADE-OFF BETWEEN FERMENTATION QUALITY AND AEROBIC STABILITY OF CORN SILAGE

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ABSTRACT

Aerobic stability (AS) temperature exerts a highly significant effect on corn silage quality, with practical consequences for feed management under warming climate conditions. A complete 3×2×2 factorial experiment tested three treatments (untreated control, Formasil® Maize Propio containing *L. buchneri* and propionic acid, homofermentative *L. plantarum* inoculant) at two fermentation temperatures (20 °C, 30 °C) followed by aerobic exposure at 20 °C and 30 °C. Twenty-three samples were analyzed after 40-day fermentation. AS at 20 °C preserved 12.7% higher NEL (6.85 vs. 6.08 MJ/kg DM; $p < 0.001$), 62.5% more lactic acid (3.60 vs. 2.21% DM; $p < 0.001$), and lower pH (4.03 vs. 4.32; $p = 0.002$). Formasil produced the highest acetic acid content (avg. 1.35% DM; $p = 0.024$) and achieved best performance at 20 °C AS (NEL 7.02 MJ/kg DM, DM losses 2.96%). *L. plantarum* showed the highest LA/VFA ratio (4.61; $p = 0.006$), indicating homofermentative pathway dominance. No butyric acid was detected in any sample. A fundamental trade-off exists between fermentation pathway optimization and temperature-dependent aerobic stability, requiring climate-specific additive selection strategies.

Keywords: lactic acid bacteria, heterofermentative inoculant, biological additives, climate warming, dry matter losses

INTRODUCTION

Corn silage is a key component of dairy cow diets worldwide, and its quality is increasingly jeopardized by warming temperatures associated with climate change. Aerobic deterioration, driven primarily by yeasts and moulds, degrades nutritional value, generates heat, and may produce mycotoxins. Temperature during aerobic exposure is a critical but often underestimated determinant of silage quality at feed-out. Biological silage additives differ in their mechanisms: homofermentative bacteria (e.g. *L. plantarum*) rapidly lower pH via lactic acid production, while heterofermentative strains (e.g. *L. buchneri*) additionally produce acetic acid and 1,2-propanediol, improving aerobic stability. The objective of this study was to quantify the effect of two contrasting AS temperatures (20 °C vs. 30 °C) on corn silage quality and to evaluate the temperature-dependent efficacy of two biological additives.

MATERIAL AND METHODS

A complete 3×2×2 factorial experiment was designed. Treatment factor: (K) untreated control, (F) Formasil® Maize Propio (*L. buchneri* + propionic acid, 1 g/t FM), (P) *L. plantarum* homofermentative inoculant (1 g/t FM). Fermentation temperature: 20 °C or 30 °C for 40 days. Aerobic stability (AS) temperature: 20 °C or 30 °C post-fermentation exposure. Corn forage from a single harvest (initial DM ~ 35%) was ensiled in LDPE vacuum bags (n = 23 bags total; some combinations with one replicate for technical reasons). Fermentation quality (lactic acid, acetic acid, butyric acid) was determined by HPLC (VVS Verměřovice s.r.o., protocols 128625–130925). Nutritional parameters (DM, crude protein, starch, NDF, ADF, NEL) were determined by NIR and standard gravimetric methods. DM losses were calculated as (35.0 − measured DM) / 35.0 × 100. VFA was defined as the sum of acetic and butyric acids (butyric acid = 0 in all samples). Statistical analysis used one-way ANOVA for treatment effects and Student's t-test for temperature effects (Statistica v.13; $\alpha = 0.05$).

RESULTS AND DISCUSSION (PRELIMINARY)

All 23 samples were free of butyric acid, confirming successful anaerobic fermentation without clostridial activity across all treatment combinations and temperatures.

AS temperature exerted highly significant effects on energy value, lactic acid content, and pH (Table 1). Silage exposed at 20 °C retained 12.7% higher NEL (6.85 vs. 6.08 MJ/kg DM) and 62.5% more lactic acid (3.60 vs. 2.21% DM) than at 30 °C. The higher AS temperature promotes accelerated yeast activity, leading to oxidation of lactic acid to CO_2 and H_2O, pH reversion, and energy losses — a process Rooke and Hatfield (2003) termed ‚fermentation reversal.' The lower pH at 20 °C AS (4.03 vs. 4.32; $p = 0.002$) reflects better preservation of the fermentation profile. DM losses and LA/VFA ratio were not significantly affected by AS temperature ($p > 0.05$), likely due to high variability and the limited number of replicates.

Biological additives significantly influenced acetic acid content ($F = 4.515$; $p = 0.024$) and the LA/VFA ratio ($F = 6.731$; $p = 0.006$). Formasil produced the highest acetic acid (mean 1.35% DM across both AS temperatures), corresponding to the heterofermentative activity of *L. buchneri* converting lactic acid to acetic acid and 1,2-propanediol. This mechanism is the basis of its antimicrobial effect against yeasts. At 20 °C AS, Formasil achieved the best performance with the highest NEL (7.02 MJ/kg DM) and lowest DM losses (2.96%), suggesting that acetic

Tab. 1: Effect of AS temperature on corn silage quality parameters. Values are mean ± SD (n = 12 for 20 °C AS; n = 11 for 30 °C AS). Significance: *** $p < 0.001$; ** $p < 0.01$; ns = not significant. VFA = acetic acid (butyric = 0); LA/VFA = lactic acid to volatile fatty acids ratio

Parameter	AS 20 °C	AS 30 °C	Diff.	Diff. (%)	p-value
NEL (MJ/kg DM)	6.85 ± 0.33	6.08 ± 0.51	+0.77	+12.7	0.0003***
pH	4.03 ± 0.18	4.32 ± 0.19	−0.28	−6.6	0.002**
Lactic acid (% DM)	3.60 ± 0.54	2.21 ± 0.71	+1.38	+62.5	0.0000***
Acetic acid (% DM)	1.22 ± 0.60	0.67 ± 0.18	+0.54	+81.0	0.008**
LA/VFA	3.48 ± 1.22	3.47 ± 1.22	+0.01	+0.4	0.977 ns
DM losses (%)	4.57 ± 3.71	2.38 ± 13.87	+2.18	+91.7	0.604 ns

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Tab. 2: Corn silage quality parameters by treatment and AS temperature. Shaded rows = Formasil. * = significantly highest within column ($p < 0.05$, Tukey HSD). K = control; LA = lactic acid; VFA = volatile fatty acids (acetic acid); homo = homofermentative; hetero = heterofermentative

Treatment	AS temp.	DM losses (%)	NEL (MJ/kg DM)	pH	Acetic acid (% DM)	LA/VFA	LA/VFA classification
Control	20 °C	7.44	6.53	4.05	0.95	3.83	homo
Control	30 °C	2.48	5.92	4.20	0.73	3.61	homo
Formasil	**20 °C**	**2.96**	**7.02**	**3.85**	**1.99***	**2.01**	**hetero**
Formasil	30 °C	0.14	5.97	4.38	0.67	3.02	mixed
Plantarum	20 °C	3.31	7.00	4.20	0.71	4.61*	homo
Plantarum	30 °C	5.24	6.45	4.40	0.60	3.88	homo

acid inhibition of yeasts is most effective at lower temperatures where yeast proliferation rates are inherently slower. At 30 °C AS, treatment differences narrowed, indicating that elevated temperature overwhelms the antimicrobial capacity of achievable acetic acid concentrations.

L. plantarum achieved the highest LA/VFA ratio (4.61 at 20 °C AS), confirming efficient homofermentative pathway dominance with minimal undesirable fermentation by-products. However, this superior fermentation profile was accompanied by reduced aerobic protection at 30 °C AS (DM losses 5.24%), because high lactic acid concentrations provide an ideal substrate for lactate-assimilating yeasts. This represents the same fundamental trade-off between fermentation quality and aerobic stability previously identified in alfalfa silage (Muck *et al*., 2018), here confirmed for corn silage under a temperature-dependent context. Each degree Celsius increase above 25 °C can approximately double the rate of aerobic deterioration (Rooke and Hatfield, 2003), which magnifies these treatment differences considerably under warm conditions.

CONCLUSION

AS temperature has a greater and more consistent effect on corn silage quality than additive type: reducing AS temperature from 30 °C to 20 °C preserved 12.7% more energy (NEL) and 62.5% more lactic acid. Formasil (*L. buchneri* + propionic acid) achieved optimal performance at 20 °C AS (NEL 7.02 MJ/kg DM, DM losses 2.96%) with the highest acetic acid content (1.35% DM), supporting aerobic stability. *L. plantarum* provided the best homofermentative profile (LA/VFA 4.61) but offered less protection at elevated AS temperatures. These results identify a fundamental trade-off: additives optimizing fermentation quality may be less protective under warm feed-out conditions, necessitating climate-specific additive selection. In warm regions or during summer feeding (>25 °C), Formasil or equivalent heterofermentative/combination additives are recommended, combined with rapid feed-out management (<24 h air exposure). In cool regions (<20 °C), either additive type performs well. All treatments effectively prevented clostridial fermentation, confirming that both biological strategies reliably protect corn silage during anaerobic storage.

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YEAST SUPPRESSION AND ENHANCED AEROBIC STABILITY IN GRASS SILAGE INOCULATED WITH *LENTILACTOBACILLUS BUCHNERI* SUBSP. *SILAGEI* NCIMB 30673

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ABSTRACT

Heterofermentative lactic acid bacteria (LAB) inoculants are used to enhance silage aerobic stability by increasing acetic acid production, which inhibits yeasts and moulds. *Lentilactobacillus buchneri* is widely used in such products, but strain-specific effects and the speed at which they provide effective protection remain poorly defined. This study evaluated a novel candidate inoculant, *L. buchneri* subsp. *silagei* strain NCIMB 30673, for its ability to improve the early aerobic stability of grass silage. Grass (33% DM) was chopped and ensiled in triplicate 1.8 L silos for 30 or 92 days, either left untreated or inoculated with *L. buchneri silagei* at 1.0×10^5 CFU/g fresh forage. At opening, silages were analysed for microbial counts, pH, fermentation products, and aerobic stability. Data were analysed by linear models with the interaction between time and treatment as a fixed effects, followed by emmeans pairwise comparisons at each time point ($p \leq 0.05$). At 30 days, inoculation with the *L. buchneri* significantly reduced yeast populations compared with the untreated control (3.16 $\log_{10}$ CFU/g reduction, $p < 0.001$). The *L. buchneri* substantially increased acetic acid and 1,2propanediol concentrations relative to untreated silage (day 30, both $p < 0.001$), indicating rapid activity within as little as 30 days. Aerobic stability measurements suggested that inoculated silage opened at day 30 remained stable for >11 days, whereas untreated silage deteriorated earlier (3 days). By day 92, both treatments had similar yeast and acetic acid levels, likely due to naturally occurring heterofermentative bacteria present in the untreated silage. The new *L. buchneri* subsp. *silagei* strain NCIMB 30673 rapidly enhanced acetic acid and 1,2-propanediol production in grass silage and substantially reduced yeast populations by day 30 post-ensiling, indicating strong antifungal activity. These findings suggest that NCIMB 30673 is a promising candidate silage inoculant to improve aerobic stability and control spoilage yeasts, even after short ensiling periods.

Keywords: *Lentilactobacillus buchneri* subsp. *silagei*, silage inoculant, aerobic stability, yeast suppression, acetic acid production

INTRODUCTION

Epiphytic yeasts and moulds can trigger aerobic spoilage in silage, especially when silage is poorly compacted, sealed or managed (Holmes and Bolsen, 2009; Borreani *et al.*, 2007; Pahlow and Muck, 2009). This can result in dry matter loss, elevated mycotoxins, and reduced dry matter intake (Tabacco *et al.*, 2011; Richard *et al.*, 2009; Whitlock *et al.*, 2000), potentially reducing animal performance (Kung *et al.*, 1998). To combat this, it is common to inoculate forage with a heterofermentative LAB, such as *Lentilactobacillus buchneri*, which converts lactic acid to acetic acid and 1,2 propanediol during secondary fermentation (Oude Elferink *et al.*, 2001). Acetic acid exhibits strong antifungal properties as undissociated acid molecules can penetrate yeast cells to help reduce undesired growth (Danner *et al.*, 2003). Although *L. buchneri* inoculation improves aerobic stability, strain-specific effects and the speed of protection remain poorly characterised. Advances in bacterial taxonomy have enabled more precise classification within the *L. buchneri* group. Recent work by Tanizawa *et al.* (2020) identified two distinct subspecies: *L. buchneri* subsp. *buchneri* and the newly described *L. buchneri* subsp. *silagei*. In this study a novel candidate inoculant, Lentilactobacillus buchneri subsp. silagei strain NCIMB 30673, isolated from aerobically stable whole-crop barley silage, was evaluated for its ability to enhance grass silage fermentation quality and aerobic stability.

MATERIAL AND METHODS

Rye Grass was harvested and chopped in the field (South Wales, UK) and transferred to the laboratory for further processing. Forage dry matter (DM) was 33% at point of ensiling. Forage was either left untreated (CON) or inoculated with *L. buchneri* subsp. silagei NCIMB 30673 (LB) at 1.0×10^5 CFU/g fresh forage. Triplicate 1.8 L glass jars per treatment were packed at 139 kg DM/m^3 and stored for 30 or 92 days at 20 ± 1 °C. After opening, silages were thoroughly mixed, and representative subsamples were taken for analysis of dry matter (DM; oven-dried at 60 °C to constant weight), microbial counts, pH, and fermentation characteristics. Volatile fatty acids and alcohols were quantified using high-performance liquid chromatography (HPLC; Prominence, Shimadzu) with a refractive index detector. Following silo opening, aerobic stability was evaluated at 20 ± 1 °C by monitoring internal temperature every 15 minutes with Squirrel data loggers (Grant). Stability was defined as the time required for the silage temperature to increase 2 °C above ambient. Statistical analyses were conducted in R (version 4.3.1). Data were analysed by two-way linear models with time and treatment as fixed effects and their interaction, followed by estimated marginal means (emmeans) contrasts to compare treatments within each time point, with significance defined at $p < 0.05$. For statistical analysis, microbial counts below the limit of detection (LOD; 2.60 $\log_{10}$ CFU/g) were reported as 0.5 x LOD (i.e. 2.30 $\log_{10}$ CFU/g).

RESULTS AND DISCUSSION (PRELIMINARY)

Time × treatment interactions were significant (linear model, all $p < 0.01$) for LAB and yeast counts, acetic acid, 1,2-propanediol, and aerobic stability indicating that the treatment effect for these parameters was not uniform over time. No significant interactions occurred for pH ($p = 0.49$), dry matter ($p = 0.058$), or mould counts ($p = 0.2$). However, pH ($p < 0.01$) and dry matter ($p < 0.001$) were significantly affected by treatment alone.

At Day 30, LB treatment had significantly increased LAB counts (pairwise emmeans, $p = 0.015$; Table 1), reduced yeast counts by 3.16 $\log_{10}$ CFU/g ($p < 0.001$; Table 1) and increased concentrations of both acetic acid and 1,2-propanediol (both $p < 0.001$; Table 1) relative to CON. We observed a significant improvement aerobic stability in LB silage at day 30 post-ensiling (11.9 vs 3.0 days, $p < 0.001$; Table 1). We also observed elevated pH ($p = 0.030$; Table 1) and reduced dry matter ($p < 0.001$; Table 1) in LB treated silage at day 30. Together, these results point to a secondary lactic acid fermentation from LB, as reported for other *L. buchneri* strains previously (Oude

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Tab. 1: Fermentation characteristics means ± SE and pairwise emmeans p values. CON = Untreated silage, LB = *L. buchneri* subsp. silagei strain NCIMB 30673 treated silage

Parameter	Day 30			Day 92		
	CON	LB	p value	CON	LB	*p* value
Sample number	3	3	–	5	5	–
LAB (log^{10} CFU/g)	9.37 ± 0.04	9.56 ± 0.05	0.015	9.24 ± 0.05	8.62 ± 0.02	<0.001
Yeasts (log^{10}CFU/g)	5.86 ± 0.05	2.7 ± 0.20	<0.001	2.30 ± 0.00	2.30 ± 0.00	1.000
Moulds (log^{10} CFU/g)	2.30 ± 0.00	2.30 ± 0.00	0.109	2.30 ± 0.00	2.30 ± 0.00	1.000
Acetic acid (g/kg DM)	25.9 ± 0.73	51.29 ± 0.58	<0.001	55.21 ± 4.11	53.96 ± 2.67	0.762
1,2-propanediol (g/kg DM)	6.31 ± 0.27	36.45 ± 0.12	<0.001	38.64 ± 3.33	49.21 ± 2.69	0.011
Aerobic stability (Days)	2.97 ± 0.09	11.9 ± 0.00	<0.001	16.9 ± 0.00	16.8 ± 0.08	0.315
pH	3.96 ± 0.00	4.27 ± 0.22	0.030	4.14 ± 0.01	4.33 ± 0.01	0.068
Dry matter (g/kg)	309.9 ± 0.45	299.61 ± 0.69	<0.001	297.33 ± 1.35	291.85 ± 0.97	0.002

Elferink *et al*., 2001). The rapid generation of acetic acid at concentrations sufficient to improve aerobic stability, as seen in LB silage, would be highly desirable in a silage inoculant.

The magnitude of the treatment effect had decreased significantly for most parameters by day 92, where no significant differences were observed for yeasts, moulds, acetic acid, aerobic stability or pH (Table 1). However, significant treatment effects were observed after 92 days for LAB counts, which were higher in CON ($p < 0.001$; Table 1); dry matter, which remained lower in LB silage ($p = 0.002$; Table 1); and 1,2-propanediol, which was higher in LB silage ($p = 0.011$; Table 1). The elevated levels of 1,2-propanediol and acetic acid present in CON silage after 92 days may point to the activity of a slow acting epiphytic *L. buchneri* like bacteria reaching impactful levels over the longer time period.

CONCLUSION

In this study, the rapid generation of acetic acid in LB silage resulted in significantly lower yeast populations and higher aerobic stability after only 30 days ensiling. These results mark *L. buchneri* subsp. silagei strain NCIMB 30673 as a promising candidate silage inoculant.

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IMPACT OF ENSILING TIMING AND INOCULANT USE ON FERMENTATION DYNAMICS AND AEROBIC STABILITY IN WHOLE-PLANT MAIZE SILAGE

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ABSTRACT

Atypical ensiling conditions, such as delayed ensiling and inadequate sealing, frequently occur in practice and may impair silage quality. This study evaluated the effects of a 24 h delay before ensiling, sealing conditions during the delay, and inoculation with *Lentilactobacillus buchneri* and *Lactococcus lactis* on maize silage quality. Whole-plant maize (*Zea mays* L.; 36% dry matter) was ensiled for 60 days in 3.0 L laboratory containers with or without a commercial inoculant. Forage was ensiled immediately or after a 24 h delay, during which it was either covered or uncovered. Treatments were replicated five times, Delayed ensiling significantly impaired fermentation, increased dry matter losses, and reduced aerobic stability and hygienic quality ($p < 0.01$). Inoculation reduced temperature rise during the delay (by 8.1–8.2 °C; $p < 0.01$), lowered silage pH, decreased ammonia-N, alcohols, and butyric acid, and increased lactic and acetic acid concentrations ($p < 0.01$). Inoculated silages also showed lower dry matter losses during fermentation and aerobic exposure and reduced yeast and mold counts ($p < 0.01$). Minimizing aerobic exposure and using LAB-based inoculants can mitigate the negative effects of delayed ensiling.

Keywords: aerobic exposure, ensiling challenge, *Lentilactobacillus buchneri, Lactococcus lactis,* maize silage

INTRODUCTION

During practical silage production, forage may remain exposed to oxygen for extended periods due to long transportation distances from the field or delays in filling and sealing the silo. Such exposure prolongs plant respiration, reduces the availability of soluble carbohydrates, and may negatively affect subsequent fermentation processes (Bruning *et al*., 2018; Michel *et al*., 2017). The presence of oxygen in the ensiled mass promotes the activity of aerobic microorganisms, leading to aerobic deterioration, nutrient losses, and reduced silage quality. During this phase, undesirable microorganisms metabolize plant substrates, which delays the development of lactic acid bacteria (LAB), increases pH, and favors the growth of yeasts and molds. As a result, the nutritional value of silage declines and the risk of spoilage increases, particularly during feed-out (Weiss *et al*., 2022). The use of silage additives, particularly LAB-based inoculants, has been widely recommended to enhance fermentation, suppress undesirable microorganisms, and improve aerobic stability (Puntillo *et al*., 2020). Therefore, the objective of this study was to evaluate the effects of overnight interrupted ensiling and the application of a LAB-based inoculant on fermentation characteristics, dry matter recovery, and aerobic stability of whole-plant maize silage.

MATERIAL AND METHODS

After harvesting, maize forage was transported to the laboratory for silage preparation. The chopped forage was divided into two equal portions (200 kg each), thoroughly mixed, and sampled to determine chemical composition and yeast and mold counts. One portion remained untreated, while the other was treated with an inoculant containing lactic acid bacteria (LAB) strains in a 50:50 ratio of *Lentilactobacillus buchneri* DSM 22501 and *Lactococcus lactis* DSM 11037 (Novonesis, Horsholm, Denmark). The inoculant was applied at 1.5×10^5 colony-forming units (cfu) per gram of fresh crop. A portion of each treatment was immediately ensiled into 3 L mini-silos, while another portion (30 kg per treatment) was placed in 80 L plastic boxes (70 × 40 × 28 cm). The forage was compressed to fill 2/3 (60 L) of each box, achieving a density of 174 ± 3 kg/m^3, and left for 24 h either uncovered or covered to simulate farm conditions where ensiling may be delayed overnight. After 24 h, the forages were sampled for chemical composition and yeast and mold counts before being placed into mini-silos. The experiment followed a completely randomized design with six treatments and five replicates: T0C-non-inoculated, promptly ensiled; T1C-non-inoculated, 24 h delay, uncovered; T2C-non-inoculated, 24 h delay, covered; T3I-inoculated, promptly ensiled; T4I-inoculated, 24 h delay, uncovered; and T5I-inoculated, 24 h delay, covered. For mini-silo preparation, the forage was weighed to achieve a density of 200 kg DM/m^3 and packed into 3 L glass jars. After 60 days fermentation, mini-silos were opened, weighed to determine fresh weight and DM losses, and sampled for chemical and microbiological analyses. Aerobic stability was evaluated during a 7 and 17-day exposure period by monitoring temperature, yeast and mold counts, pH, chemical changes, and fresh weight loss. and data were analyzed using SAS MIXED procedures. Significance was declared at $p < 0.01$.

RESULTS AND DISCUSSION

The data in Table 1 show that inoculated silages, whether promptly ensiled (T3I) or ensiled after a 24 h delay either uncovered (T4I) or covered (T5I), had 2.1–2.5% higher ($p < 0.01$) DMc compared with non-inoculated silages (T0C–T2C). Inoculated silages also showed 34.1–36.2% lower ($p < 0.01$) dry matter (DM) losses regardless of ensiling time or covering conditions. Non-inoculated silages ensiled after a 24 h delay (T1C and T2C) had significantly lower ($p < 0.01$) crude protein and starch contents than the other treatments. The LAB count in inoculated silages (T3I–T5I) was 1.1–1.4 $\log_{10}$ cfu g^{-1} higher ($p < 0.01$) than in non-inoculated silages (T0C–T2C). At the same time, inoculated silages contained 1.0–1.2 $\log_{10}$ CFU/g fewer yeasts and 1.2–1.9 $\log_{10}$ CFU/g fewer molds ($p < 0.01$). Weiss *et al.* (2022) indicated that factors such as increased availability of WSC, air ingress and higher temperature during fermentation promote yeast growth in silage. The lowest yeast counts among inoculated silages were observed in promptly ensiled T3I, while T0C had the lowest yeast and mold counts among non-inoculated treatments. Significant differences were observed in fermentation characteristics. Inoculated silages (T3I–T5I) had lower pH values (by 0.08–0.16 units; $p < 0.01$) than non-inoculated silages. The highest pH ($p < 0.01$) occurred in the delayed, uncovered treatment (T1C). Inoculation significantly reduced ($p < 0.01$) NH_3-N, alcohols, and butyric acid concentrations. The lowest (by 26.8–39.6%; $p < 0.01$) ammonia-N values were observed in T3I and T5I. Ethanol production

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was highest (by 68–96 %; $p < 0.01$) in T1C silages. Inoculation also increased ($p < 0.01$) lactic acid, acetic acid, and 1,2-propanediol concentrations, with the largest increases in lactic acid and 1,2-propanediol in T3I and acetic acid in T4I. After 7 days of aerobic exposure, promptly ensiled inoculated silage (T3I) retained 8.1% more DM and 9.2% more starch ($p < 0.01$) than delayed, uncovered non-inoculated silage (T1C). Inoculated silages showed 58.7% to 2.4-fold lower DM losses and 2.6–3.0% higher metabolizable energy than non-inoculated silages ($p < 0.01$). Aerobic stability was also significantly improved, with inoculated silages remaining stable for 165.6–250.8 h and reaching peak temperatures 3.0–4.6 °C lower ($p < 0.01$). During 7 and 17 days of aerobic exposure, yeast and mold counts in inoculated silages remained significantly lower than in non-inoculated treatments.

Tab. 1: Chemical and microbial characteristics and aerobic stability of maize silage ensiled into mini-silo under the and fermented for 60 days (data given in g/kg DMc unless stated otherwise)

Item	T0C	T1C	T2C	T3I	T4I	T5I	SE	p-Value
DMc, g/kg	348.8^{bc}	345.8 ^{c}	347.4^{c}	356.2^{a}	354.5^{ab}	355.2^{a}	3.085	<0.01
Crude protein	92.7^{ab}	88.4 ^{c}	89.9^{bc}	93.8^{a}	90.6^{abc}	91.6^{abc}	1.147	0.01
WSC	13.6	11.9	11.5	13.8	16.2	12.7	2.189	0.335
Lactic acid	46.59^{bc}	38.36^{d}	39.59^{cd}	60.54^{a}	49.78^{b}	51.6^{b}	2.320	<0.01
Acetic acid	16.6^{c}	18.38^{c}	17.42^{c}	20.63^{b}	24.70^{a}	22.60^{b}	0.642	<0.01
Ethanol	26.09^{b}	30.87^{a}	27.50^{b}	15.52^{c}	17.93^{c}	17.60^{c}	0.952	<0.01
1,2-propanediol	1.93^{d}	2.35^{d}	2.11^{d}	8.89^{a}	4.37^{c}	6.84^{b}	0.209	<0.01
Ammonia-N, g/ kg N	52.68^{b}	63.01^{a}	55.37^{ab}	38.03^{c}	40.35^{bc}	38.56^{c}	3.208	<0.01
pH	3.88^{bc}	3.98^{a}	3.90^{b}	3.80^{d}	3.82^{cd}	3.80^{d}	0.020	<0.01
DM loss	61.63^{a}	69.61^{a}	64.54^{a}	40.04^{b}	44.39^{b}	42.54^{b}	2.863	<0.01
Yeast, log_{10} CFU/g	2.38^{b}	3.30^{a}	3.10^{a}	1.40^{c}	2.07^{b}	1.99^{b}	0.143	<0.01
Mold, log_{10} CFU/g	2.23^{b}	3.18^{a}	3.03^{a}	1.00^{c}	1.28^{c}	1.16^{c}	0.096	<0.01
Aerobic stability test								
Aerobic stability, h	144.0^{c}	146.4^{c}	171.6^{c}	394.8^{a}	312.0^{b}	363.6^{ab}	26.020	<0.01
Highest temp., °C	27.72^{a}	28.14^{a}	27.34^{a}	23.12^{c}	25.12^{b}	23.44^{bc}	0.634	<0.01
Yeast, log_{10} CFU/g	8.21^{a}	9.00^{a}	8.22^{a}	2.64^{b}	2.88^{b}	2.78^{b}	0.357	<0.01
Mold, log_{10} CFU/g	6.47^{a}	6.18^{a}	6.42^{a}	1.95^{b}	2.11^{b}	2.03^{b}	0.306	<0.01
pH	5.22^{a}	5.36^{a}	5.28^{a}	3.90^{c}	4.14^{b}	4.10^{b}	0.052	<0.01
DM loss	148.93^{a}	153.22^{a}	133.58^{a}	62.94^{c}	89.09^{b}	78.92^{b}	10.575	<0.01

T0C-non-inoculated promptly ensiled; T1C-non-inoculated with ensiling pause for 24 h uncovered; T2C-non-inoculated with ensiling pause for 24 h covered-up; T3I-inoculated and promptly ensiled; T4I-inoculated with ensiling pause for 24 h uncovered; T5I-inoculated with ensiling pause for 24 h covered-up; DMc-dry matter corrected for the loss of volatiles; WSC-water soluble carbohydrates; letters a,b,c in rows descri^{b}e significant differences between treatments at $p < 0.01$.

CONCLUSION

The use of a microbial inoculant significantly improved maize silage fermentation, nutrient preservation, and aerobic stability despite delayed ensiling. Inoculation increased LAB populations enhanced lactic and acetic acid production, and reduced pH, ammonia-N, alcohols, and undesirable microorganisms. As a result, inoculated silages exhibited lower dry matter losses, higher metabolizable energy, and improved stability during aerobic exposure, effectively mitigating the negative effects of delayed ensiling

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EFFECTS OF ADDING SUGARCANE MOLASSES TO GRASSES FROM STADIUM TURFS AND AGRI-PARKS ON SILAGE FERMENTATION

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ABSTRACT

Batches of freshly mowed grasses for 1. *Cenchrus clandestnus* mixed with perennial ryegrass, collected from a stadium in Limpopo Province, 2. *Panicum maximum*, collected from agri-park in Kwa Zulu Natal (KZN), 3. *Cenchrus clandestnus*, collected from a stadium in the Gauteng Province (GP), 4. *Dactylis glumerata*, collected from an agri-park in Mpumalanga Province (MP), and 5. a mixture of *Digitaria eriantha* and *Cenchrus clandestnus*, collected from a stadium in the North West (NW) province of South Africa. The mowed grass was mixed with sugarcane molasses at 5% and ensiled in 210 L drums for 60 days. Mixing the grass with sugarcane molasses improved (P<0.05) the sugar content of the grass but the terminal pH of the silage was high in most of the silages except for the mixture of *Cenchrus clandestnus* and perennial ryegrass that had lower (P < 0.05) that resulted in increased lactic acid production. Silage can be produced from grass that are collected from stadiums with the aid of sugarcane molasses, but care should be taken not to ensile grass that was treated with pesticides

Keywords: drums, fermentation, forages, mowing, stadium, provinces

INTRODUCTION

Shortage of animal feeds is a challenge faced by emerging livestock farmers in South Africa, especially during the drought months. These farmers lost so many livestock during the drought of 2015-2017 in South Africa. Fortunately, there is a lot of grasses from stadium turfs and agri-parks that are mowed and dumped. These grasses contain nutrients such as protein, fibre, etc which can benefit livestock production if used for animal feeding. In order to have enough forage for animal feeding during droughts, mowed grasses can be ensiled to curb feed shortages. However, most of the mowed grasses are legumous in nature and may be high in buffering capacity and contain less sugar to promote silage making (McDonald *et al*., 2010). With the low content of water-soluble carbohydrate (WSC) from these mowed grasses, the addition of sugarcane molasses has been recommended (Zhang *et al*., 2013). Therefore, the present study evaluated the ensiling potential of mowed grasses from stadiums and agri-parks in some parts of South Africa.

MATERIAL AND METHODS

Batches of mowed grasses for 1. *Cenchrus clandestnus* mixed with perennial *yegrass*, collected from a stadium in Limpopo Province, 2. *Panicum maximum*, collected from agri-park in Kwa Zulu Natal (KZN), 3. *Cenchrus clandestnus*, collected from a stadium in the Gauteng Province (GP), 4. *Dactylis glumerata*, collected from an agri-park in Mpumalanga Province (MP), and 5. a mixture of *Digitaria eriantha* and *Cenchrus clandestnus*, collected from a stadium in the North West (NW) province of South Africa. The grasses were mowed with a grass mower (Red rhino ride-on lawn mower, model RLM-TTM86H) in a 2week intervals, which were repeated 3 times before the collection of samples. The mowed grass was then collected immediately after the third cut (i.e. 3rd interval) of the season for ensiling. This allowed us to collect samples of <5 mm length for good compaction. Sugarcane molasses was added to the grass at 5% of the total biomass collected. The mixture of grass with sugar cane molasses were ensiled in 210 L drums that were lined with plastic bags. Samples of the freshly mixtures

Tab. 1: Fermentation characteristics of grasses from stadium and agri-parks ensiled for 60 days (n =3)

Grass type	Province					SEM	P
	Limpopo	KZN	MP	GP	NW		
	Cenchrus clandestnus + perennial ryegrass	*Panicum maximum*	*Dactylis glumerata*	*Cenchrus clandestnus*	*Digitaria eriantha* mixed with *Cenchrus clandestnus*		
Pre-ensiled forages (day 0)							
DM g/kg	331.6^{c}	347.7^{c}	216.2^{d}	512.2^{a}	417.4^{b}	0.964	0.001
WSC g/kg DM	124.17^{a}	50.17^{b}	69.63^{b}	10.54^{d}	82.88^{b}	6.22	0.001
pH	6.295^{bc}	6.353^{ab}	6.425^{a}	6.268^{c}	6.340^{bc}	0.0242	0.004
Fermentation characteristics after 60 days of fermentation							
DM g/kg	456.0	419.1	487.4	526.3	456.2	30.2	1.111
WSC g/kg DM	53.88^{a}	10.17^{b}	12.61^{b}	7.92^{b}	49.71^{a}	3.33	0.001
Lactic acid g/kg DM	116.66^{a}	41.89^{b}	27.26^{b}	33.23^{b}	72.61^{b}	15.15	0.004
pH	3.883^{d}	4.285^{bc}	4.520^{ab}	4.742^{a}	4.197^{c}	0.103	0.001

$^{a-d}$Means with different letters in a row differ significantly ($P < 0.05$)

DM = Dry matter; WSC = water soluble carbohydrates, KZN = Kwa-Zulu Natal; MP = Mpumalanga; GP = Gauteng Province; NW = North West

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were collected prior to ensiling for the analysis of dry matter (DM), WSC and pH. After 60 days of ensiling, the drums were opened, samples collected and analysed for DM, WSC, pH and lactic acid (LA).

RESULTS AND DISCUSSION

According to Table 1, mixing the grasses with sugarcane molasses improved ($P < 0.05$) the sugar content of the forages, consistent with Zhang *et al.* (2013) when sugar cane molasses was added to Urtica cannabina forage at ensiling. Lunden-Pettersson and Lindgren (1990) recommended an amount of 60–70 g WSC/kg DM in a forage to achieve a well-preserved silage. All the grasses of this study had WSC content of more than 60 g/kg DM, sufficient for proper fermentation, except for the grasses from KZN and GP provinces. It is well established that forages from legumes have a high buffering capacity, leading to higher final pH (> 4.00) of the silage (McDonald *et al.*, 2010). This is one of the reasons we experienced higher silage pH in the study except for the silage from the mixture of Cenchrus clandestnus and perennial ryegrass. This silage had higher ($P < 0.05$) content of LA which was supported by the low ($P < 0.05$) residual WSC and pH contents of the silage compared to other silages. The increased LA content from the silage of Cenchrus clandestnus and perennial ryegrass mixtures is consistent to Sucu and Filya (2006). However, the higher ($P < 0.05$) residual WSC from this silage may lead to poor aerobic stability since higher residual WSC in a silage serves as food for aerobic microbes (Weinberg *et al.*, 1993).

CONCLUSION

Silage can be produced from stadiums and agri-parks grasses with the addition of sugarcane molasses at ensiling. The nutritive values of these silage and animal feeding trials should be determined.

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ACCURACY OF THE PREDICTION OF THE FEED VALUE OF UNDRIED GRASS AND MAIZE SILAGES BY A HANDHELD NIRS DEVICE

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ABSTRACT

Mobile NIRS devices are increasingly available for various applications. Their use for prompt feed ration adaptation seems attractive. Thus one model was tested for its reliability in terms of the prediction of feed value parameters of moist silages. Dry matter content turned out to be the best predictable parameter.

Keywords: predictive quality, on-farm tool, near-infrared spectroscopy, forages

INTRODUCTION

Efficient livestock production depends on diets that accurately matches animal nutrient requirements. However, the nutritional composition of forages and compound feeds can vary considerably due to differences in harvest timing, storage conditions, processing and environmental factors. Conventional wet-chemistry analyses provide reliable compositional data but are labour-intensive and time-consuming, limiting their suitability for rapid on-farm decision making (Williams & Norris, 2001). Near-infrared spectroscopy (NIRS) offers a rapid, non-destructive and cost-effective alternative for estimating key feed quality parameters such as dry matter (DM), crude protein (CP) and fibre fractions (Reeves, 1999; Cozzolino & Labandera, 2002). Recent technological advances have enabled the miniaturisation of NIR sensors into portable handheld devices that allow farmers and nutritionists to assess forage quality directly on the farm (Cherney *et al.*, 2021; Zhu *et al.*, 2022). However, handheld instruments generally operate over a narrower spectral range and measure fresh, untreated samples, which can reduce prediction accuracy compared with laboratory spectrometers (Fernández Pierna *et al.*, 2022; Rukundo *et al.*, 2021). Factors such as moisture content, particle size and sample heterogeneity may therefore influence calibration performance and model reliability. Despite the growing availability of portable NIR sensors, independent evaluations under practical farm conditions remain limited. The present study evaluates the performance of the PAL One (Trinamix GmbH, Ludwigshafen, Germany) handheld NIRS device for estimating feed value parameters in grass silage, maize silage and cereal grain samples and compares device predictions with laboratory NIRS analyses.

MATERIAL AND METHODS

Two sampling campaigns were conducted in August and September 2024. In total, 195 samples were analysed, including approximately 100 grass silages, 40 maize silages and 55 cereal grain samples (wheat, barley, rye). The first campaign was carried out at the LKS Lichtenwalde laboratory, where freshly delivered samples from farmers were scanned using a PAL One handheld NIRS device immediately after registration and sample splitting. The second campaign was conducted at the Lehr- und Versuchsgut Köllitsch in September 2024, where samples were collected directly from silos and feed stocks. All samples were analysed in their fresh state without drying or grinding.

For each sample, one portion was sent to the LKS laboratory for reference analysis, while the remaining portion was analysed with the PAL One device. The handheld spectrometer operates in reflectance mode within approximately 1400–2400 nm and transfers spectral data to a cloud-based application where chemometric models generate predicted nutrient values. To reduce sample heterogeneity, the sensor was moved across several areas of the sample surface during scanning and replicate spectra were averaged.

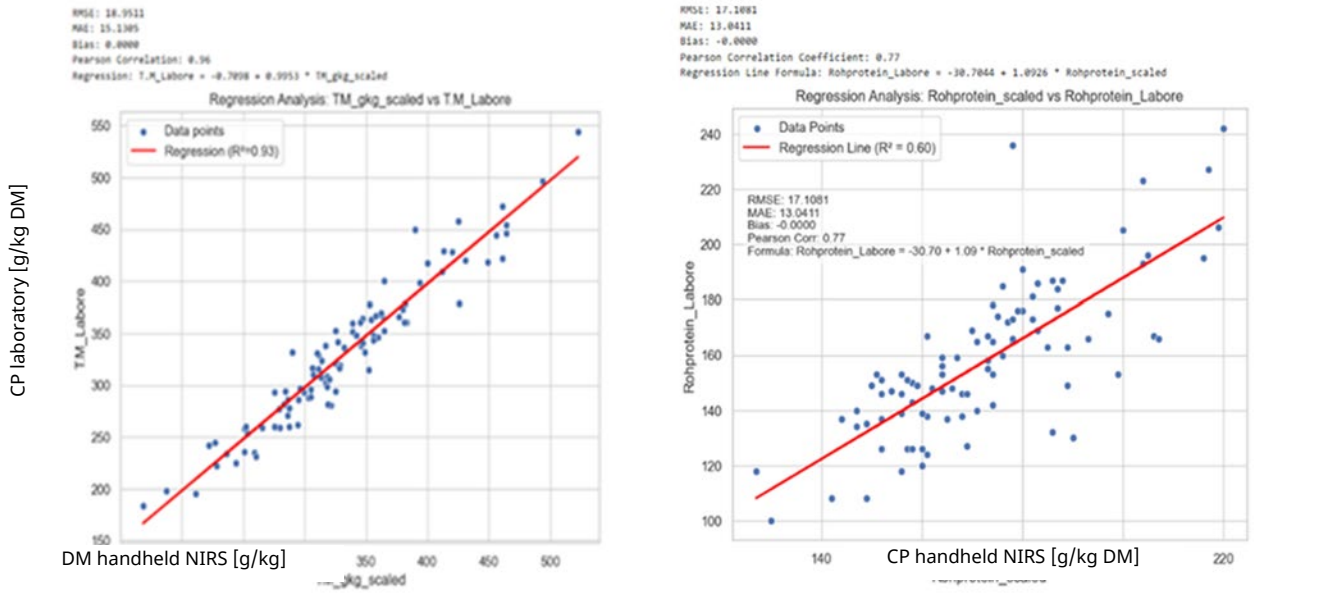


Fig. 1: Regression between handheld NIRS predictions for DM (left) and CP (right) in grass silage

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Tab. 1: Regression performance between handheld NIRS predictions and laboratory reference values

Feed type	Metric	DM	CP	ADF	NDF	ELOS	Sugar	Ash
Grass silage	R^2	0.93	0.60	0.41	0.29	0.28	0.16	0.07
Grass silage	RMSE	18.9	17.31	20.88	36.64	48.58	39.85	17.87
Maize silage	R^2	0.52	0.00	0.04	—	0.01	—	0.00
Maize silage	RMSE	20.88	5.67	19.19	—	26.16	—	2.46
Cereal grain	R^2	0.00	0.16	—	—	—	—	0.30
Cereal grain	RMSE	12.77	15.42	—	—	—	—	1.62

Reference analyses

Laboratory reference analyses were conducted at the LKS mbH feed testing laboratory using a FOSS XDS Rapid Content Analyzer (400–2500 nm). Prior to analysis, samples were dried at ≤ 60 °C to constant mass and milled to 1 mm particle size. DM was determined in two stages with a final drying at 103 °C for 4 h in a forced air drying oven. Spectral measurements were processed using ISIScan software, and nutrient concentrations were predicted using WinISI calibrations based on VDLUFA wet-chemistry reference methods (VDLUFA 1976). Additional measurements were performed at a second laboratory (Betriebsgesellschaft für Umwelt und Landwirtschaft) using a FOSS NIRS 5000 spectrometer (1100–2500 nm) equipped with an autocup sampler. Both laboratories operate according to ISO 12099:2018 standards for NIRS calibration and validation.

Statistical analysis

All statistical analyses were conducted in Python using pandas, scikit-learn, and SciPy. Data were first inspected with heat maps and scatterplots, and obvious errors or outliers were removed. For NDF, values reported below the detection limit ("<") were replaced with the stated limit. Descriptive statistics were calculated for DM, CP, NDF, ADF, sugar, ash, and ELOS. Agreement between handheld and laboratory measurements was assessed by linear regression using R^2, RMSE, MAE, and bias. Correlation matrices were used to examine relationships among variables. Depending on normality, paired t-tests or Wilcoxon signed-rank tests were applied to compare handheld and laboratory results. Bland–Altman plots were used to evaluate agreement, while residual plots, Q–Q plots, and histograms were used to check regression assumptions.

RESULTS AND DISCUSSION

Regression results varied substantially depending on feed type and parameter (Table 1). The strongest relationship between handheld NIRS predictions and laboratory reference values was observed for dry matter in grass silage ($R^2 = 0.93$; RMSE = 18.9 g/kg), while crude protein showed a moderate correlation ($R^2 = 0.60$; RMSE = 17.31 g/kg) (Fig. 1).

In contrast, other parameters in grass silage, such as ADF, NDF, ELOS, sugar and ash, showed considerably lower predictive accuracy. For maize silage, only dry matter exhibited moderate agreement between device and laboratory measurements ($R^2 = 0.52$), whereas the remaining parameters showed weak relationships. In cereal grain, prediction performance was generally low, with the highest correlation observed for ash ($R^2 = 0.30$) (Table 1).

CONCLUSION

The handheld NIRS device showed high predictive accuracy for dry matter in grass silage and moderate performance for crude protein. The results indicate that the predictive performance strongly depends on the analysed parameter. Handheld NIRS technology can therefore serve as a rapid screening tool for limited on-farm forage evaluation, while laboratory analysis remains necessary for precise nutrient determination.

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CORRELATION BETWEEN CRUDE NUTRIENTS IN FRESH GRASS AND PROPYLENE GLYCOL CONCENTRATIONS IN GRASS SILAGE TREATED WITH A MIXTURE OF HOMO- AND HETEROFERMENTATIVE LACTIC ACID BACTERIA

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ABSTRACT

Various strains of homo- and heterofermentative lactic acid bacteria (LAB) are used as silage inoculants. Homofermentative LAB convert water-soluble carbohydrates (WSC) into lactic acid, while heterofermentative LAB convert WSC into lactic and acetic acid. Some LAB strains (e.g. *Lentilactobacillus buchneri*) also produce alcohols such as propylene glycol (PG). In dairy cattle, PG is frequently used as glucogenic feed additive, to prevent ketosis at the onset of lactation. Even though high concentrations of PG in silages have been reported (up to 40 g/kg DM; Driehuis *et al*., 2001; Nishino *et al*., 2003), relatively little is known about compositional traits of grass, contributing to high PG yields. Therefore, this study was designed to identify compositional traits of grass that were associated with particularly high yields of PG in silages, inoculated with a mixture of homo- (*Lactiplantibacillus plantarum*, *Lacticaseibacillus rhamnosus*) and heterofermentative (*Lentilactobacillus buchneri*) LAB.

Keywords: bacterial inoculant, grass silage, *Lentilactobacillus buchneri*, propylene glycol

INTRODUCTION

Based on their metabolic pathways and fermentation products, lactic acid bacteria (LAB) can be categorised as obligate homofermentative, facultative heterofermentative, or obligate heterofermentative. *Lentilactobacillus buchneri* is an obligate heterofermentative bacterium and frequently part of the native epiphytic microbiome (Daeschel *et al*., 1987). Inoculation with *L. buchneri* can result in increased levels of acetic acid and 1,2-propanediol (= propylene glycol [PG]) in silages (Driehuis *et al*., 2001), since L. buchneri can convert one mole of lactic acid into approximately 0.5 mol acetic acid, 0.5 mol PG and trace amounts of ethanol, under strictly anaerobic conditions (Oude-Elferink *et al*., 2001). The main reason for using *L. buchneri* as a silage inoculant is its ability to improve aerobic stability by suppressing the growth of moulds and yeasts, primarily through the production of acetic acid (Muck, 1996; Driehuis *et al*., 1999; Ranjit *et al*., 2002). However, relatively little attention has been given to the fact that *L. buchneri* also produces propionic acid (PG), although PG is frequently used as a glucogenic feed supplement to prevent ketosis in postpartum dairy cows. Therefore, silage containing sufficiently high concentrations of PG could potentially mitigate the effects of negative energy balance at the onset of lactation. The present study was designed due to the lack of information concerning the maximum achievable PG concentrations. The aim was to examine the extent to which a silage inoculant containing a specially-selected *L. buchneri* strain would lead to the accumulation of PG in grass silage.

MATERIAL AND METHODS

This study consisted of 18 laboratory-scale ensiling experiments. Seven first, two second, six third, and three fourth cuts were ensiled over the course of two harvest seasons. The grass was inoculated with a mixture of homofermentative (*Lactiplantibacillus plantarum* and *Lacticaseibacillus rhamnosus*) and heterofermentative LAB (*Lentilactobacillus buchneri*) at a concentration of 3.0×10^5 CFU/g of fresh grass. Quadruplicate mini silos (1.5 L) were ensiled for each harvest time point (18 time points × 4 mini silos = 72 mini silos in total). Silos were stored for 90 d at 20 °C. The freshly cut grass was analyzed for ash, dry matter (DM), organic matter (OM), crude protein (CP), neutral detergent fiber (aNDFom), acid detergent fiber (ADFom), fat, sugar, and enzyme-soluble organic matter (ESOM). To determine ensiling quality, pH and fermentation end products, including PG concentrations were measured. To explore any associations between the chemical composition of the grass samples and the concentration of PG in the silages, a correlation analysis (PROC CORR) between the DM, OM, CP, aNDFom, ADFom, fat, sugar and ESOM content of the grass before ensiling and the PG concentration (mean of quadruplicate samples per harvest date) in the silages was conducted (SAS, version 9.4). As measure of correlation between the respective parameters, the Pearson correlation coefficient (r) is reported.

RESULTS AND DISCUSSION

The DM and OM content of the grass before ensiling, as well as the aNDFom, ADFom, fat, sugar and ESOM, were all correlated with PG ($P < 0.05$; Table below). Increases in OM ($r = 0.553$), fat ($r = 0.789$), sugar ($r = 0.648$) and ESOM ($r = 0.612$) content of the

Tab. 1: Correlations between the dry matter, organic matter, crude protein, aNDFom, ADFom, fat, sugar, and enzyme-soluble organic matter (ESOM) content of grass before ensiling (d 0) and the propylene glycol (PG) content of grass silage (d 90), after inoculation with a mixture of homo-and heterofermentative lactic acid bacteria (*L. plantarum*, *L. rhamnosus*, *L. buchneri* at 3×10^5 CFU/g fresh matter; n = 18).

Category	r	P-value
Dry matter	−0.705	0.001
Organic matter	0.553	0.017
Crude protein	0.632	0.005
aNDFom[1]	−0.545	0.019
ADFom[2]	−0.571	0.013
Fat	0.789	<0.001
Sugar	0.648	0.004
Enzyme-soluble organic matter	0.612	0.007

[1]Neutral detergent fiber assayed with a heat stable amylase and expressed without residual ash

[2]Acid detergent fiber expressed without residual ash

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grass before ensiling were all associated with an increase in PG in the mature silages. As expected, the WSC content was closely associated with high PG yields. Sugars are the primary energy source of LAB (McDonald *et al*., 1991). The positive correlation between CP and high PG yields can be explained by the higher buffer capacity of grass with a higher CP content. As a result, the critical pH at which LAB growth is negatively affected is reached later in the fermentation process. This allows *L. buchneri* to convert more lactic acid into acetic acid and PG over a longer period of time. Increases in DM (r= –0.705), aNDFom (r= –0.545) and ADFom (r= –0.571) of the grass before ensiling were associated with a reduction in PG in the silages. Increases in DM, aNDFom and ADFom are characteristics of older, more mature grass lacking fermentable carbohydrates for LAB.

CONCLUSION

High concentrations of PG in grass silage treated with a mixture of *L. plantarum*, *L. rhamnosus* and *L. buchneri* are closely associated with low DM and high sugar contents of the grass at the time of ensiling. High concentrations of PG in grass silage (19.9 g/kg DM) can be achieved, if the right bacterial inoculants are used and the composition of the forage is suitable. Feeding experiments involving dairy cows experiencing a negative energy balance are needed to determine whether the intake of PG through specifically inoculated grass silage can be sufficiently high, to effectively reduce the risk of ketosis.

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DATABASE FOR SILAGE ADDITIVES SELECTION

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ABSTRACT

Selection of optimal silage additives is critical for conservation quality. With 144 products on the Czech market, VÚŽV has maintained additive records since 1989 and developed an AI-assisted decision support system. The 2026 database includes 81 biological (56.3%), 55 chemical (38.2%), and 8 combined additives (5.5%), a net reduction of 8 products vs. 2025. Biological additives divide into homofermentative (34 products, 42.0%), heterofermentative (7, 8.6%), and combined (40, 49.4%). Multi-strain formulations dominate (49.4% contain ≥ 3 cultures; max. 6 strains) and 20% include enzymes (xylanase up to 716,810 nkat/g). Chemical additives are led by propionic acid (58%) and formic acid (47%); only 27% declare precise composition and 5% reach active ingredient concentrations >90%. The web application *Selection of Silage Additives* (www.vuzv.cz) provides AI-based personalised recommendations, application guidance, and ROI quantification via an Economic Efficiency Calculator. The database is updated within project *AISilageFeeding* (NAZV QL26010434).

Keywords: lactic acid bacteria, decision support system, artificial intelligence, economic efficiency

INTRODUCTION

The Czech silage additive market has grown from 7 products in 1989 to a current portfolio of 144. With products across three major categories and 24 distribution companies, evidence-based selection is increasingly supported by digital tools. This contribution reports the 2026 database analysis and the AI-assisted web platform developed to guide practical additive selection.

MATERIAL AND METHODS

Data were collected annually in January from 24 manufacturers and distributors via standardised questionnaires covering bacterial strain identity (updated per EU Regulation 2024/1196), CFU/g, enzyme activities, chemical composition, packaging, application rates, and target forage types. Statistical analyses used Python 3.x (pandas). The web application is hosted at www.vuzv.cz; the Economic Efficiency Calculator estimates ROI from DM loss savings, improved feed intake, and milk yield response.

RESULTS AND DISCUSSION

Market overview (Table 1):

Tab. 1: 2026 silage additive market structure in the Czech Republic (n = 144)

Category	N products	Market share (%)	vs. 2025	Key trend
Biological	81	56.3	−1	Multi-strain, enzymes
Chemical	55	38.2	+1	Propionic acid dominant
Combined	8	5.5	0	Stable niche
TOTAL	144	100	−8	Portfolio consolidation

The 2026 database registered 144 products—a net decrease of 8 vs. 2025, driven primarily by VOLAC Agro Best discontinuing four bacterial products (Agros Clamp, Agros XL40, Agros Hi-Dri, Agros Maize), Joachim Behrens Scheessel removing Harvest International Plus, and EW Nutrition discontinuing three Biotronic chemical products. Biological additives remain the largest category (56.3%), followed by chemical (38.2%) and combined (5.5%).

Biological additives:

The most notable shift vs. 2025 is the growth of combined biological products (40 products, 49.4% of biological)—pairing homofermentative LAB with *Lentilactobacillus buchneri* or *Levilactobacillus brevis* in a 2:1–3:1 ratio—at the expense of pure heterofermentative products (7 products, 8.6%). Strain complexity continues to increase: 49.4% of biological products contain ≥3 cultures (max. 6 strains). Enzymatic enrichment (20% of products) features xylanase up to 716,810 nkat/g and β-glucanase up to 333,400 nkat/g. A key taxonomic update: the strain previously classified as *Lactobacillus kefiri* DSM 19455 (EW Nutrition) was reclassified as *Lentilactobacillus buchneri* DSM 19455 per EU Regulation 2024/1196.

Chemical additives:

Propionic acid (58% of chemical products) now surpasses formic acid (47%) in prevalence, reflecting growing emphasis on aerobic stability for maize and high-DM silages. Formic acid retains broad utility for rapid pH reduction. Composition transparency remains limited: only 27% of chemical products declare quantitative composition, and only 3 products (5%) achieve active ingredient concentrations >90%—an important consideration for economic efficiency comparisons.

AI-assisted selection platform:

The web application *Selection of Silage Additives* at www.vuzv.cz uses AI filtering to match user-specified conditions (forage type, DM%, harvest method, storage duration) to the full 144-product database, generating ranked recommendations with application guidance, packaging availability, and distributor contacts. The Economic Efficiency Calculator estimates ROI across DM loss reduction (1–3%), improved voluntary intake (0.2–0.5 kg DM/cow/day), and production response (0.3–0.8 kg milk/cow/day). Both tools are embedded in project *AISilageFeeding* (NAZV QL26010434), which will further develop predictive AI models for fermentation outcome prediction and personalised additive recommendations.

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Tab. 2: Biological and chemical additive key data, 2026 (n = 81 biological; n = 55 chemical)

Parameter / Category	N	Share (%)	Notes
BIOLOGICAL ADDITIVES (n = 81)			
Homofermentative	34	42.0	*Lacticaseibacillus*, *Lactiplantibacillus*, Pediococcus, Enterococcus—rapid pH drop
Heterofermentative	7	8.6	*Lentilactobacillus*, *Levilactobacillus*—acetic acid, propandiol, aerobic, stability
Combined (homo + hetero)	40	49.4	2:1–3:1 homo:hetero ratio; fermentation quality + aerobic stability
With enzymes	16	20.0	Xylanase up to 716,810 nkat/g; β-glucanase up to 333,400 nkat/g
≥ 3 bacterial strains	40	49.4	1 strain: 18.5%; 2 strains: 32.1%; ≥ 3 strains: 49.4%; max. 6 strains
CHEMICAL ADDITIVES (n = 55)			
Propionic acid	32	58	Most prevalent; antifungal; aerobic stability; surpasses formic acid in 2026
Formic acid	26	47	Rapid pH drop; broad antimicrobial activity across forage types
Declared composition	15	27	Only 3 products (5%) reach active ingredient concentration > 90%

CONCLUSION

The 2026 Czech silage additive database documents a consolidating market of 144 products. Key findings: (1) combined biological products now dominate biological additives (49.4%); (2) heterofermentative single-type products declined to 8.6%; (3) multi-strain formulations prevail (49.4% with ≥3 cultures; max. 6); (4) propionic acid (58%) surpasses formic acid (47%) among chemical additives; (5) composition transparency remains limited (27% declare quantitative composition; only 5% reach >90% active ingredient). The AI-assisted selection platform at www.vuzv.cz provides evidence-based decision support, complemented by an Economic Efficiency Calculator. Integration into project *AISilageFeeding* (QL26010434) will enable closed-loop optimisation of silage management from field to feed-out.

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EVALUATION OF NIRS-ESTIMATION FOR ENZYME-SOLUBLE ORGANIC MATTER (ESOM) IN GRASS SILAGES COMPARED TO THE CELLULASE REFERENCE METHOD

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ABSTRACT

Enzyme-soluble organic matter (ESOM) of 167 Austrian grass silages was measured using the cellulase reference method, also predicted by near-infrared spectroscopy (NIRS) and a general linear model (GLM) based on chemical parameters. NIRS calibration showed much higher accuracy (R^2 = 0.90) than GLM (R^2=0.57). Results indicate that NIRS is a reliable and cost-effective method for estimating ESOM in grass silage.

Keywords: near-infrared spectroscopy, in vitro digestibility, cellulase-method, calibration

INTRODUCTION

In the German feeding recommendations for dairy cows (GfE 2023), the cellulase method developed by deBoever *et al.* (1986) is a recognized standard method in which the enzyme soluble organic matter (ESOM) is measured in order to calculate the digestibility of organic matter (OMD). According to Shenk and Westerhaus (1994), near-infrared spectroscopy (NIRS) provides a rapid, cost-effective analysis of nutrient composition with good estimation accuracy, which can be applied to the increasing demand for efficiency in livestock feeding. Using a subset of grass silage samples, deBoever *et al.* (1996) showed that NIRS equations based on partial least squares analysis (PLS) were better at explaining the variance in OMD than multiple linear regression using nutrient parameters. At AREC Raumberg-Gumpenstein, 167 grass silage samples from farms with extensive to intensive grassland management were analysed for ESOM in order to evaluate the results of estimation from NIRS and General Linear Model (GLM) in terms of variance and estimation error.

MATERIAL AND METHODS

The grass silage samples ([total] n = 167 from: [AREC 2017-2025] n = 128 and [Austrian silage project 2024 (ASP) described by Resch (2025)] n = 39) were oven-dried at 50°C for two days and then ground to 1 mm particle size. Dry matter content was determined using a BRABENDER apparatus (type MT-C) for 10 g of sample at 130 °C for 30 minutes. The enzymatic analysis-procedure is described in VDLUFA-Method 6.6.1 (VDLUFA, 2018). For the preparation of samples with cellulase (Onozuka R-10, 1 U/mg) and pepsin (≥2.0 FIP-U/mg), we use special glass frits (GTWA497) with a filter base of porosity 2 and GL32 screw cap. ESOM data presented below refer to dry matter. The NIRS measurements were performed using a Unity spectrometer (type SpectraStarTM XT) with a wavelength range of 880 to 2,600 nanometer (nm) and a resolution of 1 nm. The cuvette diameter was 86 mm. The chemometric evaluation of the spectroscopy data was performed using ZEISS SL Calibration Wizard software. For NIRS calibration and GLM statistics, 41% of the total samples (AREC: n = 48; ASP: n = 21) with measured ESOM values were used and 59% for validation. NIRS calibration was performed in the same way as in deBoever *et al.* (1996) using PLS statistics. Linear analysis of regression factors was carried out by procedure GLM (Statgraphics Centurion XVII) at confidence level of 95%. The following parameters – contents based on dry matter – are required for GLM calibration and subsequent GLM ESOM-estimation: crude protein (CP), hemicellulose (aNDFom minus ADFom), cellulose (ADFom minus ADL), ADL, crude lipid (CL) and crude ash (CA).

RESULTS AND DISCUSSION

The ESOM contents of the 167 grass silages were normally distributed and ranged from 440 to 817 g/kg DM, with an average value of 609 g/kg DM. In contrast, the mean ESOM value of the calibration subset was 626 g/kg DM and that of the validation subset was 597 g/kg DM (Table 1). With GLM, we achieved an R^2 of 0.7 to 0.77, which is comparable to deBoever *et al.* (1996), who achieved an R^2 of 0.73, using the Weender parameters for prognosis of OMD. The mean value of the GLM estimation results was the same as the ESOM measurement in the calibration set, but there was a slight ESOM underestimation in the validation set. The mean estimation error for GLM was 21.2 and 32.5 g/kg DM, respectively. The difference in PLS calibration for the NIRS estimate was less about the mean ESOM value, but more about the mean estimation error.

GLM formula (1) total sample set (R^2 = 0.70); formula (2) calibration subset (R^2 = 0.77)

$$\text{ESOM} = 1021.04 + 0.147\text{CP} - 0.6634\text{Hemicellulose} - 0.6822\text{Cellulose} - 2.0108\text{ADL} + 1.8964\text{CL} - 1.1498\text{CA} \quad (1)$$

$$\text{ESOM} = 950.969 + 0.191\text{CP} - 0.7336\text{Hemicellulose} - 0.4785\text{Cellulose} - 2.1032\text{ADL} + 3.1286\text{CL} - 1.3052\text{CA} \quad (2)$$

Tab. 1: Descriptive statistics of grass silage samples for calibration and validation with regard to enzyme-soluble organic matter (ESOM) in g/kg DM, as well as statistics on ESOM estimation using NIRS or GLM

	Descriptive Statistics – Wet Chemistry						NIRS – Estimation				GLM – Estimation			
Source of variation	**n**	**$\bar{x}$**	**med**	**SD**	**min**	**max**	**$\bar{x}$**	**$\bar{x}^R$**	**RSD**	**R^2**	**$\bar{x}$**	**$\bar{x}^R$**	**RSD**	**R^2**
Total Sample Set	167	609	603	60.4	440	817	609	12.6	11.2	0.97	609	27.0	33.7	0.70
Calibration Subset	69	626	623	56.4	486	817	627	7.7	7.3	0.92	626	21.2	28.5	0.77
Validation Subset	98	297	587	60.7	440	769	595	16.1	12.1	090	592	32.5	24.0	0.57

n = number of samples, $\bar{x}$ = aritmetic average, med = median, SD = standard deviation, min = minimum, max = maximum, $\bar{x}^R$ = average of residuals (mean estiamation error), RSD = standard deviation of residuals

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In the calibration set, the NIRS estimation error for ESOM was 7.7 g/kg DM, which was three times lower than the GLM estimation error. For validation, the NIRS estimation error for unknown samples rose to 16.1 g/kg DM, but was still half that of the GLM prognosis (Table 1). In the regressive XY plot – observed ESOM vs. predicted ESOM via NIRS – the explained variance (R^2) for NIRS validation was 0.90 and for GLM validation only 0.57.

CONCLUSION

We found that NIRS performed reasonably well in estimating ESOM for a very diverse set of grass silage samples from Austria. This confirmed similar results on NIRS reported by deBoever *et al.* (1996), Nousiainen *et al.* (2004), Gibaud (2007) and Spiekers *et al.* (2011). The accuracy of NIRS predictions significantly exceeds that of GLM predictions based on chemical parameter from nutrient analysis. Therefore, the cost-effective NIRS technology is recommended for ESOM in grass silage. This finding is of great importance for the implementation of the new feeding recommendations for dairy cows according to GfE (2023), because ESOM is a central parameter for the calculation of OMD and metabolisable energy (ME). For grass silage analysing laboratories, the use of NIRS with good calibrations can make a valuable contribution to reducing wet chemical ESOM analysis to a few reference measurements, that are needed to improve NIRS calibration.

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THE EFFECT OF HARVEST DATE OF ALEXANDRIAN CLOVER GROWN AS A CATCH CROP ON THE QUALITATIVE PARAMETERS OF SILAGE

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ABSTRACT

The aim of the work was to assess the quality parameters of silage produced from alexandrian clover (*Trifolium alexandrinum* L.), grown as a catch crop, with respect to the growth phase of the stand. The silages were experimentally created using the VacSy system. Chemical analyses were performed on the silages and the digestibility of nutrients was determined using the in situ method. The content of nitrogenous substances gradually decreased from 210 to 160 g/kg dry matter with the aging of the stand. A tendency towards a slight increase in the NDF content was found in the deflowered stand. From the point of view of nutrient digestibility, there was also a decrease in both the dry matter digestibility and the NDF digestibility in the deflowered stand.

Keywords: clover, silage, yield, chemical composition, digestibility

INTRODUCTION

Alexandrian clover is (*Trifolium alexandrinum* L.) a valuable, high-yielding annual forage legume and well adapted to a wide range of environments for grazing and forage production (Singh *et al*., 1989; Yucel *et al*., 2018). Martiniello *et al.* (1996) found that it is a suitable companion crop for barley, annual ryegrass, triticale and oats. Alexandrian clover is an appropriate species for rhoughage production which yields 12–14 t/ha dry matter (Yucel *et al*., 2016). As a legume, Alexandrian clover can fix more than 200kg N/ha/year (Giambalvo *et al*., 2011). Additionally, unlike other legume species, Alexandrian clover does not cause bloat in ruminants (Simms *et al*., 1991) and therefore can be fed alone. However, the effect of including fresh Alexandrian clover, either singly or as part of a mixed forage sward, in the diet of dairy cows on metabolic processes, MY, and milk quality is poorly documented (Enriquez-Hidalgo *et al*., 2020). Enteric methane emissions of ruminants are reported to increase with increasing proportion of forage in the diet (Archimède et a., 2018). Also roughage and silage quality of alexandrian clover grown as catch crop in Czech republic conditions is not enough check.

The objective of this study was to evaluate the effect of growth phase of Alexandrian clover grown as catch crop, on chemical composition and digestibility of silages.

MATERIAL AND METHODS

Alexandrian clover was grown as a catch crop, after harvest of pea for silage production. Forage of clover was harvested at three growth phases (full flowering, end of flowering, faded), wilted for 24 hours, cut for 20 mm and ensiled. Forage was harvested from three places of growth where dry matter (DM) yield was detected. Experimental silages were made using VacSy system to vacuumed bags (3 bags per harvest place, three places per harvest; amount 600 g per bag). Silages were stored for 8 weeks and then opened and dried at 55 °C for 48 hours. Dried silages were ground through 2 mm sieve (samples for in situ method) and 1 mm sieve (samples for chemical composition). Silages were analysed for ash, crude protein (CP), ether extract (EE), crude fibre (CF), neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL). Ruminal digestibility of DM, OM and NDF was detected by in situ method using Holstein cows and incubation for 24 hours in bags with pore size 51 µm. Results were evaluated with GLM procedure of SAS where growth phase was used as fixed effect and repetition as random effect. Significance of differences were tested by Tukey´s post hoc test.

RESULTS AND DISCUSSION

Table 1 shows the dry matter yields of Alexandrian clover forage in individual stages of maturity, where up to a 3.5-fold increase is evident in the deflowered stand compared to the full flowering stand. In the youngest stand, the stand also failed to wilt to a sufficient level suitable for ensiling. The silage was at its optimum at the second harvest. The content of CP gradually decreased from 210 to 160 g/kg DM as the stand aged, however, compared to commonly grown clovers in the Czech Republic, this range is still relatively narrow. According to the available literature (Broderick *et al*., 2001; Vanhatalo *et al*., 2009) and our experience, for example, in the case of red clover, the range of CP content in silages ranges from 120 to 220 g/kg DM and in alfalfa silages from 110 to 250 g/kg DM. The relatively balanced contents of fiber and its fractions are interesting, where no differences were found among harvests for fiber and lignin, a tendency for a slight increase in the NDF content was found in the third harvest and there was a difference in the ADF content between the first and second harvest. The CF content of our silages corresponds to the data presented by Jabbar *et al.* (2022). There was no difference in the digestibility of dry matter, OH or NDF between the first and second harvest. There was a decrease of approximately five percent in the third harvest. Similar effect of later harvest on DOM was detected also by Vanhatalo *et al.* (2009) for red clover silages.

CONCLUSION

From our findings, we can recommend harvesting rather in the end of flowering growth of the alexandrian clover when grown as a catch crop, especially due to the low yield in very young growth and, conversely, the reduced quality in the growth that has completely faded.

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Tab. 1: Effect of growth phase on yield, chemical composition and ruminal digestibility of alexandrian clover silages

Growth phase	Full flowering	End of flowering	Faded	P
DM yield; t/ha	0.47[c]	1.23[b]	1.66a	<0.01
Chemical composition				
DM; g/kg	210[c]	323[a]	272b	<0.01
OM; g/kg DM	852[b]	885[a]	875a	<0.01
CP; g/kg DM	213[a]	195[b]	160c	<0.01
EE; g/kg DM	22.6[a]	17.2[b]	17.5b	<0.01
CF; g/kg DM	221	204	216	0.288
NDF; g/kg DM	350	344	366	0.073
ADF; g/kg DM	301[a]	261[b]	280ab	<0.01
ADL; g/kg DM	88.8	60.4	70.0	0.121
Ruminal digestibility				
DDM; %	76.2[a]	76.1[a]	71.9b	<0.01
DOM; %	73.4[a]	74.1[a]	69.5b	<0.01
DNDF; %	44.6[a]	43.3[a]	36.9b	<0.01

Means in the same row with different uppercase letters differed (P <0.05).

DM – dry matter; OM – organic matter; CP – crude protein; EE – ether extract; CF – crude fibre; NDF – neutral detergent fibre; ADF – acid detergent fibre; ADL – acid detergent lignin; DDM – digestibility of DM; DOM – digestibility of OM; DNDF – digestibility of NDF.

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THE ROLE OF ARTIFICIAL INTELLIGENCE AND DRONES IN SILAGE AND CATTLE NUTRITION

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ABSTRACT

A newly initiated research project (NAZV QL26010434, 2026–2030) is developing an integrated system combining artificial intelligence (AI) and unmanned aerial vehicles (UAVs) for optimisation across the entire forage production chain – from crop cultivation through ensiling to cattle feeding. The consortium (Institute of Animal Science Prague-Uhříněves, NutriVet Ltd., Brno University of Technology) addresses critical gaps in current precision agriculture by creating a closed-loop data integration platform linking field monitoring, fermentation control, and feed optimisation.

Keywords: unmanned aerial vehicles, precision agriculture, machine learning, IoT sensor networks, forage quality prediction

INTRODUCTION

Precision agriculture has revolutionised crop production through GPS guidance, variable-rate technology, and remote sensing, yet the forage conservation chain – from field to feed-out – has remained largely outside the scope of integrated digital systems. Individual components have been studied separately: UAV-based biomass estimation (Hunt *et al.*, 2019), IoT sensor networks for fermentation monitoring (Nishida *et al.*, 2021), and machine learning for silage quality prediction (Ferraretto *et al.*, 2020, Janoušek *et al.*, 2023). However, no end-to-end system linking field data to fermentation management and then to feed ration optimisation has been validated in practice.

This contribution presents project NAZV QL26010434 AISilageFeeding (2026–2030), funded by the Czech Ministry of Agriculture under the ZEMĚ II applied research programme. The project builds on earlier NAZV work (QK1810137 on precision agriculture in silage and cattle feeding) and introduces a genuinely integrated data management system.

MATERIAL AND METHODS

The project is structured as a 5-year multi-institutional effort with clearly defined roles. The Institute of Animal Science (VÚŽV) leads fermentation biology, nutritional analysis, and animal performance trials. NutriVet Ltd. provides applied expertise in cattle nutrition, ration formulation, and practical farm integration. The Faculty of Electrical Engineering and Communication at Brno University of Technology (VUT) contributes sensor hardware development and wireless protocols.

The methodological framework encompasses five integrated components described in Table 1. AI model development follows a structured three-phase approach: (a) training on historical fermentation databases and laboratory experiments (60% of dataset); (b) validation on independent data withheld from training (20%); and (c) field testing under real farm conditions (20%). Model performance is evaluated using RMSE, MAE, and R^2 metrics. Target accuracies are: nutritional parameter prediction >90%, harvest timing ±2 days, fermentation pH prediction ±0.1.

RESULTS AND DISCUSSION (PRELIMINARY)

The project was launched in early 2026. Preliminary activities focus on three foundational tasks. First, the sensor integration architecture has been designed: low-power IoT nodes measuring pH, temperature, moisture and volatile metabolite proxies, communicating via LoRaWAN with 15-minute sampling intervals and a target battery life of ≥60 days in challenging silage-pit conditions. Second, historical fermentation databases from VÚŽV (>15 years of silage records, including continuous DS1922L temperature monitoring data from recent projects) are being structured for baseline AI model training. Third, the UAV flight protocol for multispectral crop monitoring has been specified, combining RGB and multispectral (NDVI, NDRE) imaging for maize and grass forage crops.

These activities build directly on established research conducted within the consortium. The integration of precision livestock farming tools with dairy herd management has been validated in practice (Loučka *et al.*, 2023), and on-farm comparisons of portable NIR spectrometry with laboratory chemical analysis for maize quality assessment have provided both methodological insight and experimental datasets suitable for AI model training (Loučka *et al.*, 2022). Together, these studies demonstrate that digital monitoring tools can reliably capture the variability in forage quality that the AISilageFeeding system is designed to predict and optimise.

Tab. 1: Project components, technical approaches and expected outputs

Component	Technical approach	Expected output / milestone
UAV multispectral monitoring	RGB + multispectral cameras; NDVI/NDRE indices; GPS-tagged data collection	Optimal harvest timing model; targeted additive application maps
Wireless sensor networks	pH (±0.05), temperature (±0.2 °C), moisture (±2%), volatile metabolites; 15-min interval; 60-day battery	Continuous fermentation profile; anomaly alerts; data stream for ML training
Machine learning algorithms	60% training / 20% validation / 20% field testing; RMSE, MAE, R^2 metrics; target >90% accuracy	Predictive models for nutritional parameters, harvest timing (±2 days), fermentation pH (±0.1)
Dynamic feed optimization	Real-time silage quality data + individual animal records (milk yield, lactation stage, health)	Personalized TMR formulation; ≥5% feed cost reduction; ≥15% additive savings
Integration platform	Cloud-based data aggregation from UAVs, sensors, labs, dairy management systems	Unified dashboard; economic ROI models per farm size; functional sample

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Tab. 2: Project timeline, partner responsibilities, and key deliverable milestones

Year	Component	Technical approach	Expected output / milestone
2026	Fermentation assessment methodology; baseline database	Sensor samples; UAV integration; network architecture	AI fermentation methodology (6/2026); sensor testing (9/2026)
2027	Full fermentation monitoring; silage quality database	Optimised sensors; data measurement; economic analysis	First predictive models (9/2027); ≥5% feed cost reduction demonstrated
2028	Semi-pilot experiments; feed ration trials	Model validation; functional prototype build	Integrated system samples; >90% prediction accuracy validated
2029–2030	On-farm validation; animal performance assessment	Commercial-scale testing; economic modelling	ROI models; utility model patents; extension publications

The innovation of the AISilageFeeding system lies in its end-to-end data linkage. UAV-derived pre-harvest data (crop maturity indices, spatial variability maps) will inform not only harvest timing but also fermentation management decisions – for example, directing higher additive doses to zones with elevated moisture or disease risk. In turn, fermentation sensor data will drive real-time feed ration adjustments, connecting silage quality monitoring to dairy herd nutrition management. This closed-loop architecture is conceptually distinct from existing partial systems such as Dairy Precision Farming (Netherlands) or John Deere GrowSmart (USA), which address individual phases in isolation.

Economic modelling, initiated in Year 1, projects that integration of AI-assisted harvest timing and targeted additive application alone could reduce additive costs and improve NEL on farms >500 ha. For a herd of 100 dairy cows consuming 15 kg DM silage/day, a 0.3 MJ/kg DM improvement translates to approximately 0.4–0.6 kg additional milk per cow per day, representing a substantial economic benefit during the 180-day winter feeding period. Full ROI models stratified by farm size will be delivered by end of Year 2 (December 2027).

CONCLUSION

Project AISilageFeeding (NAZV QL26010434) represents the first comprehensive attempt to integrate AI and UAV technologies across the complete forage production and utilisation chain in Czech conditions. The project addresses four critical knowledge gaps: (1) end-to-end data integration from field to feed-out, (2) predictive modelling of fermentation outcomes from pre-harvest UAV data, (3) real-time adaptation of feed rations to actual measured silage quality, and (4) economic quantification of precision forage management benefits across farm size categories. Targeting >90% prediction accuracy for nutritional parameters and ≥5% feed cost savings, the system will deliver functional on-farm prototypes by 2028 and commercially applicable tools by 2030. The closed-loop optimisation architecture – where field monitoring data inform fermentation management and fermentation outcomes drive feed formulation – is unprecedented in forage conservation research and directly responds to the challenges of climate change adaptation, resource efficiency, and precision livestock nutrition.

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LACTIPLANTIBACILLUS PLANTARUM 24-7 INOCULATED ALFALFA SILAGE ALLEVIATES SUBCLINICAL MASTITIS IN DAIRY GOATS THROUGH INFLAMMATION SUPPRESSION AND BLOOD-MILK BARRIER ENHANCEMENT

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ABSTRACT

Subclinical mastitis (SM), a prevalent yet often overlooked inflammatory disease, requires urgent attention. Building on our prior finding that feeding *Lactiplantibacillus plantarum* 24-7 inoculated alfalfa silage reduces systemic inflammation and may thus aid in SM prevention, this study further examined the underlying mechanisms. In the SM + Lp 24-7 group, inoculation markedly lowered milk fat, protein, casein, and free fatty acid content, while elevating ruminal acetate, propionate, and butyrate. Somatic cell counts decreased by over 30% on average compared to the SM group during the observation period. Furthermore, the SM + Lp 24-7 group showed increased serum levels of glutathione peroxidase (GSH-Px), total antioxidant capacity (T-AOC), ZO-1, and Occludin, along with reduced pro-inflammatory cytokines (TNF-α, IL-1β, IL-6). Collectively, these results indicate that *L. plantarum* 24-7 feed helps prevent SM through two complementary mechanisms: mitigating inflammatory responses and strengthening blood-milk barrier integrity, supporting its potential as a dietary strategy for maintaining dairy goat health.

Keywords: Subclinical mastitis, *Lactiplantibacillus plantarum* 24-7, somatic cell

INTRODUCTION

Subclinical mastitis (SM) poses a major challenge to the global dairy industry, causing significant economic losses, while conventional antibiotic use raises concerns about antimicrobial resistance. Previous research suggests that alfalfa silage inoculated with *Lactiplantibacillus plantarum* 24-7 (*L. plantarum* 24-7) may reduce mastitis risk through immunomodulatory effects, though its specific mechanisms remain unclear (Zhang *et al*., 2024). Although lactic acid bacteria-fermented feeds are known to benefit ruminant health (Li *et al*., 2022), their targeted efficacy against SM is not well established. This study, therefore investigated the effects and mechanisms of *L. plantarum* 24-7 fermented feed in an SM goat model, aiming to support the development of sustainable prevention strategies.

MATERIAL AND METHODS

Thirty dairy goats with SM were randomly divided into two groups: the SM+Lp 24-7 group (55.5% alfalfa silage containing *L. plantarum* 24-7 + 27.8% concentrate + 16.7% hay, with somatic cell counts (SCC) >500,000/mL) and the SM group (the same proportion of silage without inoculant, with SCC >500,000/mL). Additionally, 15 healthy goats served as the control group (Con group, SCC < 200,000/mL, same silage ratio without inoculant). The experiment lasted 80 days (15-day adaptation period + 60-day treatment period + 5-day sampling period). Serum inflammatory indicators were detected using ELISA kits. Significant mean differences between dietary treatments were separated using Tukey's honestly significant difference (HSD) test at a 5% probability level.

RESULTS AND DISCUSSION

Characteristics of Rumen Fermentation and Milk Quality in Dairy Goats

Feeding with L. plantarum 24-7 inoculated alfalfa silage significantly increased rumen acetate, propionate, and butyrate levels in dairy goats, indicating that this feed effectively promoted fiber degradation efficiency and energy conversion efficiency (Chai *et al*., 2023). Furthermore, the study found that milk protein and casein content were significantly reduced in the SM+24-7 group, suggesting that

Tab. 1: Effects of 24-7 silage on rumen fermentation and milk quality in dairy goats

Items	Dietary treatments			SEM	*P*-value
	Con	SM	SM+Lp 24-7		
Ruminal fermentation					
Acetate, mmol/L	43.6a	18.9c	27.1b	1.07	<0.001
Propionate, mmol/L	8.76a	5.03b	5.49b	0.233	<0.001
Butyrate, mmol/L	7.67a	4.69c	5.84b	0.125	<0.001
Milk composition					
Milk yield, kg/d	1.87a	1.32b	1.43b	0.319	<0.001
Fat, %	6.11b	7.39a	5.54b	0.183	0.001
Protein, %	4.30b	5.44a	4.57b	0.086	<0.001
Casein, %	3.37b	4.25a	3.36b	0.079	<0.001
Milk FFA, %	0.54c	1.74a	0.49c	0.03	<0.001

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this treatment may help maintain mammary gland health by inhibiting protein leakage into milk caused by impaired blood-milk barrier function (Gao *et al*., 2020).

Somatic Cell Count in Dairy Goats, Serum Antioxidant, and Inflammatory Changes

After feeding the *L. plantarum* 24-7 silage, somatic cell counts in dairy goats decreased by 36.8%, 30.6%, 29.6%, 27.8%, and 39.3% at days 7, 14, 30, 45, and 60, respectively (Figure 1A). It significantly elevated serum GSH-Px and T-AOC levels while reducing IL-1β, TNF-α, and IL-6 levels (Figure 1B). Concurrently, it markedly increased ZO-1 and Occludin levels (Figure 1C), indicating that this silage prevents SM by reducing systemic inflammation and maintaining normal blood-milk barrier function.

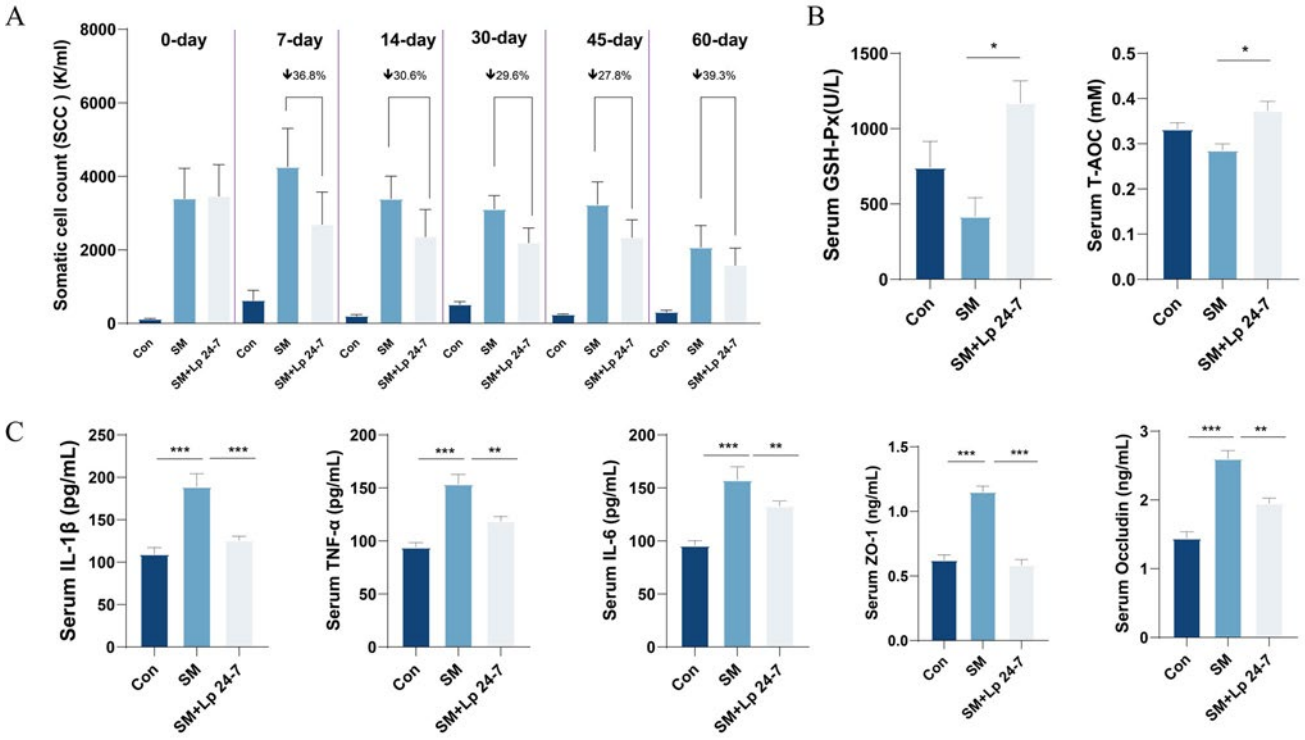


Fig. 1: Effects of 24-7 inoculated feed on SCC, inflammation, and tight junction proteins in dairy goat

CONCLUSION

Feeding dairy goats with *L. plantarum* 24-7 inoculated silage can effectively prevent SM by alleviating systemic inflammatory responses and enhancing the integrity of the blood-milk barrier. This intervention significantly reduced SCC in milk, improved the composition of ruminal volatile fatty acids, and influenced milk composition. These findings indicate that *L. plantarum* 24-7 may serve as a potential strategy for regulating host immune function and barrier health through nutritional intervention, thereby contributing to mastitis prevention and offering new insights for dairy goat health management.

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METHANE INDEX FOR GRASS SILAGES: A STANDARDIZED TOOL FOR BENCHMARKNG METHANE EMISSIONS GLOBALLY

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INTRODUCTION

Methane is a significant environmental concern in agriculture worldwide, contributing to climate change. Governments globally are addressing this issue; for instance, in 2022, the Dutch Government introduced a National Methane Strategy to reduce methane emissions by 30% by 2030 compared to 2020 levels.1 The agricultural sector, responsible for a large share of methane (CH_4) emissions, up to 78%.2

Measures like feed additives, optimized livestock diets, and advancements in breeding are being implemented to create a more sustainable sector.

This study observed significant differences in methane emissions between years and seasons, prompting the creation of a Methane Index by Eurofins Agro. This Index benchmarks methane emissions in grass silages worldwide. This innovative tool supports promotes environmental sustainability in agriculture.

MATERIAL AND METHODS

Based on research conducted by Wageningen University, two unpublished models developed by Sebek *et al.*, based on chemical parameters of silages, were utilized to predict potential methane emissions from grass silages. These models were applied to calculate the potential methane emissions for 47,422 grass silages harvested across Europe in 2022 and 2023. Additionally, to examine yearly fluctuations in methane excretion, we analyzed a dataset involving 347,185 production samples from grass silages harvested between 2015 and 2024 in the Netherlands.

In the analysis of the production samples submitted to the Eurofins Agro laboratory in the Netherlands, the ADF model was preferred; however, when ADF was not available, the Crude Fiber model was used. The results were processed using Excel, and statistical methods, including regression analysis, were employed to ensure accuracy and consistency.

RESULTS

The analysis revealed significant differences in methane emissions across various dimensions, including countries, seasons, and years. These findings underscore the complexity of methane emissions and the need for a comprehensive approach to benchmarking and reduction strategies.

The analysis of average potential methane emissions across the Netherlands, Belgium, Germany, and Denmark reveals significant variations, as shown in Figure 1. The Netherlands exhibits the lowest average emissions, while Belgium shows the highest. Additionally, Belgium's interquartile range (IQR) is the largest, indicating greater variability in methane emissions. Denmark has the lowest IQR, suggesting that methane emissions are more consistent.

The seasonal variation in potential methane excretion was analyzed, revealing distinct patterns throughout the year. As illustrated in Figure 2, methane excretion peaks during the spring season and reaches its lowest levels in autumn. This

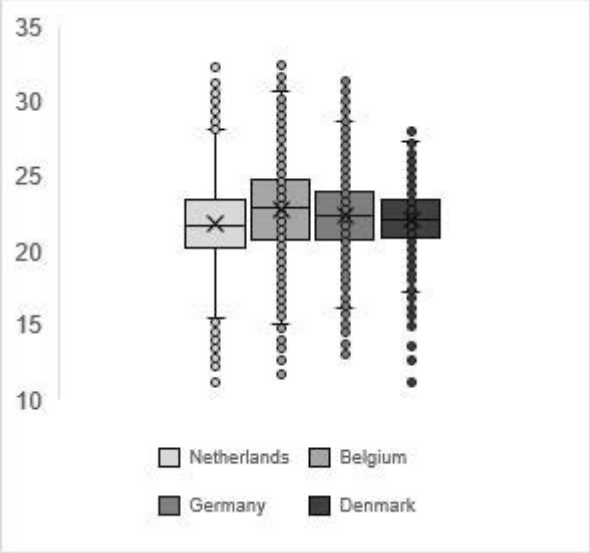


Fig. 1: Potential methane emissions in grass silages in the Netherlands, Belgium, Germany and Denmark

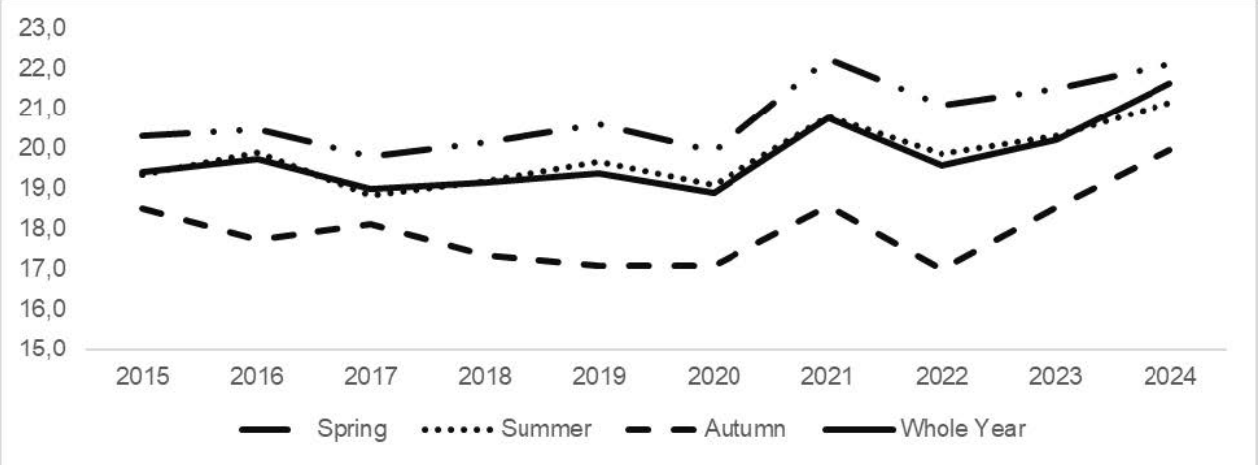


Fig. 2: Seasonal Variation of Potential Methane Emissions in the Netherlands (2015-2024)

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seasonal trend suggests that environmental or biological factors influencing methane production are more active in spring compared to autumn.

During the analysis, it became evident that benchmarking methane emissions based on country averages was not feasible due to the varying number of samples from different countries. To address this, an index was created to correct for seasonality and yearly fluctuations. This index allows for a more accurate comparison of methane emissions across different regions and time periods. The index evaluates whether the potential methane emissions of the silage, based on the Wageningen University models, are high or low.

CONCLUSIONS

The analysis of potential methane emissions across different seasons and countries revealed significant variations. The Netherlands exhibited the lowest average emissions, while Belgium showed the highest and most variable emissions. Denmark had the most consistent emissions profile. Additionally, the seasonal analysis indicated that methane emissions peaked in spring and were lowest in autumn.

The proprietary index, developed during the results analysis, proved essential for benchmarking methane emissions globally. This index corrects for seasonality and yearly fluctuations, enabling farmers to understand that high absolute methane emissions may be due to environmental conditions rather than poor management practices.

More research on a wider scale is needed to identify effective management practices that can lower methane emissions and, consequently, reduce the methane index.

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THE POSSIBILITIES OF DETERMINING THE NUTRITIONAL VALUE OF PRESERVED SORGHUM SILAGE USING NIRS TECHNOLOGY

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ABSTRACT

The aim of the paper is to present newly verified progressive methods for evaluating the nutritional value of dry feed (standard ground sample, particle size <1 mm) of preserved two-coloured sorghum silage (*Sorghum bicolor* (L.) Moench.) in terms of the following parameters: CP, fibre, fat, ash, ADF, NDF, cellulose, ADL, DMD and OMD, using near-infrared spectroscopy (NIRS). The parameters of the sorghum silage were determined using classical laboratory methods and measured simultaneously using NIRS. Calibration equations were developed using multidimensional regression methods (PLSR, MPLSR). The values of the coefficient of determination (R^2) of the calibration (validation) ranged from 0.83 to 0.96 (0.77 to 0.97) for the nutritional value parameters. Only a small amount of sample material (approx. 5–10 g) is required for analysis using NIRS. Not only is the measurement very fast (sample determination takes approx. 2–5 minutes), it is also sufficiently accurate.

Keywords: sorghum silage, NIRS

INTRODUCTION

Sorghum (*Sorghum bicolor* (L.) Moench.) is a promising crop for producing bulk feed, particularly silage for ruminants, in areas with low precipitation (arid to semi-arid regions). It thrives under such environmental conditions as well as in areas with lower soil fertility, such as hilly regions (Li *et al*., 2020; Mrkvicová *et al*., 2025). Due to its high water-soluble carbohydrate (WSC) content, sorghum is well-suited for preservation as silage (Rodrigues *et al*., 2020), which enhances its ensilability by promoting lactic acid formation. Sorghum silage can help stabilize fluctuations in roughage supplies, compensating for variations in corn silage yields and preserved forage from grasslands. Furthermore, it serves to maintain sufficient feed supplies in the event of roughage shortages (Nazlı and Polat, 2024). Regarding the analysis of sorghum silage quality using NIR spectroscopy, there is still limited information in the global scientific literature concerning calibration models for predicting quality and determining key parameters, such as crude protein (CP), fiber, acid detergent fiber (ADF), and neutral detergent fiber (NDF) (Eguchi *et al*., 2013; Kański *et al*., 2013). When formulating feed rations, understanding the nutritional value of roughage components is crucial; however, conventional laboratory analyses are often time-consuming and costly (Menšík *et al*., 2025). Within the framework of precision livestock feeding, near-infrared spectroscopy (NIRS) plays a key role, enabling the immediate quantification of nutritional parameters and forming the basis for the dynamic adjustment of feed rations (Liu *et al*., 2023).

MATERIALS AND METHODS

The basis for developing calibration equations to determine the nutritional value of sorghum silage consisted of microsilage samples from two sorghum varieties ('Latte' and 'Pampa Triunfo') harvested in 2022–2023. These were grown on experimental plots at Mendel University in Brno (Žabčice site). The sorghum varieties were cultivated in both fertilized and unfertilized plots under a one-cut regime per year. The crops were harvested using a cutter, and the material was ensiled in experimental microsilos (microtubes) containing various additives (microbial and chemical additives based on acids, acid salts, and urea), as well as untreated control variants. A total of 170 microsilage samples were obtained. Following four months of fermentation, samples were collected, dried at 60 °C, and ground to a particle size of less than 1 mm using a laboratory grinder. Laboratory analyses were used to determine dry matter (DM) content and basic silage quality parameters: crude protein (CP), fiber, fat, ash, acid detergent fiber (ADF), neutral detergent fiber (NDF), cellulose, acid detergent lignin (ADL), dry matter digestibility (DMD), and organic matter digestibility (OMD). Identical forage samples were scanned using a FOSS NIRSystems 6500 dispersion spectrometer (NIRSystems, Inc., Silver Spring, USA) located at the CARC Prague, Research Station in Jevíčko. Measurements were performed in duplicate using small ring cups. Scanning was conducted in reflectance mode across the 400–2500 nm range (visible and near-infrared regions) with a scanning step of 2 nm. WinISI II software (version 1.50; Infrasoft International, Inc., USA) was used to develop the calibration and validation equations, including graphical outputs. Partial Least Squares Regression (PLSR; Næsset *et al*., 2005) and Modified PLS (MPLSR; Nawar *et al*., 2023) methods were applied.

RESULTS AND DISCUSSION

The dataset of sorghum silage samples, comprising 170 reference values, was divided into a calibration set (n = 154; for DMD and OMD n = 56–57) and a validation set (n = 13–16; for DMD and OMD n = 6–7). The validation set was created by selecting every 10th sample from the original collection. This independent validation set exhibited forage quality parameters similar to the calibration set and was used to verify prediction accuracy. The results, verified using independent validation datasets with comparable characteristics, confirm a high degree of agreement between conventional laboratory methods and NIRS analysis. Validation of the developed calibration equations yielded coefficients of determination (R2) ranging from 0.77 to 0.97 across individual parameters (see Table 1).

CP - crude protein, ADF – acid detergent fiber, NDF – neutral detergent fiber, ADL – acid detergent lignin, DMD – dry matter digestibility, OMD – organic matter digestibility; n – number of validation samples, SEP – standard deviation of prediction, SEP (C) – standard deviation of the prediction corrected by the calibration curve constant (BIAS), BIAS – calibration curve constant, SLOPE – calibration curve slope, R2 – koeficient of determination; RPD – Ratio of Performance to Deviation

Based on the achieved coefficients of determination, the performance of the calibration models was excellent for most evaluated parameters ($R^2 \geq 0.90$), making them suitable for routine agricultural practice and monitoring the quality of plant products (Dvořáček *et al*., 2014). The Ratio of Performance to Deviation (RPD) ranged from 2.0 to 5.0. RPD values above 2.0 or 2.5 are considered applicable for standard agricultural practice and environmental quality monitoring (Pasquini, 2018; Samadi *et al*., 2020).

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Tab. 1: Validation of quality parameters of sorghum silage using NIRS technique

Parameter (%)	Validation						
	n	SEP	SEP (C)	BIAS	SLOPE	R2	RPD
CP	16	0.401	0.411	-0.045	0.887	0.94	4.0
Fibre	15	0.772	0.662	-0.431	0.881	0.77	2.4
Fat	13	0.469	0.474	-0.109	0.768	0.80	2.1
Ash	16	0.182	0.181	-0.049	0.920	0.86	3.2
ADF	14	0.874	0.888	0.176	1.235	0.89	2.0
NDF	14	1.203	0.755	0.957	0.906	0.92	3.8
Cellulose	14	0.463	0.479	0.028	1.108	0.90	3.1
ADL	15	0.345	0.346	-0.009	0.909	0.88	2.7
DMD	6	1.612	0.888	1.622	1.106	0.97	5.0
OMD	7	2.481	2.127	1.510	1.106	0.89	2.4

CP – crude protein, ADF – acid detergent fiber, NDF – neutral detergent fiber, ADL – acid detergent lignin, DMD – dry matter digestibility, OMD – organic matter digestibility; n – number of validation samples, SEP – standard deviation of prediction, SEP (C) – standard deviation of the prediction corrected by the calibration curve constant (BIAS), BIAS – calibration curve constant, SLOPE – calibration curve slope, R2 – koeficient of determination; RPD – Ratio of Performance to Deviation

CONCLUSIONS

Calibration equations were developed using near-infrared reflectance spectroscopy (NIRS) to evaluate the quality of sorghum silage across various parameters (e.g., CP, fiber, fat, ash, ADF, and NDF). Based on silage (microsilage) samples, these equations demonstrated a high degree of agreement between laboratory methods and NIRS determination (calibration set, $R^2 = 0.83–0.96$; validation set, $R^2 = 0.77–0.97$). The highest prediction accuracies were achieved for CP, DMD, ADL, ADF, and NDF content ($R^2 = 0.88–0.96$). For most evaluated parameters, the RPD values indicate that these models are suitable for routine agricultural practice and environmental quality monitoring. Key advantages of NIRS include enhanced operator safety and environmental sustainability, since the process consumes no reagents and generates no chemical waste. Furthermore, the process is rapid (taking approximately 2–5 minutes, depending on the instrument settings), accurate, and has no negative environmental impact.

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PRODUCTION AND QUALITY OF FORAGE SILAGES FROM DROUGHT-TOLERANT CROPS

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ABSTRACT

The study focuses on the production and quality of forage silages from drought-tolerant crops, with emphasis on sorghum (cv. Tarzan). The aim was to evaluate fermentation characteristics and silage quality using a biological additive (soluble Lactobacillus paracasei DSM 16245, Lactococcus lactis NCIMB 30160, Pediococcus acidilactici DSM 16243, concentration 1.25×10^{11}, carrier lactose) applied at 1 mL/kg and a chemical additive (composed of propionic acid, formic acid, and glycerol) applied at 8 mL/kg in comparison with a control. The results showed that the highest dry matter losses occurred in the chemically treated silage (1.19%), while the control and biological variants had lower losses (0.64–0.74%). The biological additive enhanced lactic acid production, resulting in a lower pH and improved fermentation. All variants showed good silage quality with low proteolysis. Sorghum silages represent a suitable alternative roughage source, particularly under drought conditions, while appropriate ensiling practices and additive selection significantly influence their quality.

INTRODUCTION

Climate change and global warming are increasingly forcing farmers to adapt crop production systems. The importance of drought-tolerant species is growing, as they can thrive under adverse conditions such as water deficiency, extreme temperatures, and poor soil quality. In drier regions, traditional crops (e.g., maize) are increasingly being replaced by sorghum, Sudan grass, and their hybrids, which provide stable biomass yields.

These crops have deep root systems, lower water requirements, and favourable nutritive value. Their silages are suitable for feed rations, support rumen function and digestion in ruminants, and contribute to the stability of livestock production even during drought periods.

Among the most promising species are sorghum, Sudan grass, and their hybrids (Hyso). They are primarily cultivated for silage and green forage production. Although they contain less starch than maize, they have higher fibre content and better drought tolerance.

Sorghum–Sudan grass hybrids combine high yield potential, good digestibility, and stress resistance. They allow multiple harvests, exhibit good fermentation stability, and are suitable for inclusion in Total Mixed Ration (TMR) diets in combination with protein supplements. Several studies (Lv *et al.*, 2023; Cattani *et al.*, 2017) have shown that, with a properly balanced ration, satisfactory milk production can be achieved without adverse effects on animal health.

The aim of this study was to evaluate the production and quality of forage silages from drought-tolerant crops, with a focus on sorghum (cv. Tarzan), which is suitable for ensiling and characterised by high biomass yield.

MATERIAL AND METHODS

The experiment with the drought-tolerant sorghum variety Tarzan was conducted at the National Agricultural and Food Centre, Department of Animal Production, Division of Animal Nutrition and Small Livestock, Lužianky (Slovakia), in the Laboratory of Feed and Biological Material Analysis. The plant material originated from an agricultural cooperative in western Slovakia.

The experiment was conducted in laboratory silos with a working volume of 1.7 L, which were stored under controlled laboratory conditions in darkness at 20 ± 2 °C. The experiment was arranged in three treatments. The first treatment served as a control without silage additives. In the second treatment, a biological inoculant, containing soluble Lactobacillus paracasei DSM 16245, Lactococcus lactis NCIMB 30160, and Pediococcus acidilactici DSM 16243 (concentration 1.25×10^{11}, carrier: lactose), was applied at a dose of 1 mL·kg^{-1}.

In the third treatment, a chemical preservative, composed of propionic acid, formic acid, and glycerol, was applied at a dose of 8 mL·kg^{-1}. Each treatment was prepared in six replicates. During fermentation, dry matter losses were monitored on day 3 and subsequently at 21-day intervals using the gravimetric method.

After 90 days of ensiling, the experiment was terminated. The silages were removed from the laboratory silos and prepared for quality and nutritional analyses, including determination of nutrient composition.

Subsequently, the following parameters were determined in accordance with Commission Regulation (EC) No. 152/2009 of 27 January 2009: dry matter, crude protein, ash, crude fibre, acid detergent fibre (ADF), neutral detergent fibre (NDF), fat, and total sugars (Luff–Schoorl method).

Fermentation quality parameters were also analysed. The pH of silage extract was measured electrometrically, while lactic acid and volatile fatty acids were determined by gas chromatography. From these data, total volatile fatty acids (VFA) and total acid content were calculated.

RESULTS AND DISCUSSION

The fermentation process of sorghum silage production was evaluated. During fermentation, dry matter losses were monitored on day 3 and subsequently at regular 21-day intervals using the gravimetric method. After 90 days of ensiling, the experiment was terminated, and the silages were removed from the laboratory silos and prepared for quality and nutritional analyses, including determination of nutrient composition.

Both the control silage and the silage treated with a biological additive showed no signs of spoilage (colour or smell changes) after 90 days, which was confirmed by nutrient composition and fermentation parameters. In contrast, silages treated with the chemical additive showed reduced quality in two replicates.

In the fresh sorghum material as well as in the differently treated silage samples, nutrient composition and fermentation parameters were analysed. The highest average dry matter losses were recorded in silage treated with the chemical preservative (1.19% after 90 days), whereas the control and biologically treated silages showed lower losses (0.64–0.74%). Dry matter content decreased by 7.5% during ensiling.

The contents of ADF, NDF, lignin, hemicellulose, and cellulose were higher in silage treated with the chemical preservative, as these additives rapidly suppress microbial activity. Consequently, no fermentation-driven degradation of hemicellulose or partial breakdown of cell walls occurred. In contrast, the use of a biological additive led to partial degradation of plant cell structures due to acidification, enzymatic activity,

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and plant-derived hydrolases, resulting in lower NDF and ADF fractions. Similar findings were reported by Xu et al. (2024), who observed a faster pH decline, higher lactic acid content, and improved fermentation stability in inoculated silages.

Total sugar content decreased markedly from 95.8 $g \cdot kg^{-1}$ dry matter to approximately 1–2 $g \cdot kg^{-1}$ dry matter due to its utilisation during fermentation. Lactic acid, produced by lactic acid bacteria, contributed to pH reduction in this experiment. The highest lactic acid content was found in the control variant (39 $g \cdot kg^{-1}$ dry matter). In the chemically treated silage, lactic acid content was lower; however, the proportion of propionic acid increased (3.7 $g \cdot kg^{-1}$ dry matter), which helps suppress the growth of yeasts and moulds. Ardiansyah *et al.* (2025) reported that chemical additives reduce lactic acid and ammonia content while improving aerobic stability and dry matter digestibility.

Butyric, isobutyric, valeric, and isovaleric acids are formed during proteolytic fermentation and protein degradation. Their low levels across all variants indicate minimal proteolysis and high fermentation quality.

Ammonia (NH_3-N) is an indicator of protein degradation during fermentation. In this experiment, NH_3-N levels were low in all variants, with the lowest value observed in chemically treated silage (5.28%), indicating effective protein preservation. Low NH_3-N values reflect efficient crude protein retention. Similarly, Diepersloot *et al.* (2021) reported that microbial inoculants improve fermentation profiles, enhance aerobic stability, and contribute to better nutrient preservation during storage.

CONCLUSION

Sorghum silages are widely used as a major roughage component in Total Mixed Ration (TMR) diets, where they are combined with protein feeds, mineral supplements, and concentrates. Long-term drought conditions create significant challenges in ensuring sufficient production of roughage from both temporary and permanent grasslands.

Owing to climate change, which adversely affects the yield and quality of traditional crops, including grasslands, increasing attention is being paid to alternative drought-tolerant species such as sorghum, its hybrids, Sudan grass, and Hyso. These crops are capable of producing high biomass yields even under dry and less fertile conditions, thereby ensuring a more stable feed supply for dairy cows and other ruminants in years with low precipitation.

When appropriate agronomic practices are applied, including optimal harvest timing, suitable dry matter content, and proper silage management, sorghum silages can provide high-quality feed, contributing to good animal health and productive performance in dairy cattle.

Therefore, further research should focus on suitable varieties, optimisation of harvest timing, and improvement of ensiling techniques to ensure high-quality sorghum silage and a stable feed supply under changing climatic conditions.

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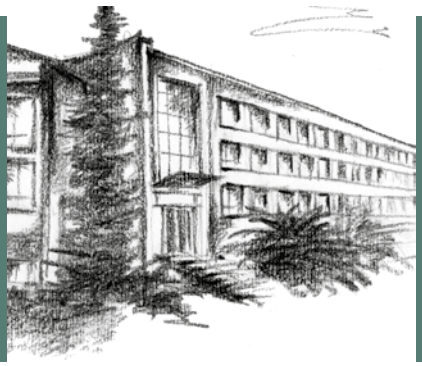


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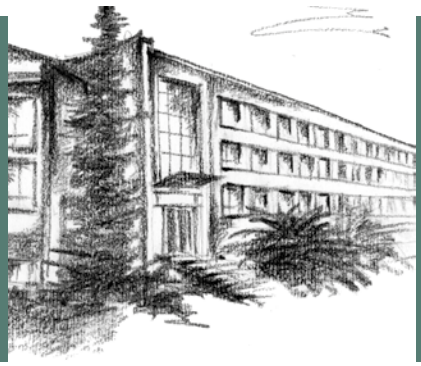
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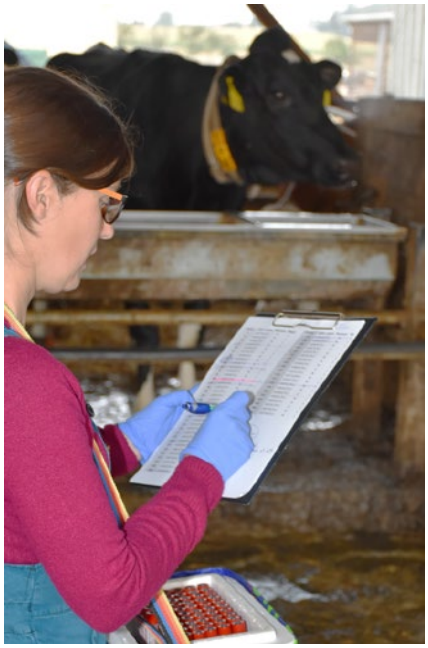


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COLLABORATION AND CONTRACT RESEARCH

- Contract research following the partners' assignment
- Services of accredited experimental stables including the provision of defined animals for experiments and carrying out experiments in line with legislation on the protection of animals
- Expert cooperation on the verification of materials, technologies and processing of livestock production

EXPERT REPORTS

- Preparation of analyses, studies and research on individual demand
- Defining genetic variants of milk protein genes for cattle, sheep and goats
- Defining genetic variants of Booroola genes for sheep
- Defining genetic variants of genes for the colouring of horses

ECONOMIC ANALYSES FOR BREEDERS

- ECOWEIGHT software for the calculation of economic weight of important traits in beef cattle, dairy cattle and sheep
- FarmProfit – economic software for the evaluation of profitability of agricultural commodity production

CONSULTANCY

- Addressing topical issues in the breeding of dairy cattle, beef cattle, goats and sheep, pigs, poultry, rabbits, horses and cervids
- E-mail and telephone consultancy
- Online consultancy: poradenstvi@vuzv.cz

TRAINING COURSES AND EXPERT SEMINARS

- Pig insemination training courses
- Training of specialists in the classification of carcasses of pigs and cattle (SEUROP)
- Training of consultants in the field of livestock production
- Expert visits and practical instruction for the training of livestock hauliers
- Invited lectures for breeders, students and agricultural business staff on specific topics

INSTITUTE OF ANIMAL SCIENCE
Přátelství 815, 104 00 Praha Uhříněves, Czech Republic
www.vuzv.cz | tel. 267 009 650 | vuzv@vuzv.cz

ECOSYL™

For consistently better silage

QUALITY.
SEE IT.
SMELL IT.
ECOSYL IT.



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Formasil®

BIOLOGICAL TOOL FOR BETTER SILAGE FERMENTATION



Experiment Formasil®

Watch the video verifying the functionality of VVS silage inoculants.

Harvest at the stage of budding – heading

High nutrient utilization and feed intake

Reduction of undesirable bacteria, yeasts, and molds

Suitable mechanization and line organization

Minimal losses and rapid pH decrease

Preservation of animal health

STOP *silage losses*

VVS®

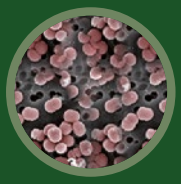
in form **on the farm**®

Feed Safety GMP+ Assurance

Formasil®

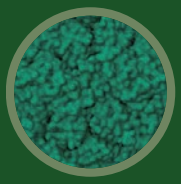
BIOLOGICAL TOOL FOR BETTER SILAGE FERMENTATION

BACTERIAL STRAINS



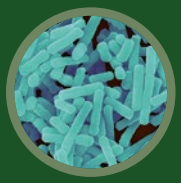
PEDIOCOCCUS PENTOSACEUS

- functions as a starter for the entire fermentation process because it has a short generation interval of 20 minutes
- it thrives in a pH range of 4.5–8, ideally at pH 5–6.5
- it is more tolerant to high dry matter content, has a wider range of optimal temperature and pH for growth, and therefore grows well even in colder periods (spring, autumn)
- it has the ability to produce bacteriocins, which inhibit the growth of certain other bacteria and eliminate Listeria
- it can also utilize five-carbon sugars, resulting in a higher production of lactic acid



ACIDIPROPIONIBACTERIUM ACIDIPROPIONICI

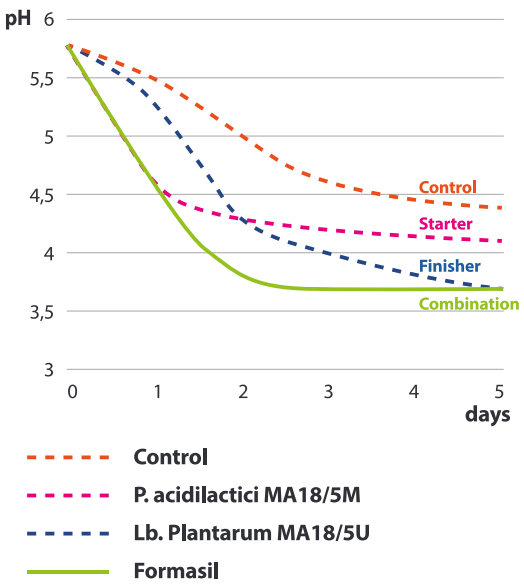
- does not act at the beginning of fermentation but only later, **increasing the aerobic stability of silage**
- it slowly converts part of the lactic acid into **propionic and acetic acids**, which have antifungal effects, suppressing the growth of molds and yeasts
- **it is not affected by the environmental pH** and can act even in well-acidified silage
- its effect appears **after approximately 6 weeks of fermentation**, when the concentration of protective acids increases
- it does not cause a pH drop, which is why it is **combined with Pediococcus**, which initiates fermentation
- it works **over a wide range of dry matter content and temperatures**



LACTOBACILLUS BUCHNERI NCIMB 40788

- does not act at the beginning of fermentation but only later, significantly increasing the aerobic stability of silage after opening
- it slowly converts part of the lactic acid into acetic acid and 1,2-propanediol, which have antifungal effects, suppressing the growth of molds and yeasts
- it is not sensitive to low pH and can act even in already acidified silage
- its effect appears after 30–60 days of fermentation
- it does not ensure a rapid pH drop, which is why it is combined with Pediococcus or other homofermentative bacteria (e.g., L. plantarum)
- it functions over a wide range of dry matter content and temperatures

Faster and more pronounced pH drop



WWW.VVS.CZ

Dr. PIEPER
Technologie- und Produktentwicklung
für Landwirtschaft
und
Umwelttechnik

BIO-SIL®

Highly Effective Lactic Acid Bacteria

For premium silage quality and improved fermentation.



- Increased milk yield
- Increased feed intake
- Improved digestibility
- For all types of forage
- Proven on leading farms
- DLG-tested

QUALITÄTSSIEGEL DLG WWW.DLG.ORG
KATEGORIE 1b, 1c, 4b, 4c (Milch)
KONTINUIERLICH GEPRÜFT
DLG-Zertifikat 6512

BIO-SIL® for delicious silage ...
Moooh!

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Phone: +49 33 91 - 68 48 0 • Fax: +49 33 91 - 68 48 10
Email: info@dr-pieper.com

www.dr-pieper.com

Dr. PIEPER

Technology for innovative farms – we develop solutions for modern silage production and dairy nutrition.

The product portfolio includes:

- BIO-SIL® and other silage additives
- modern dosing technology
- Transi-fit® for close-up diets

- ✓ *Scientifically proven*
- ✓ *Production in Germany*
- ✓ *DLG-tested quality*
- ✓ *Proven on leading farms*



PASSION AG

Cow performance is our

PASSION

High-performing dairy herds depend on two simple principles: healthy cows perform better, and high-quality forage plays a vital role in achieving this.

These principles are at the heart of the global dairy industry and form the foundation of Passion Ag's approach to forage management.

We believe superior forage quality is key to meeting the nutritional demands of today's high-producing dairy cows. That's why our forage management systems are built around products designed to help **preserve feed quality, reduce losses** and **support consistent silage performance** year-round.

Contented cows perform better, and high quality feed has a direct impact on contented cows.

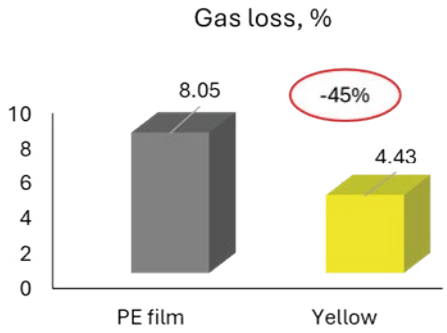


Passion YELLOW
Oxygen Barrier Film Trial Results

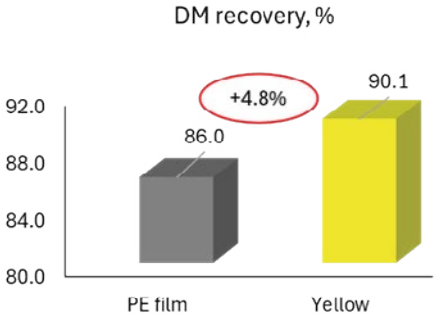
Silage quality depends heavily on the cover used. Conventional polyethylene (PE) films are inexpensive but allow oxygen ingress, risking poorer fermentation and stability. Oxygen-barrier films are designed to limit oxygen transmission and protect silage quality.

In a mini-silo trial, sorghum was ensiled with either PE (control) or an oxygen-barrier film (Passion Ag Yellow) and stored for 52 days. Fermentation, hygiene, dry matter losses, and aerobic stability were then compared to assess cover performance.

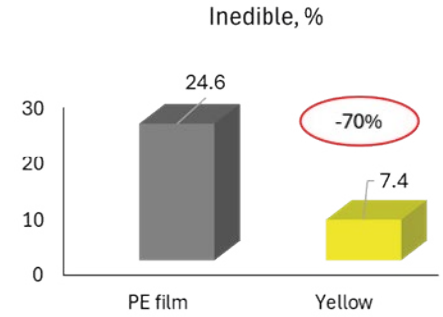
Maximize Feed Value & Reduce Waste



Almost halves gas losses vs. PE, locking in valuable nutrients.

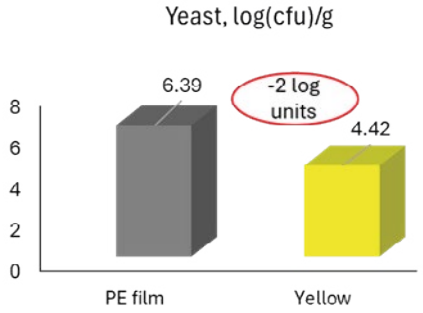


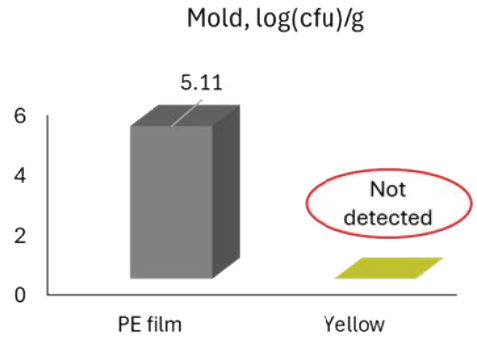
Dry matter recovery up 5%, meaning more high-quality feed for your herd.



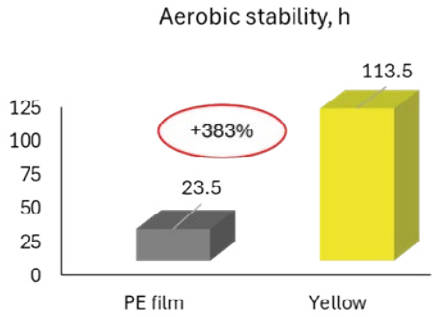
Inedible silage reduced by ~70%, so less waste and more cost-effective feeding.

Superior Silage Hygiene & Shelf Life





Yeast decreased and mold eliminated, preventing spoilage and ensuring cleaner, and safer silage.



Aerobic stability increased nearly five-fold, so feed stays fresh longer, even at the bunk.

passionag.com

Excerpt from "Effects of Oxygen Barriers on Losses, Fermentation and Microbial Counts of Sorghum Silage, F.X. Amaro et al., 2025, XX International Silage Conference (ISC), Gainesville, FL USA"



agrility a precision digital platform

- ✓ vegetation under close monitoring from sowing to harvest
- ✓ prediction of the optimal silage harvest date
- ✓ maximum milk production from high-quality silage

Breeding your profit

Limagrain Field Seeds • 2026

BALANCED!



FUNCTIONAL SILAGE MAIZE FOR CATTLE NUTRITION

- ✓ Above-standard agronomic parameters - yield, earliness, adaptability
- ✓ Higher fiber digestibility = EXTRA ENERGY
- ✓ Higher dry matter intake and better animal health
- ✓ HIGHER MILK PRODUCTION

LG Animal Nutrition

www.lgseeds.cz

Limagrain

KOMPLETNÍ PROGRAM SILÁŽOVÁNÍ S BIOFERMEM





CHEMICKÉ SILÁŽNÍ PŘÍPRAVKY:

SilaFor® **2 Plus**, Silafor® **2000 NC**
SilaPro® **Mix**, SilaPro® **NC-P**



BIOLOGICKÉ SILÁŽNÍ INOKULANTY:

AdiSil® **LG-100 Perfect**, Adisil® **Mix**, AdiSil® **Plus**
AdiSil® **Lac**, AdiSil® **M-100 Stabil**



HERMETICKÉ ZAKRÝVÁNÍ SILÁŽNÍCH JAM:

Kvalitní vícevrstvé **PE fólie**
Zátěžové tkaniny **SILOTEX**
Zátěžové pytle **SILOBAG**

KONTAKTY PRO ODBORNÉ PORADENSTVÍ PRO KVALITNÍ SILÁŽOVÁNÍ



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KOMPLETNÍ SILÁŽNÍ PORADENSTVÍ A SERVIS



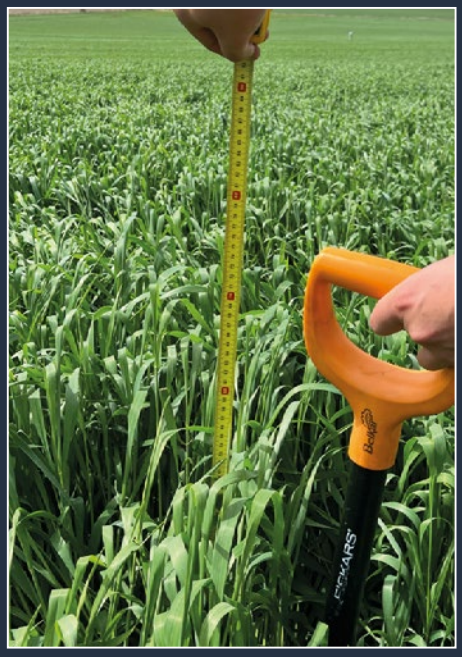


























THORSIL®



HAMMER



Top Treatment

Protecting the top layer of silage is essential for maintaining feed quality and minimizing losses during storage and feed-out.

Thorsil Hammer is specifically designed for top treatment applications, helping to improve aerobic stability and reduce heating, mould growth, and spoilage in the most vulnerable parts of the silo.

Easy to apply and highly effective at low application rates, Thorsil Hammer supports better preservation of valuable nutrients, helping farmers maintain high-quality forage and maximize the value of every tonne of silage.

Benefits of Thorsil Hammer:

- **Improves aerobic stability**
- **Reduces heating and spoilage**
- **Helps preserve nutrients and feed value**
- **Easy and safe to handle**
- **Suitable for all forage types and storage systems**

Thorsil of Sweden

www.thorsil.com
info@thorsil.com
+46 70 518 32 36



OSEVA
hybrids

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BETTER SILAGE.
BETTER PERFORMANCE.
safesil® PRO
Maximum forage protection
safesil® CHALLENGE
For the toughest conditions
safesil® SMART
Effective silage protection
Professional silage additives
for modern forage production.
Safesil helps farmers protect forage quality from harvest to feed mix. Designed for modern livestock production, Safesil supports an efficient ensiling process while helping to preserve valuable nutrients and reduce losses throughout storage and feeding.
Whether conditions are ideal or challenging, Safesil helps secure high-quality forage and protect your investment.
REDUCED DRY MATTER LOSSES
IMPROVED HYGIENIC SILAGE QUALITY
REDUCED HEATING AND SPOILAGE
EFFECTIVE IN DIFFICULT ENSILING CONDITIONS
PRODUCED IN SWEDEN
SALINITY
EXPERTS IN SALT SINCE 1830
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LABORATOŘ
POSTOLOPRTY

NABÍDKA AKREDITOVANÝCH ROZBORŮ KRMIV

CELKOVÉ ROZBORY

ROZBOR SILÁŽE, SENÁŽE
Sušina, NL, vláknina, popel, tuk, živiny (P, K, Ca, Mg, Na), KNO_3, fermentace (pH, kyseliny mléčná, octová, máselná, propionová, valerová, KVV, volný NH_3), ADF, NDF, smyslové hodnocení, výpočet energie (ME, NEL, NEV, SNL, PDIN, PDIE), škrob, cukr…

ROZBOR SENA, KRMNÝCH SMĚSÍ, ZELENÉ PÍCE
Sušina, NL, vláknina, popel, tuk, živiny (P, K, Ca, Mg, Na), KNO_3, výpočet energie…

ROZBOR KRMNÉ DÁVKY (TMR)
Sušina, NL, vláknina, popel, tuk, živiny (P, K, Ca, Mg, Na), metabolizovatelá energie pro skot…

ROZBOR KRMIV PRO BPS
Sušina, NL, vláknina, popel, N-NH_4, fermentace (pH, kyseliny mléčná, octová, máselná, propionová, valerová), škrob, cukr, P, S



DOPLŇKOVÉ ROZBORY
- ADF, NDF, ADL (lignin)
- škrob, cukr, tuk, olejnatost
- ČKT (FFA), peroxidové číslo (PV)
- mykotoxiny (DON, zearalenon, T-2 toxin, ochratoxin, suma aflatoxinů, fumonisin)
- aminokyseliny (ASP, THR, SER, GLU, PRO, GLY, ALA, CYS, VAL, MET, ILE, LEU, TYR, PHE, HIS, LYS, ARG)
- vitamíny (A, D, E a ß-karoten)
- doplňkové látky – kokcidiostatika, chemoterapeutika (robenidin, salinomycin, monenzin, narazin, nikarbazin, lasolocid, halofuginon, semduramycin, maduramycin)
- frakce dusíku (A, B1, B2, B3, C)
- stravitelnost OH, NDF, škrobu (metoda in vitro)
- stravitelnost NL metodou in vitro
- výpočet metabolizovatelné energie pro skot, prasata, drůbež, koně, králíky, kočky, psy…
- plísně, kvasinky, ethanol
- těžké kovy (As, Pb, Hg, Cd, Cr…)
- mikroprvky (Se, Cu, Zn, Mn, Fe, Mo)
- homogenita krmných směsí, křížová kontaminace
- chloridy, sírany
- rozbory minerálních směsí, masokostních a krevních mouček



PŘEDSKLIZŇOVÁ STANOVENÍ
- stanovení sušiny kukuřic
- stanovení dělených vzorků kukuřic – celková sušina, sušina a hmotnost palic a zbytku rostliny
- stanovení škrobu, cukru, NL před senážováním a silážováním
- inokulační test

ODBĚR KRMIV
- z otevřené jámy ze stěny
- ze zavřené jámy sondovací tyčí
- z vaků
- z partií vyrobených krmných směsí

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LABORATOŘ POSTOLOPRTY s.r.o.
Masarykova 300, 439 42 Postoloprty
tel.: 415 784 310, 777 225 066

Potential to turn into **your success.**



THE NEXT GENERATION OF HYBRIDS FOR SILAGE PRODUCTION

SiloExtra marks **the best choice for milk and biogas production.** With precision breeding, our hybrids have been carefully developed to excel at the two most important factors in silage: **high quality and high quantity.**

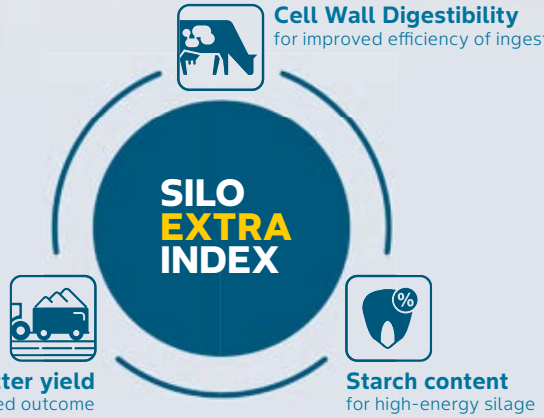
Successful livestock feeding starts with a **high dry matter yield.** On the other hand, important parameters such as **starch content** and **fiber digestibility** also help produce high-quality silage. All these characteristics evolve in different ratios as the plant develops. This makes the decision when to harvest most critical.

With their exceptional **stay-green characteristic,** SiloExtra hybrids allow you to widen your **cutting window** while **minimizing risks.**

The result: silage full of energy and digestible fiber that increases milk production.

SILO EXTRA INDEX: THE 3 KEY ELEMENTS FOR THE RIGHT PICK

To identify the hybrids that really deserve the SiloExtra label for high-quality corn seeds, we developed the **SiloExtra INDEX.** It is based on the best possible and well-balanced mixture of the three parameters of successful silage production. To make sure you can select the hybrids that really go the Extra mile.





SILO EXTRA

OUR BEST SILOEXTRA HYBRIDS FOR YOU
Browse our complete hybrid portfolio on
www.cropscience.bayer.cz

SILO EXTRA

BAYER



EASY HERBICIDE PROTECTION OF CORN

ADENGO®

Long-term residual pre-emergence and early post-emergence herbicide against annual weeds

LAUDIS®

Post-emergent herbicide for a quick solution of a wide spectrum of weeds up to 8 maize leaves

MaisTer® power

Comprehensive solutions for annual and perennial weeds, including *Fallopia convolvulus*, *Cirsium arvense*, *Elytrigia repens*

Capreno®

Post-emergence herbicide with long-term residual effect, an effective anti-resistance tool

- **for all application dates**
- **against all important weeds**
- **in all cultivation technologies**

Bayer ExpertNEWS

YouTube Bayer Expert CZ&SK

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SUSTAINABILITY IS BASIS

cropscience.bayer.cz

BAYER

Používejte přípravky na ochranu rostlin bezpečně. Před použitím si vždy přečtěte označení a informace o přípravku. Respektujte varovné věty a symboly.

novonesis



Silážní biologické řešení

SiloSolve™ FC

Dejte své siláži maximální péči se SiloSolve™ FC inokulatem od Novonesis a zvyšte si výkonnost vaší farmy

Užijte si lepší kontrolu fermentace díky unikátní technologii OxyCap™

Krmte siláž již za 10 dní, kdykoliv ji vaše krávy potřebují

Snižte plýtvání siláží až o 30% a profitujte

Zachovejte kvalitu a živiny pro podporu zdraví vašich krav

Z pole až po krmení může být až 50% siláže ztraceno

Z pole po silážní žlab
-12%

Zkažení horní vrstvy
-15%

Fermentace
-5%

Aerobní kažení
-10%

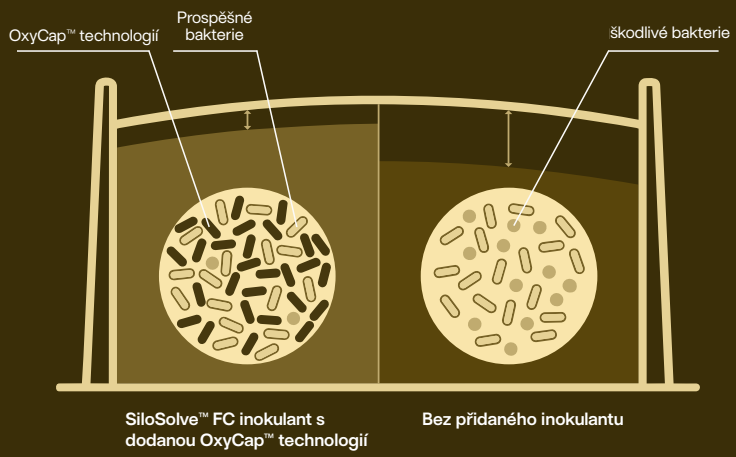
zbytky krmení
-10%

Inokulant SiloSolve™ FC s technologií OxyCap™

Získejte větší kontrolu, minimalizujte ztráty a užijte si maximální flexibilitu.

Méně odpadu, větší svěžest

Připraveno ke zkrmování již za 7 dnů



SiloSolve™ vysvětlení

Proč se více farmářů rozhodne pro SiloSolve™ FC

Funguje napříč všemi plodinami

Efektivní v širokém spektru sušin ~20–50%

Preferován > 15 miliony krav

Potvrzeno pokusy na farmách i univerzitách

Kvalitní siláž nevzniká náhodou

Přidejte do svého silážního balíčku SiloSolve™ FC inokulant s technologií OxyCap™ a kontrolujte lépe fermentaci, kvalitu krmení a výkonnost.

Pro více informací se spojte s našimi experty:

Informace zde uvedené jsou pouze pro obecné informační účely. Veškeré informace jsou poskytovány v dobré víře.Neexistuje záruka a nepřijímá se žádná odpovědnost za jejich přesnost (mimo jiné také za přímé nebo nepřímé škody v souvislosti s ušlým ziskem nebo přerušením podnikání), úplnost, správnost, shodu s předpisy, obchodovatelnost nebo vhodnost pro konkrétní účel.

novonesis

Chr. Hansen A/S, Part of Novonesis Group, Krogshoejvej 36, 2880 Bagsvaerd, Denmark
novonesis.com

Dodavatel moderních zemědělských technologií
AgriTechnika CZ, s.r.o., Husova 35, 664 51 Šlapanice
www.atcz.cz, atcz@atcz.cz, 775 047325



NIR analyzátory

+ AgriNIR: mobilní NIR analyzátor stanovující základní výživové parametry (sušina, škrob, N-látky, ADF, NDF, popel, tuky a jiné) krmiv (kukuřičná siláž, TMR, vojtěšková siláž, zelená kukuřice a jiné) během pár vteřin
+ XNIR: ruční NIR analyzátor na okamžité stanovení základních výživových parametrů krmiv přímo ve žlabu / stáji
+ DG precision feeding: NIR analyzátor stanovujíví základní výživové parametry krmiv v reálném čase přímo na krmném voze nezávisle na značce s automatickým přepočtem krmné dávky dle aktuální sušiny
+ NIR on board: NIR analyzátor stanovující základní výživové parametry zelených krmiv v reálném čase přímo na řezačce nezávisle na značce
+ EVO NIR: NIR analyzátor stanovujíví základní výživové parametry kejdy v reálném čase přímo na cisterně nezávisle na značce







Čistící kartác Actisweep

Systém ACTISWEEP V je patentovaná technologie pasivního čištění ploch od nečistot. Má jedinečné uspořádání kartáčů do tvaru V, které zabezpečují rychlé a efektivní čištění s minimální mírou prašnosti.





Supplier of modern agricultural technologies
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NIR analyzers

+ AgriNIR: mobile NIR analyzer measuring basic nutritional parameters (drymatter, starch, protein, ADF, NDF, ash, fat and others) in forages (mais silage, TMR, alfalfa haylage, green mais and others) in few seconds
+ XNIR: handheld NIR analyzer for immediate measurement of basic nutritional parameters of forages directly in the silage pit / stable
+ DG precision feeding: NIR analyzer measuring basic nutritional parameters of forages in real time directly on a feeder mixer independently on the brand with automatic recalculation of the feed rate according to the actual drymatter
+ NIR on board: NIR analyzer measuring basic nutrional parameters of green forages in real time directly on the forage harvester independently on the brand
+ EVO NIR: NIR analyzer measuring basic nutrional parameters in slurry in real time directly on the slurry tanker independently on the brand







Cleaning brush Actisweep

The ACTISWEEP V technology is a patented technology of passive cleaning of surfaces from dirt. It has an unique V shaped brushes, which ensure a quick and effective cleaning with minimal dust.





KRMIVO PRE PRODUKT
PR*DUCT FEED
PRODUKT PRE KŔMENIE

BACTÉRIOSOL®

TECHNOLOGIE MARCEL MÉZY®

koncentrovaný

Organické hnojivo umožňující humifikaci půdy.
Splňuje normu NF U44-051

Rozvíjí rychlou produkci humusu (huminových kyselin) v půdě.
Bactériosol nakrmí půdu živinami a půda nakrmí rastliny
Ukladá v půdě uhlík a minerálni prvky.

ZPŮSOB ÚČINKU

Baktériosol obsahuje aerobní mikroorganismy vyselektované na kompostech technologií Marcela Mézyho. Díky spontánním mikroorganismům živým v půdě transformované do použitelné formy pro rostliny a uskladněné v humusu a tím jsou rostlinou později využity podle potřeby. Nedochází k jejich ztrátě vyplýváním a únikem do atmosféry.

BACTÉRIOSOL VÁM UMOŽNÍ

- **Optimalizovat výnosy**
- **Přestat používat P, K a Ca, postupně snižovat N o 25%**
- **Zlepšit kvalitu půdy a na ní pěstovaných plodín**
- **Lépe odolat suchu, zlepšit vstřebávání vody do půdy a podpořit tvorbu kořenového systému**
- **Omezit ztráty živin (aj dusičnanů) do atmosféry a vyplavováním do podzemních vod**
- **Zpřístupní v jakékoli půdě fosfor, draslík, vápník a ostatní minerály**
- **Zlepšuje strukturu půdy a rozpouští dno brázdy**

APLIKACE

- Vždy rozhazovat na zem, při teplotě půdy vyšší než 8°C
- Při dostatečné vlhkosti (ranní rosa stačí)
- Doporučuje se aplikovat na podzim a na jaře, neaplikovat na půdu v období sucha a mrazu
- NEZAORAT hlouběji než 5 cm. V případě orby zaorat přibližně za 3 týdny
- Dávka 100–300 kg/ha/ročně podle druhu pěstované plodiny a cíle pěstitele
- Dávka 2–4 kg/ar/ročně do zahrady, sadu, fóliovníku a trávníku
- Nepoužívat simultánně s jinými chemickými produkty s baktericidním, fungicidním nebo herbicidním účinkem, s vápnem, superfosfátem, dezinfekcí a jinými pesticidy
- Dodržte alespoň 14denní odstup mezi aplikací Bactériosolu a jiných chemických ošetření

SLOŽENÍ

- Živé mikroorganismy vyselektované na kompostech vybraných rostlin, organická a minerální hmota
- Pro ekologické farmáře dostupný certifikovaný BACTÉRIOSOL Koncentrovaný UAB

Vliv Bactériosolu na tvorbu humusu v půdním profilu



- Pro dosažení deklarovaných účinků na hloubku ornice doporučujeme opakovat 3–4 aplikace v intervalech 5–12 měsíců.





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KRMIVO PRE PRODUKT
PR*DUCT FEED
PRODUKT PRE KŔMENIE

BACTÉRIOLIT®

TECHNOLOGIE MARCEL MÉZY®

koncentrovaný

Komplex mikroorganismů do podestýlky a hnoje na kompostování.
Splňuje normu NF U44-051

Skompostuje hnůj přímo v maštali: už nemusíte zaorávat ani nechat odležet několik měsíců. Po třech až šesti týdnech se dá kompost aplikovat na půdu.

Zlepšuje klima v maštali: sníží vylučování amoniaku až o 85 %.

ZPŮSOB ÚČINKU

- Aditivum do podestýlky, hnoje, hnojovice a na kompostování organické hmoty obsahující přírodní minerály a vybrané rostliny skompostované se spontánně narostlými mikroorganismy vyprodukovanými technologií Marcel Mézy®
- Aktivuje biologickou přeměnu hnoje, tekutého hnoje, organického odpadu vedlejších produktů a jakékoli jiné organické hmoty na humus (kompost)
- Dá se použít na hlubokou podestýlku, do lehoven, do hnojovice a do digestátu podle vašich technologií

BACTÉRIOLIT VÁM UMOŽNÍ

- Sníží vylučování minerálů do podzemních vod a ovzduší
- Snižuje infekční nábor patogenních mikroorganismů v prostředí zvířat jako aj amoniakální zápach v maštalích
- Menší úniky amoniaku do ovzduší
- Přírodněji kompost bez mechanické úpravy
- Snížení tvorby povrchové kůry při hnojovici a digestátu
- Přestat používat P, K, Ca a méně dusíka na půdu s Bacteriolitem v aplikovaném hnoji



APLIKACE

- **Frekvence aplikace závisí od efektu, jaký chcete dosáhnout:**
  - **Skompostování hluboké podestýlky– v jedné až dvou aplikacích v dávce 1až 2 kg/m³ hnoje, hnojovice, digestátu.**
  - **Do kompostu 30 až 60 g na 100 litrů (1–2 hrsti na 100 litrů).**
  - **Při aplikaci Bacteriolitu stačí posypat na hnojovou kopu, tak aby hnojová kopa byla upravená do tvaru dlouhé štrúdlě ne vyšší jak 1,5 metra a ne širší jak 3 metry, nakolik Bactériolit umí skompostovat hnůj až do hloubky 1metr, viď obrázek dolu.**
  - **Ozdravení prostředí – raz týdně v malých množstvích 2 až 6 kg na VDJ/měsíčně (VDJ – je velká dobytčí jednotka 1VDJ =1kráva).**

- **Třeba přizpůsobit aplikovanou dávku tak, aby se při rozhazování ošetřeného hnoje (kompostu) na půdu, aby se dodržela koncentrace 30–40 kg koncentrovaného Bacteriolitu na ha. Je to přibližně 1– 2 kg Bacteriolitu na 1m³ hnoje.**



Při aplikaci ošetřeného hnoje na půdu:

- **Nepoužívat simultánně s jinými chemickými produkty s baktericidním, fungicidním nebo herbicidním účinkem, s vápnem, superfosfátem, dezinfekcí a jinými pesticidy, s minimálním odstupem 14dní.**
- **Pro dodržení opakování můžete kombinovat s Bactériosolem.**

- **Pro dosažení deklarovaných účinků na hloubku ornice doporučujeme opakovat 3–4 aplikace v intervalech 5–12 měsíců.**





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YOUR CORN SILAGE MUST-HAVE

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Protect your silage from losses, spoilage and heating with MAGNIVA Forage Inoculants.

Why lose 13% of your corn silage, when you can save it by using MAGNIVA inoculant?* Join the thousands of successful farmers who'll be investing in MAGNIVA to ensure maximum yield and profits this year.

*Impact of MAGNIVA Platinum 1 on Corn Silage: Reduction in Yeast Levels, Enhanced Aerobic Stability, and 13% Decrease in Dry Matter Losses. Heather M. Dann[1], Cari A. Reynolds[1], Sarah Y. Morrison[1], Catherine S. Ballard[1], Richard A. Scuderi[2], and Richard J. Grant[1]. [1]William H. Miner Agricultural Research Institute, Chazy, NY, USA. [2] Lallemand Specialties Inc., Milwaukee, WI, USA International silage conference 2025

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LALLEMAND ANIMAL NUTRITION LALLEMAND

STOP MAKING SILAGE



Stop letting spoilage, dry matter loss, waste and guesswork chew through your margins.

MAKE SILAGE THE MAGNIVA WAY

MAGNIVA Forage Inoculants deliver:

- Less wastage: ***≥13% reduction in DM losses***
- Better palatability: ***Increased DMI of 0.9 kg/cow/day***
- More Milk: ***2.2 kg/c/d of energy correct milk (ECM)***
- Improved fat yields ***by 90 g (from 1.91 Kg to 2.0 Kg)***
- Improved NDF digestibility ***by 5.4%***

Get the Advantage at MAGNIVA.com

MAGNIVA
FORAGE INOCULANTS

LALLEMAND ANIMAL NUTRITION LALLEMAND

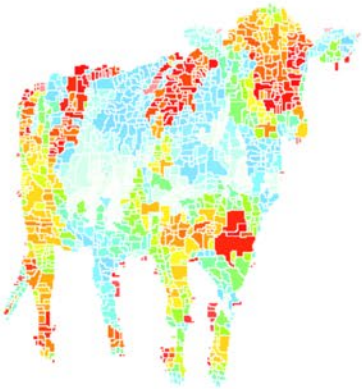


CHEMICAL LABORATORY

Chemical analysis of feeds and fermentation processes, hybrid maize selection, AgriNIR system implementation, NDF digestibility analysis, aerobic stability measurement, screening for mycotoxins and alhocols, clostridia detection, CSPS (Corn Silage Procesing Score), PSPS (Penn State Particle Separator).

SALE OF PRESERVATIVES

Biological: Ecosyl100
Chemical: Safesil, Biogas MIX
Biological - chemical: L. plantarum together with citric acid



SALE OF SUPPPLEMENTS FOR ANIMALS

Valibiom Mix:
Combination of probiotic microorganisms and herbal in one product. This product supports a healthy gut microbiome and natural animal immunity.
Debass: Stabilizes rumen pH.

DRONE APPLIATIONS FOR PRECISION AGRICULTURE

Crop growth monitoring, determination of optimal harvest timing, storage assessment, and creation of yield maps.
A precision application of biological products such as BACTÉRIOLIT and the Nourivit product line using agricultural spraying drones.

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Accurate insights from space

DRONE MONITORING

High - resolution data from fields

NUTRITIONAL ANALYSIS

Better feed quality through precise analysis

NEW IN **2026**

Agracio and NutriVet together support the future of data-driven forage production. Our technology helps determine the optimal harvest timing with greater accuracy. Advanced analytics turn complex data into practical recommendations for farmers.

The result is better silage quality and lower losses. More efficient harvesting. Better feeding.



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THE FUTURE IS CONNECTED

Space and agriculture innovation

AI

FYTO LASER

Laser seed stimulation

More energy for your seeds



How the service works

Laser seed stimulation takes place directly at the customer's site. Our equipment is fully mobile; we travel directly to farms and agricultural enterprises, where we laser-treat the seeds on-site. The service is suitable for both small and large growers. Alternatively, it is possible to order already laser-treated seeds through our business partners with whom we have established cooperation.

Our mission

We are a Czech technology company specializing in non-chemical laser seed treatment. Our mission is to support sustainable agriculture using modern and environmentally friendly technologies that naturally improve germination, vitality, and crop resistance and contribute to stable yields – without the use of chemical substances.

Non-chemical treatment.

FYTOLASER is an innovative technology of laser seed stimulation without the use of chemicals. Treatment with laser light takes place before sowing and activates biochemical processes in the seed

What Fytolaser treatment brings you

Increase in seed value, higher germination,
higher field emergence, elimination of defective seedlings.

Support of the root system,
stronger and deeper root system, dense root hairs, resistance to abiotic stresses, more efficient nutrient utilization.

Acceleration of early growth stages,
faster plant development and biomass increase, higher chlorophyll content, greater leaf coverage (LAI – Leaf Area Index)

Phytosanitary effect
reduction of pathogens

Why choose non-chemical treatment

Establishment of uniform and well-developed crop stands ready for performance

Increase in yield and product quality

Favorable cost efficiency

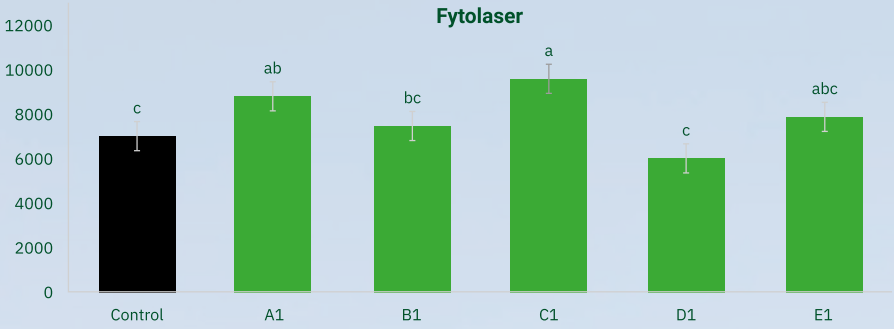
High nitrogen use efficiency (NUE – Nitrogen Use Efficiency)

Physical method without chemicals, friendly to soil and the environment

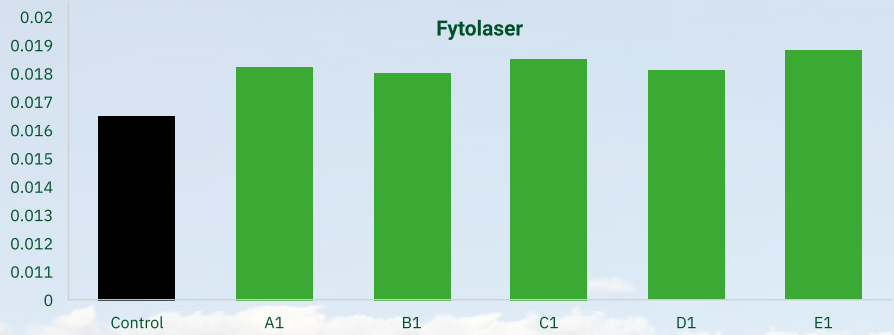
Proven technology

CATRIN-CRH: research center for new biotechnologies, Palacký University Olomouc

Leaf area – parameter AREA_PX



Growth rate – parameter RGR



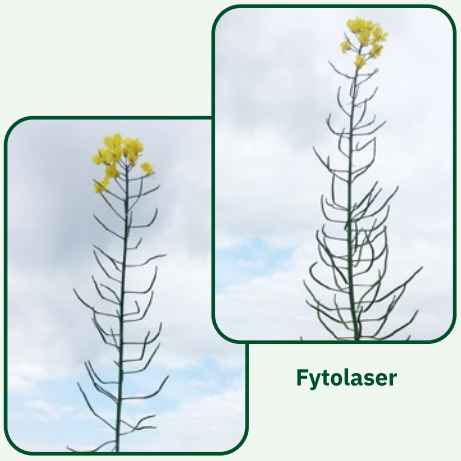
Statistically significant effect of the **FYTOLASER** method (exposure **A1–E1**) on:

→ leaf area of experimental plants (parameter **AREA_PX**)

→ relative growth, i.e. growth rate (parameter RGR)

The basis of the success of the **FYTOLASER** method is a **high root potential**, which fundamentally influences yield-forming factors.

Rapeseed pods



Rapeseed root system



Best response to FYTOLASER

- Rapeseed
- Spring cereals
- Poppy
- Soybean
- Sunflower
- Maize
- Sorghum
- Sugar beet

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CONFERENCE PROCEEDINGS

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