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USE OF MICROBIAL ADDITIVES IN ALFALFA SILAGE PREPARATION (a review)

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Alfalfa poses challenges for ensiling because of its elevated protein levels, low amounts of water-soluble carbohydrates, low dry matter content, and high buffering capacity. As a result, there has been a recent push to improve silage production using additives. In recent years, silage additives have been employed to enhance the quality of alfalfa silage. Bacterial additives are employed to enhance the quality of crop silage, with a particular emphasis on hay silage. A primary objective of incorporating lactic acid bacteria into silage is to inhibit the proliferation of undesirable microorganisms, including Clostridium and Enterobacteriaceae. This is achieved by swiftly elevating the hydrogen ion concentration to a threshold that is inhospitable for the growth of these detrimental bacteria. Recent insights into the functions of bacterial additives in crop silage suggest significant potential for enhancing silage, not just as a fermented feed, but also to deliver probiotic substances that can benefit animal health. This article provides a comprehensive overview of the silage preparation process and critically assesses a range of studies concerning the quality of silage, as well as the impact of bacterial additives on alfalfa silage. The quality of silage can be enhanced by incorporating different bacterial inoculums, which help during fermentation, storage, and feeding by improving fermentation processes, encouraging beneficial microbial diversity, and inhibiting harmful microorganisms. Alfalfa is the most important forage, and microbial additives can enhance its silage preparation in a cost-effective and environmentally friendly way.

Key words: *alfalfa, bacterial additive, buffer capacity, silage, wilting.*

Introduction. High-quality silage is achieved by reducing the activity of plant enzymes and harmful microorganisms while promoting the growth of lactic acid bacteria. Alfalfa (*Medicago sativa*) is the most widely grown forage globally, known for its exceptional nutritional value, high yield potential, and other beneficial characteristics, earning it the title of "queen of forage". For several decades, alfalfa has been highly significant, not just as a nutritious fodder crop for dairy farming, but also for its positive role in various health and environmental issues (Fig. 1) [73].

Typically, alfalfa is stored as dry fodder after harvesting, but in recent years, the production of alfalfa silage has gained popularity among ranchers. This shift is attributed to several factors: minimizing the loss of leaves and nutrients in the field post-harvest, reducing storage delays caused by adverse weather, and the fact that silage is more compatible with mechanization in industrial livestock operations [22; 33]. Various attributes of forage, including the types of silage plant species, dry matter levels, water-soluble carbohydrates, buffering capacity (which indicates resistance to pH fluctuations), and the interplay of these elements on the microbes found in the fodder, influence the outcome of the fermentation process. Alfalfa, in particular, tends to lose a significant portion of

its nutritional value during the ensiling process because of its low levels of soluble carbohydrates and its hollow stems (Fig. 2) [33; 135].

Fig. 1. Uses of Alfalfa as a multipurpose crop [73].

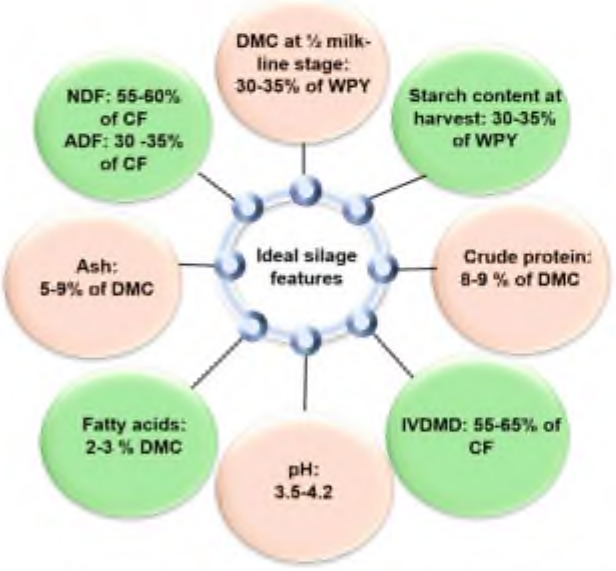

Fig. 2. Optimum level of quality parameters for good silage [62].
DMC = dry mass content; NDF = neutral detergent fiber; ADF = acid detergent fiber;
CF = crude fiber; IVDMD = In Vitro Dry Matter Digestibility;
WPY = Water-Soluble Carbohydrates.

Concerning general optimum levels for good silage (Fig. 2), the following can be stated:

– Dry Mass Content (DMC): Typically, the ideal DMC for good silage ranges from 30 % to 35 %. Silage that is too wet can lead to poor fermentation, while silage that is too dry can be difficult to compact and ferment properly.

– Neutral Detergent Fiber (NDF): Optimal levels for NDF in silage should be between 40 % to 60 % of dry matter. NDF measures the total fiber content, and it affects the intake potential of the silage by animals.

– Acid Detergent Fiber (ADF): ADF levels should ideally range from 25 % to 35 %. Lower ADF values are generally preferred as they are associated with higher digestibility.

– Crude Fiber (CF): Although crude fiber is less commonly used as a primary parameter, it generally aligns with ADF and NDF levels, and lower values indicate better quality.

– *In Vitro* Dry Matter Digestibility (IVDMD): High IVDMD values indicate better silage quality, with optimal ranges typically being above 65 %. This parameter measures the digestibility of the dry matter content.

– Water-Soluble Carbohydrates (WSC): For good silage, the WSC content should be high (above 8–10 % of dry matter), as these sugars are essential for efficient fermentation.

– pH: Silage should have a pH between 3.8 to 4.2, which ensures proper fermentation and preservation.

– Ammonia Nitrogen (% of total N): Should ideally be less than 10 %. Higher levels indicate poor fermentation.

For several decades, the addition of carbohydrate sources and bacterial additives have been studied to improve the quality of silage. Citrus pomace, tomato pomace, apple pomace, sugar beet pomace, and residues from pistachio peeling are among the by-products of the transformation and agricultural industries, which are introduced as a potential source for animal feed [135; 140]. These materials are produced in the respective factories depending on the season of fruit production and are mostly thrown away without use, which causes environmental pollution when being mismanaged, such as CH_4 release, and disposal costs. Since adding carbohydrate sources can enhance fermentation but does not stop proteolysis — caused by heterolactic fermentation and a gradual drop in pH [3] — the combined use of microbial additives and an appropriate carbohydrate source can lead to im-

proved fermentation and silage with greater nutritional value. Wilting is used to enhance the dry matter content of forage prior to ensiling. Silage that has a very low dry matter content is typically linked to higher effluent production and *clostridium* fermentation, whereas silage with a high dry matter content does not pack efficiently and significantly decreases aerobic stability. The ideal dry matter level for ensiling alfalfa varies based on the type of silo design, as well as environmental and management factors [23; 126].

The low critical pH that inhibits the growth of *Clostridium* is directly influenced by the moisture levels in the plant. In most cases, when ensiling materials have high moisture content, except for those with significant soluble carbohydrate levels, *Clostridium* fermentation occurs, resulting in silage that is of poor quality and nutritional value. Increased humidity leads to a reduction in the optimal intake of dry matter and generates a substantial amount of wastewater, which is highly nutritious and was found to be 200 times more environmentally harmful than human sewage [36; 83; 138]. Wilting enhances the lactate to acetate ratio by promoting a more uniform and restricted fermentation, thereby boosting the nutritional quality of silage. Even in the absence of additives, wilting can lead to improved silage quality by optimizing the fermentation process [142]. In the United States and Europe, bacterial additives are frequently utilized for preserving silage. These products consist of uniform strains that generate lactic acid, including *Lactobacillus plantarum*, *Enterococcus faecium*, and *Pediococcus* spp. The use of these additives leads to a quicker reduction in pH, a lower final pH, an increased ratio of lactate to acetate, and reduced levels of ethanol and ammonia nitrogen, ultimately resulting in a 1 % to 2 % enhancement in the recovery of nutrients from silage [50].

Commercially available heterogeneous additives, including *Lactobacillus buccaneri* and bacteria that produce propionic acid, generate additional volatile fatty acids. This process helps inhibit fungal growth and safeguards silages that are prone to spoilage in aerobic environments [50; 78]. The effectiveness of bacteria in silage is primarily influenced by the moisture level of the silage and is restricted by high dry matter content. Since the nutritional quality of ensiled legumes is significantly affected by the degree of protein breakdown during the ensiling process, there has been a recent focus on enhancing the quality of fodder to create feed with optimal nutritional value for livestock. Proper management of alfalfa silage is crucial for producing high-quality silage [103].

History of silage preparation

Silage is a material produced through the controlled fermentation of moist agricultural products. The process of making silage and the location where it occurs are both referred to as silage (Fig. 3) [70].

Fig. 3. Silage preparation in a traditional trench silo (Source: https://www.fao.org/4/x6512e/X6512E05.htm).

The modern techniques for producing fodder silage were developed by the French farmer Goffart, who released his initial book on corn silage preparation in 1877. Additionally, artwork from ancient Egypt, dating back to 1000 to 1500 years before Christ, indicates that the Egyptians had knowledge of silage preparation for the preservation of agricultural goods. In the Mediterranean area, airtight storage of fodder has played a crucial role in the preservation of agricultural products [47; 89].

Suitable plants for making silage

Silage can be produced from a variety of plants (Fig. 4). Some plants are specifically grown for silage production, while others are used for silage due to an excess supply. Ideal characteristics for plants intended for silage include an appropriate level of fermentable substances, particularly water-soluble carbohydrates. Additionally, the materials should have

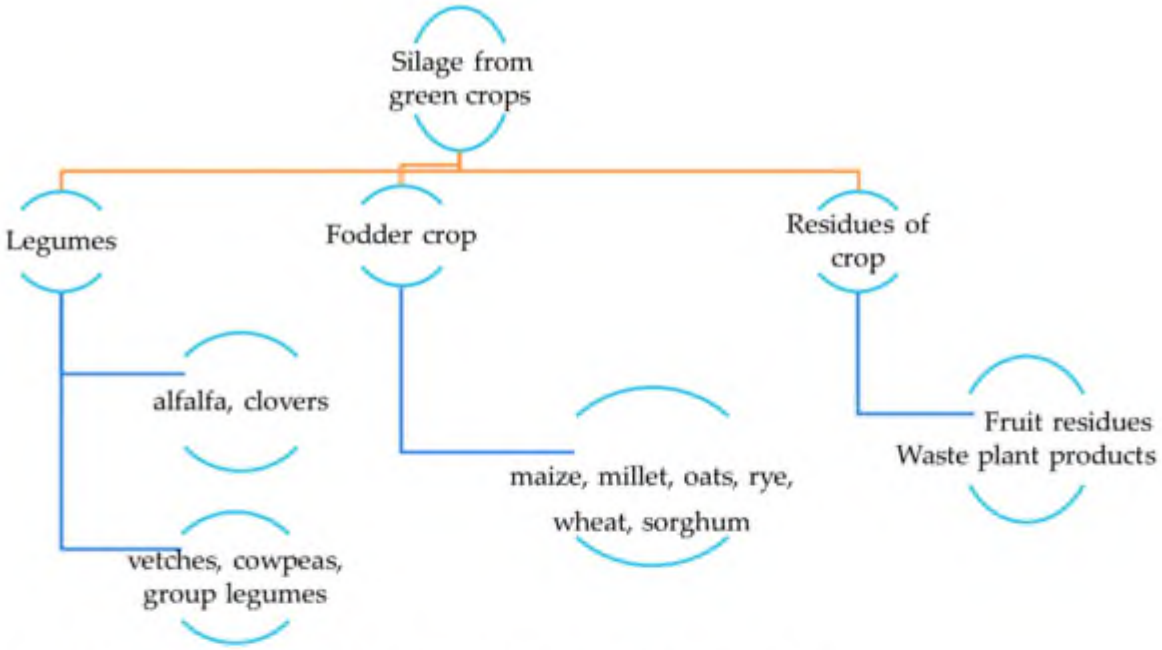

Fig. 4. Sources of silages production from various crops [116].

a relatively low buffering capacity and contain more than 20 % dry matter. The physical structure of the plants should also allow for easy compaction into silage after harvesting [21; 25; 89]. The quality and nutritional content of silage fodder is influenced by various biological and technical factors, such as the type and species of the agricultural product, its maturity stage and dry matter content at harvest, the size of the chopped fodder pieces, the rate of silage discharge, the weather conditions during harvest, and the use of additives, among others [104; 136].

The amount of fiber carbohydrates

The primary goal of producing silage is to preserve the nutritional quality of the core agricultural product as effectively as possible. This preservation is accomplished through acidification and the establishment of an oxygen-free environment, which serves as the primary substrate for the bacterial fermentation of water-soluble carbohydrates. Bacteria convert fermentable carbohydrates into organic acids, particularly lactic acid and acetic acid [23; 70; 136].

Carbohydrates found in plants are divided into two main types: structural carbohydrates and non-structural carbohydrates. Structural carbohydrates encompass hemicellulose, pectin, and fibrous polysaccharides like cellulose [38]. In grass (*Gramineae*, *Poaceae*) forage, hemicellulose features a primary xylan chain made up of D-xylose units, along with side chains that include methylglucuronic acid, as well as some glucose, galactose, and arabinose. Pectin is made up of branched chains of D-galacturonic acid units linked by (1-4) α bonds. Fibrous polysaccharides consist of cellulose, which is a linear polymer of D-glucose units. The sugars present in these structural carbohydrates are not readily available to lactic acid bacteria for fermentation. However, they can become accessible through hydrolysis by plant enzymes or enzymes added during the silage preparation process [27; 32]. Legumes have lower levels of water-soluble carbohydrates than grasses and contain minimal dry matter. The primary polysaccharide found in temperate grasses is fructan, whereas legumes primarily contain starch. Starch does not dissolve in cold water and is not classified as a water-soluble carbohydrate, making it unsuitable for most lactic acid bacteria [23; 25].

Forage protein amount

In plant growth, around 75 % to 95 % of nitrogen is found as true protein. In silage, this percentage may drop to between 30 % and 50 %. The primary cause of this issue is not microbial activity; rather, the activity of protease enzymes is significant in this context. Plant proteases serve various functions and different species exhibit varying stability based on pH and temperature. Legume forages like clover and alfalfa contain high levels of degradable crude protein. In contrast, byproducts from cereals, such as wheat straw, rice straw, and corn straw, are low in crude protein [23; 53].

Factors affecting the breakdown of proteins

Dry Matter

When plants wilt, the pH remains relatively stable, so any reduction in protein breakdown is linked to the rise in dry matter. Slight wilting of materials can actually enhance protein degradation by preventing acidification [18; 23; 119].

pH reduction

If the pH drops gradually, the breakdown of proteins will rise. Plant protease enzymes function effectively at pH levels above 4 [18; 23]. This contradicts the assertion that plants do not possess active protease enzymes at pH levels below 4 [83]. Current knowledge indicates that numerous plant proteases have optimal pH levels that are lower than this threshold. The activity of these enzymes declines steadily between pH 4 and 6 [81; 82].

Temperature

Since plant protease enzymes function best at elevated temperatures, therefore raising the temperature in silage enhances their activity [18; 23; 82].

Plant Species

The influence of species on the rate of protein breakdown remains uncertain. The extent of protein degradation while materials are stored in the silo is influenced by the proteolytic potential and the rate of pH decline. Proteolytic potential refers to the overall activity of the protease enzyme, as well as the availability and affinity of the materials. This trait varies among different species and is likely influenced by crop management practices and environmental conditions [18; 23; 82].

Time

The activity of proteolysis diminishes over time during the process of silage preparation. In the case of both corn and alfalfa, proteolytic activity takes place within the first hour of the initial day following silage preparation, and it declines after five days. This reduction in proteolysis over time is not influenced by the dry matter content of alfalfa [18].

Natural process of forage fermentation and silage production

Once the plant is harvested from the field, its cells remain active, and the microorganisms in the silo generate CO_2 and heat. The process of turning fresh fodder into preferred silage involves several fermentation stages, which take approximately 21 days to complete (Fig. 5) [12; 60].

Respiration stage of the plant

The respiration process in plant cells and microorganisms within the silo generates water, carbon dioxide, and heat. This phase is referred

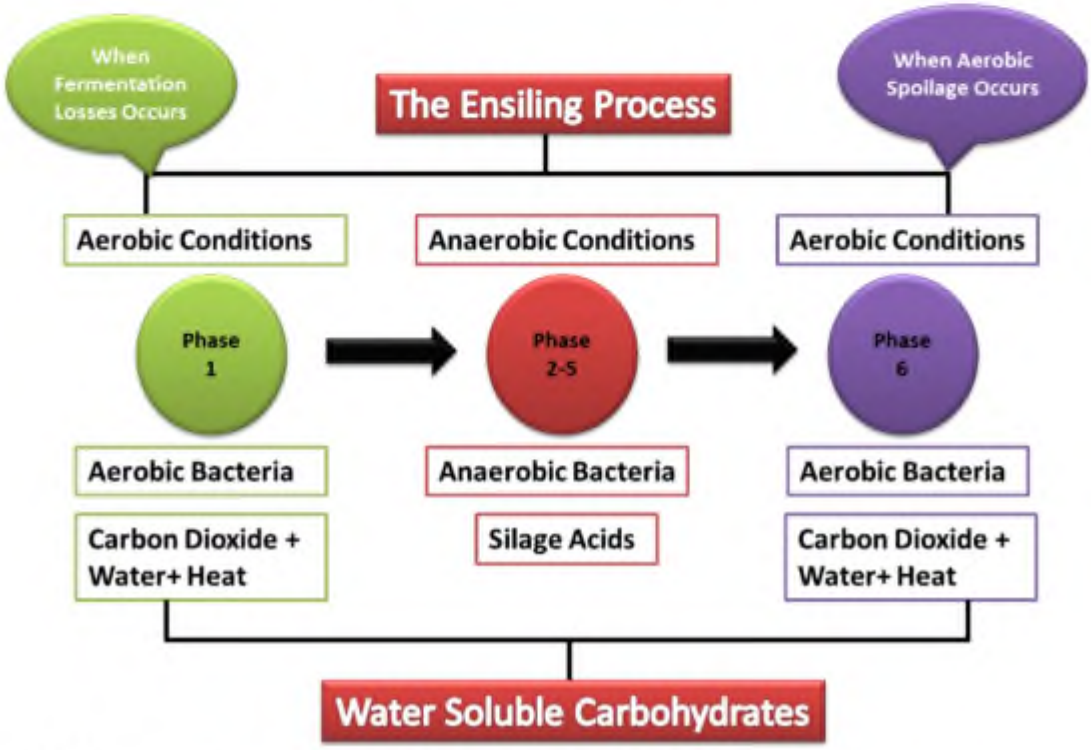

Fig. 5. The ensiling process [5].

to as aerobic respiration since it requires oxygen. As carbon dioxide levels rise, the rate of cellular respiration diminishes and ultimately ceases. The heat generated by aerobic bacteria raises the temperature of the silage. Typically, this respiration phase lasts between 3 to 5 hours, depending on the oxygen availability in the silo [12; 60].

Acetic acid production stage

This phase starts when the silage runs out of oxygen, leading to the growth of anaerobic bacteria. Bacteria that produce acetic acid utilize the carbohydrates in the silage, transforming them into acetic acid, which initiates the acidification process and lowers the pH from 6 to 5. The reduction in pH results in a decline in the population of acetic acid-producing bacteria. A swift drop in pH restricts the function of enzymes that break down proteins. This fermentation stage typically lasts between one to two days [12; 60; 70].

Lactic acid production stage

This phase starts when the quantity of acetic acid-producing bacteria from the earlier phase declines. Lowering the pH of the silage environment enhances the growth of lactic acid-producing bacteria, leading to the decomposition of carbohydrates and the formation of lactic acid, ethanol, mannitol, and carbon dioxide [12; 60].

Peak stage of lactic acid production

This is the longest phase of fermentation, and is the third stage. Lactic acid production begins in this stage, reaching its highest levels here. This phase lasts for about two weeks until the pH decreases sufficiently to inhibit the growth of all bacteria. The fodder mass stabilizes in roughly 21 days, and fermentation ceases, assuming no air enters. If fermentation is successful during this stage, the anticipated pH for silage will range from 4.5 to 3.5, depending on the moisture level of the forage [12; 60]. When the conditions in the silo are not ideal, it creates an environment conducive to the growth and activity of *clostridia*, which are harmful bacteria. These anaerobic bacteria generate butyric acid. Both *clostridia* and coliforms have the ability to decompose amino acids, transforming them into volatile fatty acids, amines, and ammonia [35; 74].

Silage fermentation occurs naturally in anaerobic environments thanks to the bacteria found in plants, but the efficiency of this fermentation can vary based on the specific types of lactic acid bacteria present in the feed. Factors such as the speed of pH decline, the quantity of sugar that remains unutilized by bacteria, the preservation of true protein, and the levels of lactic acid, acetic acid, and ethanol all influence the quality of the silage [31, 50].

Effective factors in silage preparation

The effective preservation of fodder and other products relies on suppressing microbial activity, particularly that of bacteria. Ideal conditions for storing silage involve eliminating air from the silo and ensuring anaerobic conditions. Under these circumstances, the population of lactic acid bacteria increases as they utilize the plant's internal sugars, producing significant amounts of acid that lower the pH to around 4. Preserving fodder can be challenging because it can create favorable conditions for the growth of harmful bacteria like *clostridia*. When harvested, the pH of the forage ranges from 6 to 7, but with proper fermentation during ensiling, the pH can drop to 4 or lower [35; 74].

The pH of silage drops as lactic acid and other organic acids are generated by lactic acid bacteria. Accelerating the pH reduction is crucial for preserving the nutritional quality of the silage. A decrease in pH also leads to a reduction in the populations of *clostridia* and coliform bacteria [35; 82]. In addition to reducing the pH and inhibiting microbial activity, other factors are also effective in preserving silage, such as the way of filling the silo, the size of the chopped fodder, proper compression of fodder, and proper structure. Silage and proper silage closure are noted [12; 60; 135].

Effective microorganisms in the ensiling process

The bacteria that are typically sought after in the ensiling process belong to the lactic acid-producing group, which includes species such as *Streptococcus*, *Lactobacillus*, and *Pediococcus*, among others (Fig. 6) [20;103].

These bacteria generate lactic acid by fermenting water-soluble carbohydrates and can utilize organic acids like citric and malic acid found in plant cells as a substrate for mixed fermentation. A high concentration of water-soluble carbohydrates enhances the activity of lactic acid-producing bacteria. Conversely, a low concentration of these carbohydrates promotes

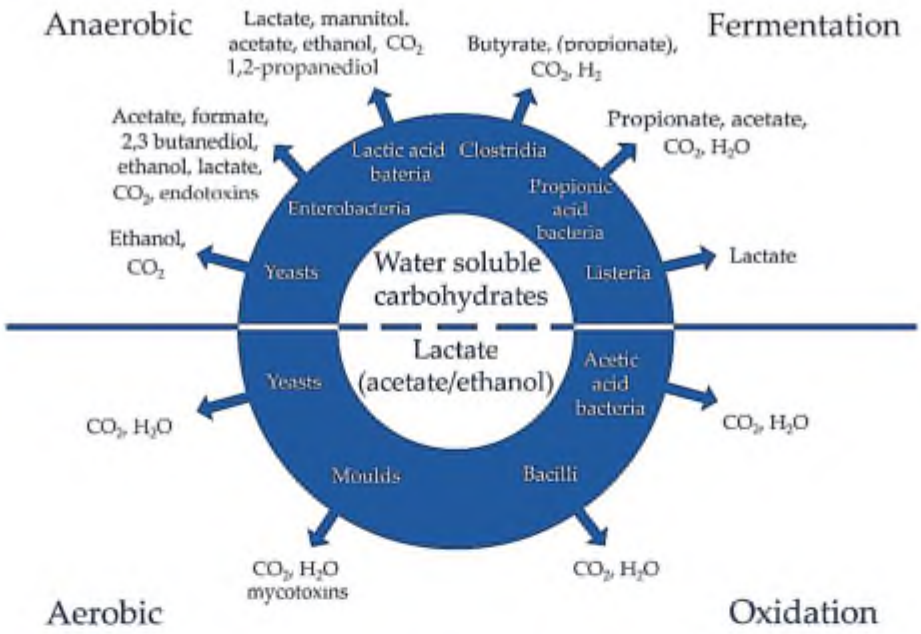

Fig. 6. Main groups of microorganisms involved in the fermentation process [135].

the activity of clostridial bacteria, which are undesirable as they thrive in anaerobic environments and lead to silage spoilage by producing butyric acid. These unwanted bacteria compete with lactic acid producers for water-soluble carbohydrates and yield less acidic byproducts such as acetate, butyrate, propionate, and ethanol. Clostridia growth is more pronounced in plants with high moisture content but is suppressed at a pH of 4 or lower, which also inhibits most plant protease activity [12]. The main objective of using silage additives is to promote the growth of lactic acid bacteria during fermentation, leading to the creation of high-quality silage. These additives facilitate the desired fermentation process, restrict unwanted fermentation, and enhance the nutritional value of the silage [20; 103].

Silage additives can be classified into various groups: Fermentation stimulators, which include microbial inoculants like enzymes (cellulase, hemicellulase) and molasses; fermentation inhibitors, such as formic acid, formaldehyde, and sulfuric acid; nutrients like ammonia and urea; moisture-absorbing substances; and agents that prevent aerobic spoilage [139]. The microbial mass that can be used consists of both dry and living microbes, which remain inactive until they are hydrated. This microbial material is available in two forms: liquid and dry. For the liquid form, the microbes need to be activated before they interact with the fodder to minimize their dormant period and maximize their fermentation capability. In the dry form, the microorganisms are activated by the moisture in the fodder, resulting in a slower initial fermentation rate. It is important to follow the manufacturer's guidelines when using these products [23].

The most commonly used microbes for microbial impregnation are lactic acid bacteria, such as *Lactobacillus*, *Epenococcus*, and *Enterococcus* species. These bacteria enhance lactic acid production, leading to a quicker drop in pH and inhibiting the growth of undesirable fermentation microorganisms in silage. The high natural bacterial populations in silage can hinder microbial impregnation from achieving a competitive edge. In contrast, alfalfa and clover have lower natural bacterial levels, making their microbial inoculation more effective than that of corn silage [23]. Kung et al. [72] discovered that microbial impregnation improved the fermentation process in both grass and legume silage.

The process of microbial impregnation in silage preparation aims to lower the pH in the early phases of fermentation, maintain plant carbohydrates through uniform fermentation, and protect plant protein by minimizing proteolysis and deamination [36; 50; 116]. As a result, we anticipate that treated silage will enhance animal performance [83]. The second type of additives consists of fermentation inhibitors that prevent undesirable fermentation. These products typically contain a mix of acids. The goal of using these acids is not to halt the fermentation process, but rather to enhance the

natural fermentation by introducing moderate levels of acids [116].

The importance of the alfalfa plant in animal nutrition

Alfalfa and various other perennial leguminous plants are significant forage crops due to their capacity to generate a substantial quantity of high-quality fodder. No other group of forage products can offer a superior balance of energy, protein, and minerals for high-yielding livestock [33; 122].

Traditionally, legumes have been utilized as natural grazing and dry feed, but in recent years, the practice of ensiling these products has become a popular method of preservation [18], particularly in areas with high rainfall that restricts the production of dry fodder. Nonetheless, it is important to take into account certain adjustments to the livestock feeding strategy, as the chemical and physical characteristics of forage can alter during the ensiling process. By fully grasping this process, it is possible to convert nearly all agricultural products into silage without negatively impacting livestock production [25; 45]. Alfalfa can typically be fermented into stable silage, as long as its limitations are clearly recognized. Key issues with using alfalfa as silage fodder include insufficient water-soluble carbohydrates, a high buffering capacity, hollow stems, and low dry matter content [52].

Final products of fermentation

The ultimate fermentation products are indicative of the predominant microorganisms present during the silage process and significantly influence the overall quality of the silage. Microorganisms involved in silage fermentation facilitate the conversion of water-soluble carbohydrates into organic acids. The end products of this fermentation process can arise from various soluble sugars [103]. Lactate serves as a marker for bacterial fermentation characterized by the production of uniform lactic acid during the fermentation process. In contrast, the presence of a combination of acetate, propionate, and lactate suggests the prevalence of heterogeneous fermentation. Elevated levels of butyrate and ammonia in silage are indicative of fermentation by *Clostridium* spp. Additionally, ammonia nitrogen is generated through the action of plant enzymes and enterobacteria, as well as through the processes of nitrite and nitrate regeneration [52].

The effect of maturity on soluble carbohydrate content

Generally, the concentration of sugars in alfalfa diminishes as the plant matures. Raguse and Smith [115] documented a 15.3 % reduction in the total quantity of non-structural sugars. Additionally, it has been observed that there is a decline of 51.8 % in sucrose levels, 19.2 % in glucose levels, and 14.8 % in fructose levels from the budding stage to the 50 % flowering stage, a phenomenon that some researchers attribute to a reduction in the leaf-to-stem ratio. Other scholars have suggested that this decline can be ascribed to two primary factors: first, a decrease in photosynthetic activity of the leaves as the plant ages, and second, an increase in shading resulting from higher plant density, which further diminishes photosynthesis. Furthermore, the stage of maturity also influences the content of soluble carbohydrates post-harvest, particularly in relation to plant respiration (Fig. 7). Geronimo and Beevers [46] indicated that 40 % to 60 % of the reduction in respiratory rate is mitigated by the maturation process.

Forage quality typically indicates the overall nutrient content that livestock can obtain from forages. Several factors influence forage quality, which can be divided into three categories: plant factors, animal factors, and environmental factors. Gaining a clear understanding of how these factors interact with forage quality can assist in selecting the right forages and supplements to meet animal needs, ultimately leading to improved livestock performance and economic benefits [63]. Amer et al. [6] determined that the nutritional value of forage sorghum silage is better than that of forage millet silage when both are harvested at the same point in their physiological growth.

The effect of ensiling on soluble carbohydrates

During the ensiling process, soluble carbohydrates undergo conversion into organic acids. It is widely recognized that lactic acid bacteria lack the capability to hydrolyze starch during this phase of ensiling. According to Smith [115], a minimum concentration of 6 % to 7 % water-soluble carbohydrates, relative to dry matter, is necessary to achieve a silage pH of 4. However, in practical applications, this optimal level of soluble carbohydrates may not be readily available in the field. The concentration

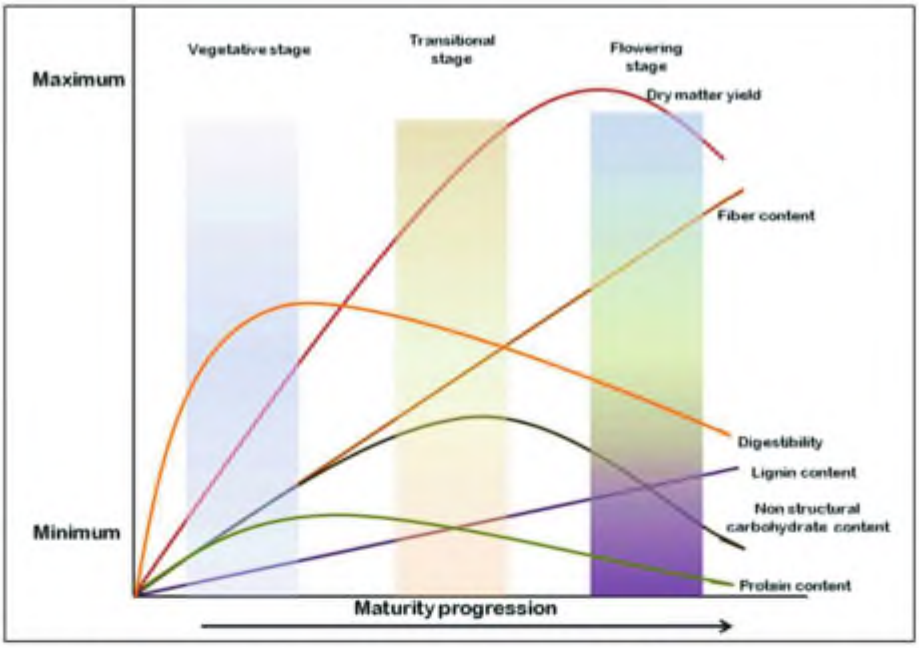

Fig. 7. Maturity influencing forage nutritional quality [63].

of soluble carbohydrates in alfalfa fodder typically ranges from 2 to 7 percent of dry matter, with variations influenced by the stage of maturity and prevailing weather conditions.

Buffering capacity in silage fodder

The buffering capacity of plant materials is closely associated with their ability to resist changes in pH, which is a critical consideration in the ensiling process. It is widely believed that organic acids and their corresponding salts play a predominant role in the buffering capacity observed under silo conditions. Conversely, some researchers propose that inorganic ions contribute significantly to the buffering activity of legumes. Recent studies lend support to the hypothesis that organic acids are the primary contributors to this buffering capacity. The concentration of organic acids in grass forage typically ranges from 2 % to 6 %, while in legume forage, it is generally higher, approaching 6 % to 8 %. Additionally, proteins appear to have a minimal impact on the buffering capacity of forage materials [25; 33].

The effect of wilting on buffering capacity

Wilting diminishes the buffering capacity of fodder in comparison to fodder that is ensiled immediately, a phenomenon attributed to the transformation of organic acids into water and carbon dioxide during the respiration process. The rate of water loss during the wilting stage influences the quantity of residual organic acids, subsequently affecting the buffering capacity; a gradual loss of water can further deplete the levels of remaining organic acids. Research indicates that the buffering capacity declines by approximately 150 % as the maturity stage progresses from pre-budding to 50 % flowering [91; 126].

Effect of wilting on silage quality

Wilting is applied to optimize the amount of dry matter of forage before ensiling. Silage with very low dry matter is often associated with increased effluent production and clostridium fermentation. Whereas, silage with high dry matter does not compress well and greatly reduces aerobic stability [33]. The optimal dry matter for ensiling alfalfa depends on the type of silo construction, environmental and management conditions. Ishler et al. [57] suggested that the amount of dry matter suitable for silage is 30 to 35, 40 to 45, 45 to 60 percent for tower, oxygen-free and bag silos, respectively [113].

The moisture content of ensiled plants significantly influences both the total bacterial population and the rate of fermentation. The process of wilting tends to inhibit bacterial proliferation, whereas the addition of water to forage promotes bacterial growth, particularly among *lactobacilli* and gram-negative bacteria. Furthermore, wilting may impact the relative proliferation of both homogeneous (homofermentative) and heterogeneous (heterofermentative) lactic acid bacteria. Generally, silages of inferior quality are produced under two specific

conditions: first, when silages possess low dry matter content, which diminishes ammonia production due to clostridial fermentation; and second, when silages exhibit excessively high dry matter content. In the latter scenario, although fermentation activity is reduced, there is a pronounced increase in the growth of yeasts and fungi, leading to elevated heat production. This heat generation subsequently contributes to an increase in the formation of insoluble nitrogen in acid detergent (ADIN) and results in the degradation of certain amino acids, including methionine, cysteine, and tyrosine [127]. McDonald et al. [83] conducted a study examining the qualitative alterations in lactobacilli during the ensiling process of gramine and red clover forage under varying humidity conditions. Their findings indicated that after a 142-day ensiling period, 75 % of the total lactobacilli present in silage with high humidity and 98 % of those in silage with low humidity were classified as heterogeneous types.

Wilting elevates the energy demands associated with the maintenance and growth of microbial populations, while also prolonging the digestion of plant cells. Additionally, it diminishes plant respiration and enzymatic activity. Notably, although wilting does not decrease the overall level of proteolysis, it has the potential to mitigate this process under optimal conditions [4; 22]. Owens et al. [105] found that fertilization not only failed to decrease the levels of non-protein nitrogen but actually resulted in an increase. The process of wilting the plant inhibits the release of plant effluent, which is significant for two reasons: first, it avoids the polluting effluent; second, the leachate released in this manner affects both the environment and the nutritional quality of silage [48].

The research conducted by Whiter and Kung [132] demonstrated that lactic acid-producing bacteria exhibit greater resistance to variations in osmolarity compared to other microbial species and are relatively unaffected by humidity levels. Their findings revealed that the ratio of lactic acid to acetic acid in dry matter at 30 % concentration was between 2.6 % and 3 %, while at 54 % dry matter concentration, this ratio exceeded 8.5 %. This observation suggests a notable increase in the ratio of homogeneous to heterogeneous fermentation processes.

Kung et al. [71] conducted a study on alfalfa fodder with varying dry matter content of 30 %, 40 %, 50 %, and 60 %. Their findings indicated that the pH level increased at 60 % dry matter concentration. Additionally, they observed a reduction in lactic acid levels when dry matter exceeded 40 %, while the concentration of remaining soluble sugars increased. This phenomenon was attributed to the stimulation of microbial growth due to higher moisture levels, which in turn led to an increased consumption of soluble carbohydrates. Furthermore, to establish stable conditions during the silo's stable phase, a greater production of lactic acid was necessary to inhibit bacterial activity. The study also revealed a general decline in crude protein content with increasing dry matter, which was linked to leaf loss during the harvesting and silage processes. Moreover, the wilted treatments exhibited a decrease in dissolved nitrogen, ammonia nitrogen, and free amino acids, which was associated with reduced clostridial and enzymatic activity. Gou et al. [49] studied the optimal harvest timing for ensiling alfalfa by collecting first and second cuts at the budding stage (BS), initial flowering stage (IFS), and full flowering stage (FFS) during 2016 and 2017. After harvesting, the alfalfa was ensiled for 45 days. Their findings indicated that, in terms of nutritional quality, fermentation properties, and in vitro rumen digestibility, the ideal harvest stages were BS for the first cut and IFS for the second cut. Additionally, they found that alfalfa silage from the first cut was superior to that from the second cut at the same maturity stage.

Wilting has been shown to enhance the concentration of residual soluble carbohydrates in comparison to non-wilted forage through several mechanisms. Firstly, the process of wilting decreases water activity, which in turn inhibits microbial growth [28]. Additionally, during the wilting process, a significant number of bacteria are eliminated relative to unharvested fodder, thereby limiting fermentation [71]. Consequently, the introduction of bacteria to wilted forage appears to be essential. Furthermore, wilting reduces the buffering capacity of the forage, which diminishes the quantity of lactic acid required to establish stable conditions, ultimately preventing further fermentation by maintaining these stable conditions [33].

In the study conducted by Whiter and Kung [132], alfalfa was evaluated at two distinct levels of dry matter content, specifically 30 % and

54 %, utilizing two varieties of soluble and dried microbial additives. The findings indicated a significant decline in the proliferation of lactic acid bacteria within the silage characterized by higher dry matter on the second day, with a return to baseline levels observed by the eighth day. Notably, no significant differences were detected between the two experimental groups after the fourteenth day. Furthermore, the concentration of acetic acid in the dry matter remained unaffected by the introduction of bacterial additives. This experiment revealed that acetic acid-producing bacteria exhibit activity during the initial phases of ensiling, and their growth is not inhibited in less moist fodder, where the pH decreases at a more gradual rate, indicating that the additives do not influence their levels. Additionally, it was observed that the population of lactic acid bacteria, such as Lactobacillus plantarum, diminishes when water activity decreases from 0.987 to 0.949. Ridla et al. [111] conducted a meta-analysis to evaluate the impact of wilted and unwilted silage on various parameters. They demonstrated that wilting prior to ensiling greatly enhanced silage quality by boosting dry matter and water-soluble carbohydrates, while also decreasing dry matter losses, butyric acid, and ammonia levels. Notably, wilting did not significantly affect pH, crude protein, or in vitro dry matter digestibility.

Silage epiphytic population

These microorganisms are inherently found on the fodder itself, with lactic acid bacteria, enterobacteria, Clostridium species, yeasts, fungi, and aerobic bacteria being particularly significant in determining the quality of silage. The diversity of aerobic bacteria is influenced by various factors, including plant species, different plant parts, climatic conditions, seasonal variations, the process of wilting, and mechanical crushing [49, 51]. This group of microorganisms requires aerobic respiration, utilizing molecular oxygen as an oxidizing agent to generate the energy necessary for their metabolic processes. Aerobic bacteria are prevalent during the growth phase of plants and continue to engage in fermentation and respiration for several hours post-harvest and ensiling. They remain metabolically active and are capable of utilizing over 100 different types of organic compounds, which can lead to a reduction in nutritional value by approximately 1 % to 2 % during the initial stages of the ensiling process (Table 1) [134].

This issue can be mitigated by promptly establishing an anaerobic environment. Upon the opening of the silo, microbial activity resumes, leading to aerobic degradation [33]. Research conducted by Woolford [134] identified bacilli as the primary agents of aerobic decomposition during the feeding phase. However, more recent studies have indicated that certain species of acetobacter also play a crucial role in initiating this decomposition process, particularly under low pH conditions where they metabolize fermentation byproducts. This metabolic activity not only diminishes the nutritional quality of silage but also poses a potential health risk to livestock and individuals handling these materials due to the production of endotoxins [134].

Enterobacters

This group encompasses a diverse family of bacteria characterized as gram-negative, non-spore-forming, facultatively anaerobic, and

Table 1. *The substrate being fermented by epiphytic microorganism* [22; 83]

Organism	Substrate	Products
LAB (Ho)	Glucose	2 lactate
LAB (He)	3 Fructose	1 lactate, 1 acetate, 2 mannitol, 1 CO
Enterobacteria	2 Glucose	1 lactate, 1 acetate, 1 ethanol, 2 CO_2
LAB (He)	Glucose	lactate, 1 ethanol, 1 CO_2
LAB (Ho/He)	2 Citrate	1 lactate, 3 acetate, 3 CO_2
LAB (Ho/He)	Malate	1 lactate, 1 CO_2
Yeasts	Glucose	2 ethanol, 2 CO_2
Clostridia	2 Lactate	1 butyrate, 2 CO_2, 2 H

LAB: lactic acid bacteria; HO, homofermentative; HE: heterofermentative.

typically motile, although some may exhibit non-motility. These bacteria, which are rod-shaped and metabolize soluble carbohydrates, are commonly referred to as coliforms or acetic acid-producing bacteria, although the latter designation is often misapplied [33, 83]. Research findings regarding the population dynamics of these bacteria during placentation have been inconsistent, some studies report an increase in their numbers, while others indicate a decrease. These variations are largely contingent upon the initial population size prior to the onset of placentation.

The process of chopping forage enhances the proliferation of specific microbial populations. The abundance of these bacteria typically equals or exceeds that of lactic acid bacteria [25]. Under anaerobic conditions, these microorganisms exhibit a pronounced requirement for fermentable carbohydrates. As lactic acid bacteria proliferate and pH levels decline rapidly, enterobacteria experience a significant reduction in numbers. However, if the decrease in pH is delayed or if formic acid is introduced, these bacteria demonstrate resilience. Enterobacteria primarily generate acetic acid, lactic acid, carbon dioxide, hydrogen, and trace amounts of ethanol and 2,3-butanediol.

The population of Enterobacteriaceae exhibited an increase during the initial days of the ensiling process, ultimately reaching a peak concentration of 10^8 to 10^{10} CFU per gram in both grass and leguminous fodder. Although these bacteria possess limited proteolytic activity, they are capable of deaminating or decarboxylating certain amino acids, and a majority of species are known to regenerate nitrate. Enterobacter species are particularly notable for their capacity to generate substantial amounts of ammonia during ensiling, which plays a crucial role in the decomposition of nitrate under conditions conducive to regeneration within the silage. This biochemical process is essential for determining the final chemical quality of the silage and serves to inhibit the proliferation of Clostridium by facilitating the production of nitrate and nitric oxide derived from nitric oxide [135; 137].

In the initial phase of the ensiling process, there is a notable increase in the population of enterobacteria, which coincides with a peak in nitrate concentration. This period is characterized by the maximum decomposition of nitrate, which may serve as an effective anti-clostridial agent of endogenous origin, particularly under conditions of elevated pH when *Clostridium* spores begin to proliferate. However, in regions where fodder is processed into silage, the relationship between nitrate levels and the suppression of *Clostridium* activity remains inadequately substantiated. The significant reduction in Clostridium activity is more commonly attributed to decreased water activity and increased osmolality within the silage, rather than the presence of elevated nitrate concentrations. Generally, the presence of enterobacteria is considered undesirable, as these microorganisms compete with lactic acid bacteria for nutrient uptake and can produce endotoxins. Furthermore, they may contribute to ammonia formation through protein degradation and nitrate reduction during the ensiling process, which can enhance buffering capacity and inhibit pH decline. Ultimately, the rapid decline of enterobacterial populations serves as a more reliable indicator of high-quality silage production than any other metric [18], [135].

In the study conducted by Kizilsimsek et al. [66], it was demonstrated that the concentration of enterobacters in fodder was notably elevated, corroborating the findings of Lin et al. [145]. The bacterial counts remained consistent across treatments during the initial six hours of ensiling. However, at the twelve-hour mark, a treatment incorporating additives in the form of fresh culture exhibited a reduction in bacterial numbers, and by twenty-four hours, both types of additives significantly decreased the enterobacters compared to the control treatment. Prior research has indicated that in inoculated treatments, the decline of these microorganisms occurs at an accelerated pace. The authors referenced Kung et al. [72] to explain these observations, attributing the results to the sensitivity of enterobacters to low pH levels.

Clostridium

These bacteria are characterized as gram-positive, spore-forming, typically motile, obligate anaerobes, and exhibit a rod-shaped morphology. They are categorized based on their substrate utilization into two distinct groups: 1) saccharolytic bacteria, which primarily metabolize sugars with minimal activity on proteins, and 2) proteolytic bacteria, which ferment amino acids. Certain species possess the capability

to utilize both fermentation substrates [25]. In unharvested fodder, the concentration of these microorganisms can reach approximately 100 per gram of fresh material; however, this number significantly increases following harvesting and processing. It is important to note that these bacteria are not epiphytic; their presence in silage is attributed to contamination from soil and fecal matter during the harvesting process or from precipitation entering the silage environment. Due to their obligate anaerobic nature, these bacteria become active in the subsequent stages of ensiling, when oxygen is absent and anaerobic conditions prevail [33].

A pH level exceeding 5, which is conducive to the activity of Clostridium, also provides an optimal environment for the functioning of plant proteolytic enzymes. The action of these enzymes facilitates the release of amino acids, which Clostridium subsequently utilizes in three distinct processes: 1) deamination, leading to the formation of organic acids and ammonia; 2) decarboxylation, resulting in the production of amines and carbon dioxide; and 3) ongoing reactions that yield organic acids, carbon dioxide, ammonia, and alcohols [83]. Clostridium fermentation enhances proteolytic activity, leading to the generation of water-soluble nitrogen, which can be categorized into two components: ammonia nitrogen and non-ammonia nitrogen. The presence of ammonia nitrogen significantly reduces the quantity of metabolizable protein, potentially impacting milk production, milk urea nitrogen levels, and nitrogen use efficiency in dairy production [52]. A direct correlation exists between the concentration of clostridia and the quality of silage, with significant clostridial activity potentially resulting in a reduction of up to 50 % in the nutritional value of the silage. Several factors that may influence the proliferation of these bacteria include: 1) temperature, 2) the proportion of dry matter, 3) the level of soluble carbohydrates, 4) the buffering capacity of the feed, and 5) the efficiency of silo closure and compaction [70; 89].

In addition to losses incurred during fermentation, spores from Clostridium species may contaminate milk and impede the coagulation process in hard cheese production [83]. Certain Clostridium species, such as *Clostridium butyricum*, are known to cause botulism in silage that has been contaminated with soil, posing a risk to livestock. Given that these bacteria exhibit sensitivity to acidic pH levels, their proliferation can be mitigated through the application of organic and mineral acids. However, organic acids demonstrate greater efficacy than mineral acids in inhibiting the growth of these microorganisms. Furthermore, the incorporation of microbial additives represents an additional strategy to curtail the proliferation of these bacteria. These additives not only facilitate the production of lactic acid and other volatile fatty acids but also inhibit microbial growth. Additionally, they can produce antibiotic compounds, such as nisin, which serves as a food preservative and further suppresses the growth of these bacterial species [134].

Clostridia thrive in humid environments; however, when the dry matter content of a plant reaches 30 % and the plant is wilted, the proliferation of these bacteria is inhibited. Clostridia exhibit a higher sensitivity to water activity compared to lactic acid bacteria [132]. Furthermore, the critical pH level conducive to Clostridium growth is directly correlated with water activity levels. Water activity, alongside dry matter or moisture content, serves as a more precise indicator of the water available for microbial growth during the fermentation process. It influences all four phases of microbial growth: 1) the duration of the incubation period, 2) the growth rate, 3) the stabilization or stationary phase, and 4) the rate of bacterial mortality [121; 132].

The buffering capacity significantly influences the proliferation of these bacteria, as the buffering capacity of the plant increases, the quantity of lactic acid required to lower the pH to a critical threshold that inhibits the growth of *Clostridium* also rises. A primary challenge associated with legumes is their elevated buffering capacity coupled with a low concentration of water-soluble carbohydrates, which typically results in the dominance of Clostridium, unless the plants are ground prior to ensiling or suitable additives are employed. The relationship between temperature and Clostridium growth is complex, as the heat generated within silos is primarily due to the oxidation of sugars, leading to a depletion of soluble carbohydrates and a subsequent reduction in lactic acid production. Furthermore, it appears that elevated temperatures may promote the growth of *Clostridium* [102].

It is widely accepted that Clostridia are strictly anaerobic organisms, which suggests

they do not contribute to aerobic spoilage. However, Jonsson [61] demonstrated that the introduction of air can indirectly promote the proliferation of *Clostridium thyrobutyricum*. In his study, silage produced under optimal conditions within laboratory silos exhibited a significant increase in *Clostridium* spores and butyric acid when exposed to air and subjected to aerobic degradation. This phenomenon was observed both on the surface of the silage and in deeper regions characterized by low pH levels. It is hypothesized that *Clostridia* thrive in localized microenvironments created by aerobic microorganisms, such as yeasts and certain *Bacillus* species, which utilize lactic acid and amino acids. This growth is likely facilitated by a reduction in lactic acid concentration, a decrease in oxygen levels, and an increase in pH, ultimately resulting in conditions conducive to *Clostridium* proliferation.

In a study conducted by Vissers et al. [125] to corroborate the findings of Jonsson [61], the researchers examined the concentration of *Clostridium* spores and its correlation with aerobic stability. The initial experiment revealed that the quantity of spores ingested by livestock is influenced by small portions of silage that contain a high density of spores. Elevated concentrations of spores are typically found in regions where mold is present. Specifically, the areas exhibiting mold on the surfaces of grass silage and corn silage account for approximately 21 % and 19 %, respectively, of the regions with elevated spore levels. In the subsequent experiment, it was demonstrated that the concentration of butyric acid-producing bacterial spores is predominantly found within the upper 50 cm of silage, and an increase in spore numbers is associated with indicators of aerobic instability. Furthermore, high concentrations of yeasts, as well as areas exhibiting temperatures 5 degrees Celsius above ambient or pH levels exceeding 4.4, are generally correlated with a significant presence of spores. The researchers posited that the elevated levels of butyric acid-producing bacterial spores in corn silage are associated with regions where oxygen infiltration occurs, resulting in aerobic decomposition. This process contributes to the formation of anaerobic conditions and an increase in pH at the silage surface, thereby facilitating the proliferation of butyric acid-producing bacteria in these specific areas [125].

Yeasts and molds

Yeasts are characterized as unicellular organisms, whereas molds consist of multicellular filamentous structures. The majority of fungal species are aerobic, requiring oxygen for their growth; however, yeasts possess the capability to generate energy in anaerobic environments. The proliferation of yeasts and molds is enhanced by the incorporation of silage, and their populations tend to rise following the growing season [41]. Yeasts are known to metabolize sugars into alcohol [33], which is linked to organoleptic quality issues in milk. This fermentation process can lead to alterations in rumen fermentation dynamics, resulting in increased concentrations of rumen acetate and caproic acid, ultimately diminishing the palatability of the feed. Furthermore, yeasts possess the capability to utilize organic acids [69] and can produce mycotoxins, rendering them an undesirable component in silage. They play a significant role in aerobic degradation and can be classified into two categories: 1) lactate fermenters and 2) sugar consumers.

According to Woolford [134], the presence of lactate-degrading yeasts at concentrations of 10^5 CFU per gram of material increases the likelihood of silage spoilage. Subsequently, molds appear to contribute to the further degradation process. The anaerobic and acidic conditions characteristic of silos are highly unfavorable for yeast proliferation. Short-chain fatty acids, such as propionate and acetate, exhibit inhibitory effects on yeast growth, with unsaturated fatty acids demonstrating even greater efficacy. This enhanced effectiveness is likely attributed to the more rapid penetration of these compounds into yeast cells, where they subsequently lower the intracellular pH by releasing protons, leading to the rapid destruction of the yeasts. Under aerobic conditions, the energy required to extrude protons is derived from the oxidation of various substrates, whereas in anaerobic conditions, this energy is obtained through the fermentation of sugars, a process that is notably less efficient. Most yeasts require oxygen for alcohol production, and in its absence, the growth of many yeast species is significantly impeded [25].

The yeasts is influenced by several key factors: 1) the volume of air that enters the silo during the ensiling process, 2) the specific type of silage plant utilized, and 3) the application

of silage additives. For instance, the use of formic acid has been shown to enhance yeast populations, whereas bacterial additives that generate heterogeneous lactic acid tend to diminish their numbers [42; 131]. Additionally, research conducted by Lindgren et al. [76] indicated that the yeast populations present in alfalfa and grass silages are more abundant than those of *Torulopsis* and *Rhodotorula* aerobic species.

Lactic acid producing bacteria (LAB)

This group possesses the capability to synthesize lactic acid and was first recognized in 1900. In 1919, Oral Genus characterized true lactic acid-producing bacteria as an epiphytic assemblage of gram-positive, non-spore-forming, non-motile, rod-shaped, and spherical microorganisms that primarily ferment carbohydrates and certain alcohols into lactic acid. The genera of epiphytic lactic acid-producing bacteria include *Lactobacillus*, *Streptococcus*, *Enterococcus*, *Pediococcus*, *Lactococcus*, and *Leuconostoc* (Fig. 8) [109].

Bacteria involved in the fermentation of hexoses can be categorized into three distinct groups based on their metabolic pathways. The first group, known as compulsory homogeneous fermenters, exclusively produces lactic acid. The second group, referred to as optional heterogeneous fermenters, primarily generates lactic acid but also possesses the capability to ferment pentoses into both lactic acid and acetic acid. The third group, termed heterogeneous obligate fermenters, is characterized by its ability to convert hexoses into a range of products, including lactic acid, acetic acid, ethanol, and carbon dioxide [33].

The population of lactic acid bacteria present on a living plant is relatively low and is influenced by various factors, including plant species, growth stage, environmental conditions, seasonal variations, and the processes of planting or crushing. Notably, environmental conditions, seasonal changes, and the act of crushing appear to be the most significant determinants of this bacterial population [109].

Muck [90] indicated that the population of lactic acid bacteria can be estimated based on various meteorological factors, including air temperature, sunlight exposure, precipitation, and relative humidity. The bacterial count on fresh plant material ranges from 10^3 to 10^5 and is significantly affected by the processes of harvesting and crushing, a phenomenon referred to as inoculation by crushing, which can lead to an approximate 100-fold increase in lactic acid-producing bacteria. Notably, lactic acid bacteria are characterized by the absence of the catalase enzyme, rendering them incapable of detoxifying peroxides. The mechanical disruption of plant cells during harvesting and crushing releases compounds such as superoxide dismutase

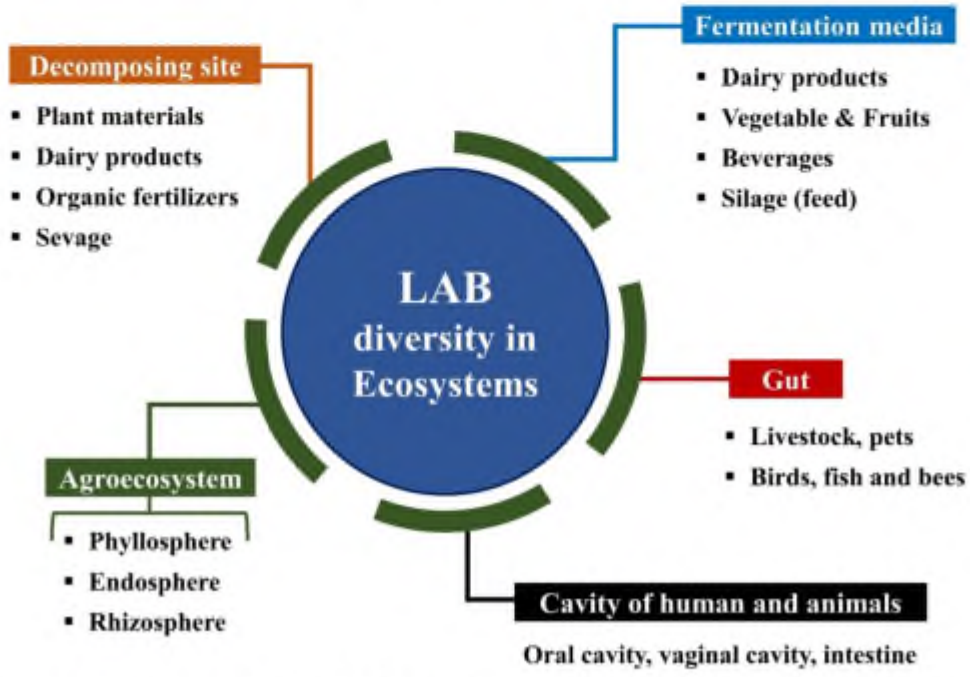

Fig. 8. The presence and activity of LAB in various ecological niches: A broad application in agriculture, environmental science, and health functions [109].

and manganese, which become accessible to the bacteria and exhibit effects analogous to those of catalase

In the early phases of the ensiling process, the growth of *lactococci* and *lactobacilli*, including *Lactobacillus plantarum*, occurs alongside aerobic microorganisms such as yeasts, fungi, and aerobic bacteria, facilitated by the presence of air among the plant materials. As fermentation advances, an anaerobic environment is established, leading to the predominance of lactic acid-producing bacteria, particularly *Lactobacillus plantarum*, which exhibit a high tolerance to acidic conditions. It appears that the various species identified on the plant are frequently heterogeneous lactic acid-producing species [92]. But some researchers have identified homogeneous fermenters as predominant, highlighting the interdependent influence of plant species, growing season, and climatic conditions on the microbial composition of silage forage plants. Recent findings further elucidate this phenomenon, indicating that when hexose availability is restricted, specific species of lactic acid bacteria can utilize lactic acid as an energy source to produce acetic acid under anaerobic conditions. This metabolic process results in an elevation of pH, which subsequently fosters the proliferation of microorganisms, including non-beneficial organisms such as Clostridium and Enterobacter [92]. Lactic acid bacteria exhibit proteolytic capabilities; however, their action on amino acids appears to be restricted. Furthermore, it is believed that certain lactic acid bacteria are capable of fermenting two specific amino acids, namely serine and arginine. Additionally, some bacterial species, including *Lactobacillus plantarum*, possess the ability to reduce nitrate to ammonia or nitrogen oxide [128].

Propionic acid producing bacteria

These bacteria serve as a natural source for the production of propionic acid. Their advantageous properties, including the synthesis of propionic acid, bacteriocins, and vitamin B12, as well as their capacity to thrive and proliferate in the rumen, have led to their application as fermentation enhancers in silos and in high-moisture food products. Furthermore, these bacteria possess a diverse array of peptidases, enabling them to degrade various amino acids; however, significant variations exist among different strains [76].

Propionic acid is extensively utilized in the preservation of seeds, dry matter, fodder, and silage. Research conducted by Moon et al. [88] demonstrated the synergistic effects of acetic acid, lactic acid, and propionic acid in combating acid-resistant yeasts. In addition to propionic acid, certain bacteria are known to produce antimicrobial proteins, referred to as bacteriocins. These bacteria exhibit stability at temperatures below 85 degrees Celsius and within a pH range of 3 to 9. The presence of propionic acid and other volatile fatty acids inhibits cellular growth, which may be attributed to their suppressive effects on the absorption of amino acids and other essential compounds necessary for cellular proliferation, as well as their role in obstructing ATP production, which relies on the electron transport chain.

Contrast between lactic acid and propionic acid producing bacteria

Lactobacilli are capable of synthesizing lactic acid from sugars, while propionic acid-producing bacteria generate propionic acid from both sugars and lactic acid. The extent to which lactic acid producers stimulate propionic acid producers is contingent upon the specific bacterial strain involved. Research conducted by Perez-chaia et al. [106] identified an inhibitory effect of lactic acid-producing bacteria on propionic acid production, which was attributed to a rapid decline in pH levels. Furthermore, lactic acid-producing bacteria generate both D and L isomers, whereas propionic acid-producing bacteria exhibit a preference for L-lactate over D-lactate, which serves as a stimulant for their activity. The interaction between propionic acid-producing and lactic acid-producing bacteria extends beyond the dynamics of lactate production and consumption. Lactobacilli in mixed cultures derive advantages from the metabolic activities of propionic bacteria, particularly due to the production of carbon dioxide. Additionally, the proteolytic activity of lactic acid bacteria leads to the release of amino acids, which enhances the growth conditions for propionic acid-producing bacteria. However, it is important to note that excessive proteolysis resulting in an overabundance of amino acids can inhibit the growth of propionic acid-producing bacteria.

Observations indicate that these mutual interactions between the two bacterial species predominantly occur under conditions of low glucose and soluble sugar availability in the environment [56; 128].

In this context, lactic acid-producing bacteria exhibit an inhibitory effect on other lactic acid-producing bacteria. This phenomenon is likely attributable to the infiltration of propionic acid into the cells of lactic acid-producing bacteria, resulting in the release of protons within the cellular environment. In an effort to mitigate this issue, the cells attempt to extrude protons via the H+ATPase pump, a process that necessitates energy expenditure. However, the limited availability of soluble sugars to supply the requisite energy leads to a decline in both the growth rate and biomass production of lactic acid-producing bacteria [106]. It is important to note that the growth constraints faced by these bacteria, compounded by their low tolerance to acidic conditions, have contributed to a reduction in their application as silage additives [110; 129].

Effect of microbial additive on silage forage

The application of bacterial additives to enhance fermentation processes in silage has reached a historical peak (Table 3). Currently, a diverse array of these products, featuring various bacterial formulations, is available in the commercial market for this specific purpose. The quality of silage fermentation is contingent upon the size, diversity, and activity of the lactic acid-producing bacterial population present in the fodder. This population typically ranges from 10 to 10^2 colony-forming units per gram of material, escalating to levels of 10^6 or 10^7 during the harvesting and crushing phases. Nevertheless, a significant proportion of these bacteria are heterogeneous and predominantly belong to the *Leuconostoc* species, which may not be the most effective organisms for facilitating dominant fermentation processes (Fig. 9, 10) [50; 95].

Since 1950, homogeneous lactic acid bacteria have been effectively utilized as additives in silage. These bacteria are added to support the growth of the epiphytic bacterial population that generates lactic acid, allowing them to outcompete other microbes. This leads to an increased production of lactic acid in a shorter timeframe, which shortens the fermentation process by minimizing proteolysis and the formation of volatile organic acids and ethanol, ultimately enhancing feed preservation [66].

Over the past decade, advancements in the selection and upkeep of bacterial strains have resulted in the creation of commercial products that contain a sufficient quantity of uniform lactic acid-producing bacteria, with a minimum concentration of 10^6. This level is essential in fresh grass to promote the prevalence of lactic

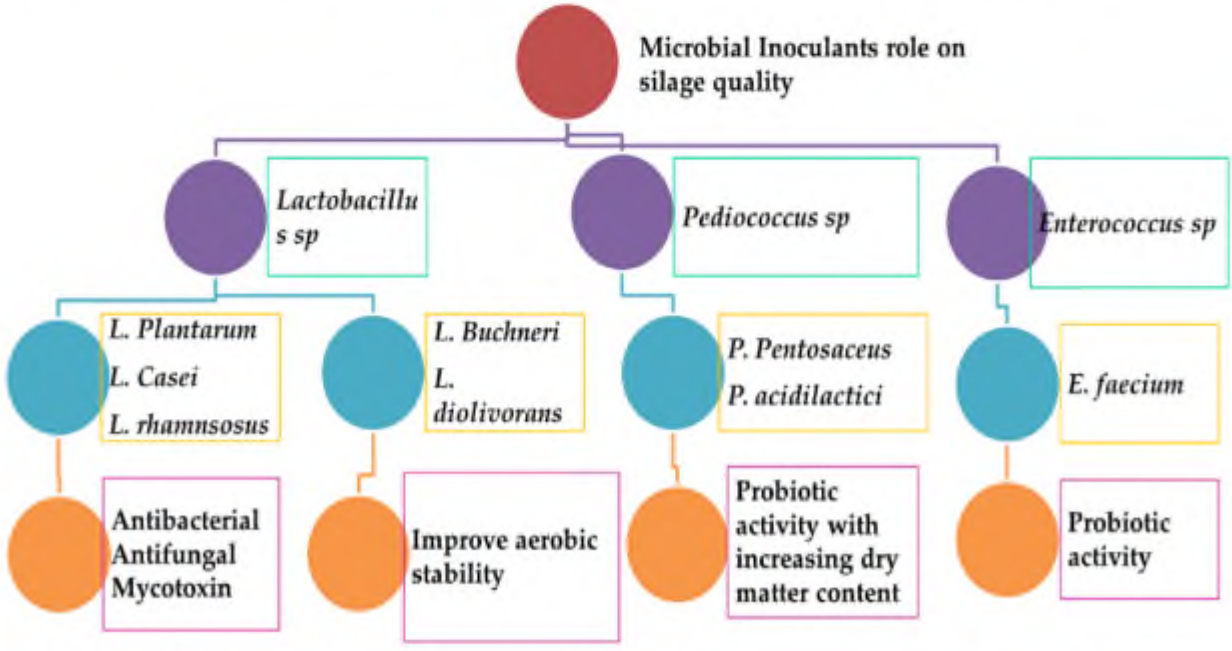

Fig. 9. Role of lactic acid bacteria on silage production and preservation [116].

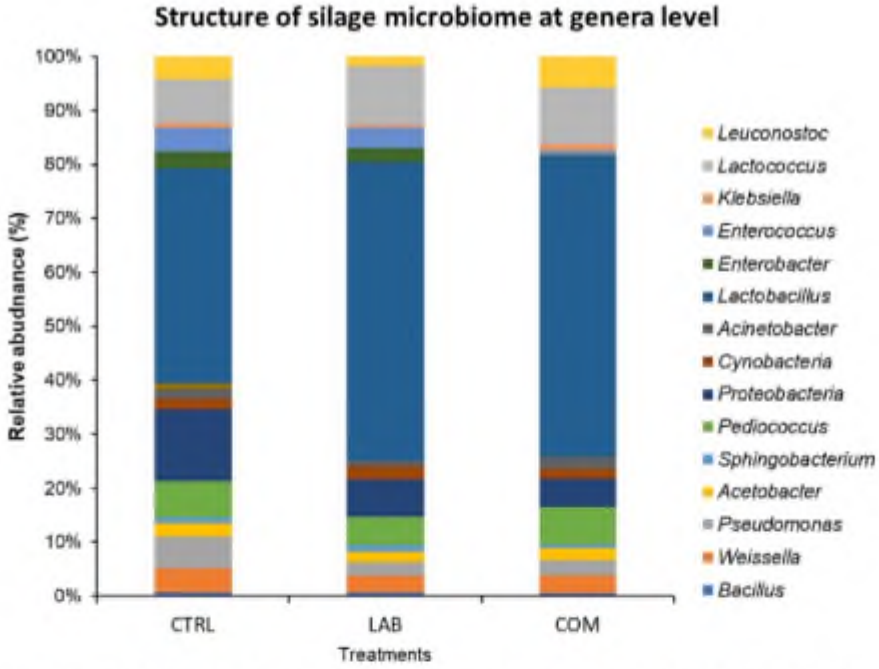

Fig. 10. Comparison structure of silage microbiome at genera level between control and inoculant addition [112].

Table 3. *Effect of LAB inoculants on silage quality*

Silage type	LAB inoculant	Additive composition	Effect on silage preservation	References
Alfalfa	*L. buchneri*	orange pulp	LAB can improve the aerobic stability and quality of silage in laboratory silos and also, OP and LAB might improve silage quality and cause better silage management in the farm.	[15]
Alfalfa	*L. buchneri*	orange pulp	Supplementation silage with orange pulp and bacterial inoculant increased in vitro DM digestibility in all incubation times. There is highly relationship between in situ and in vitro DM digestibility.	[14]
Alfalfa	*L. plantarum* (1×10^6 CFU/g)	cellulase (20 mg kg^{-1})	*L. plantarum* showed the highest in vitro digestibility of dry matter and highest abundance of natural LAB compared to cellulase-treated silage. Overall silage quality of alfalfa was improved.	[75]
Alfalfa	*L. buchneri*, *L. plantarum*, *L. buchneri* + *L. plantarum* (1×10^6, 2×10^6 CFU/g)	–	Decreased pH, increased the production of lactic and acetic acids, reduced the number of yeasts and molds, inhibited *Enterobacterium* and *Klebsiella pneumoniae*, stabilized silages during aerobic exposure.	[143]
Lucerne, oat, sorghum, Whole-crop corn	*L. plantarum*, *Limosilacto-bacillus fermentum* (1×10^6 CFU/g)	–	Silages were well preserved based on their pH and DM values, enhanced aerobic stability of maize silage.	[107]
Alfalfa	*L. buchneri* (3×10^8 CFU/g)	molasses, orange pulp	Supplementation treatments with inoculant had a significant effect on gas production and increased gas production volume	[17]

Continuation of table 3

Silage type	LAB inoculant	Additive composition	Effect on silage preservation	References
Alfalfa	*B. subtilis*, *L. buchneri* (1×10^6 CFU/g)	–	Inhibited the growth of *Enterococcus* after 3 d of aerobic exposure, improved silage fermentation quality, aerobic stability, and bacterial community during ensiling.	[10]
Alfalfa	*L. plantarum*, *L. casei*, *E. faecium*, *P. acidilactici* (1×10^5 CFU/g)		Increased the organic acids content, bacterial species number, and relative abundances following fermentation, resulting in a general pH and mycotoxin reduction.	[11]
Alfalfa, Chinese rye grass	*B. amyloliquefaciens*, *B. licheniformis*, *B. subtilis*, *Paenibacillus xylanexedens*, *B. cereus*, *B. flexus*, *E. faecium*, *B. pumilus*	–	With a low pH and high lactic acid concentrations, the silages were successfully preserved, loss of water-soluble carbohydrates, starch, and hemicellulose in both silages with prolonged ensiling, activity of microbial amylase was detected all through the ensiling process.	[99]
Alfalfa	*L. buchneri*	fresh whey (3 %)	Fresh whey and bacterial inoculation with the fermentable carbohydrates leads to rapid reduction of pH, limiting the proliferation of yeasts, increased aerobic stability and improved silage quality	[16]
Alfalfa	*L. plantarum* (1×10^5 CFU/g)	–	Better silage quality-lower pH, greater lactate-to-acetate ratio, higher ruminal levels of *L. plantarum* and, in some cows, a detectable shift in bacterial community composition	[86]
Alfalfa	*L. plantarum*, *L. buchneri* (1×10^6 CFU/g)	–	Hastened the inhibition of *E. coli* during ensiling, prevented the growth on silage contaminated with the pathogen after ensiling.	[100]
Alfalfa	*L. plantarum* (1×10^6 CFU/g)		Higher lactic acid and acetic acid concentrations.	[29]
Alfalfa	*L. brevis*, *L. citreum*, *L. hilgardii*, *L. plantarum* (1×10^5 CFU/g)		Positively affected fermentation properties and silage quality parameters.	[40]
Alfalfa	*L. plantarum* (1×10^5 CFU/g)	–	Improved ruminal fermentation and milk production.	[87]
Alfalfa, corn straw	*P. pentosaceus*, *P. acidilactici*, *L. acidophilus*, *L. plantarum* (2×10^5 CFU/g)	–	Improved silage characteristics and fiber degradation in alfalfa, while having no detectable effect on corn silage.	[30]
Alfalfa	*L. plantarum*, *L. pentosus*, *P. pentosaceus* (1×10^5 CFU/g)		Efficiently regulated enterobacteria and mold populations, the chemical properties of the silage were improved by an increased index of in vitro dry matter digestibility.	[97]

End of table 3

Alfalfa	*L. pentosus*, *L. pentosus* + *L. brevis* + *P. acidilactici* (1×10^6 CFU/g)		Presented greater residual WSC and the least pH.	[140]
Corn hybrid	*L. buchneri* (4×10^5 cfu/g), *P. pentosaceus* (1×10^5 CFU/g)	molasses (3 %)	Molasses increased ethanol and lactate concentration but did not improve aerobic stability, while LAB inoculants made the fermentation more heterolactic and improved corn silage's aerobic stability	[55]
Rice straw	*L. plantarum* (1×10^6 CFU/g)	molasses (4 %)	Molasses improved rice straw silage's fermentation quality and in vitro digestibility.	[144]
Wilted rice straw	*L. bulgaricus*, *L. helveticus* (1×10^6 CFU/g)	Acetic acid (5 %) – molasses (40 %)	Treatment with chemical additives increased the concentrations of CP, WSC, acetic acid, and lactic acid reduced the concentrations of ADF and NDF but did not effectively inhibit the growth of spoilage organisms as seen in the treatment with LAB	[98]
Whole-crop maize	*L. plantarum*, *L. paracasei*, *P. pentosaceus* (1.5×10^{11} CFU/g)	Formic acid (42.5 %), propionic acid (10.0 %), ammonium formate (30.3 %), benzoic acid (2.2 %)	The concentrations of WSC were higher for chemical additives compared to LAB on days 3, 5, 10, and 90 of fermentation, and silage had fewer LAB populations than LAB treated silages regardless of the days of fermentation.	[123]
Whole-crop corn	*L. acidophilus* – *L. plantarum* (1×10^6 CFU/g)	Formic acid, acetic acid, propionic acid ($6\ ml \cdot g^{-1}$)	Silages treated with LAB showed increased lactic acid content and decreased pH after 45 days, while higher levels of acetic acid and increased abundance of *Acetobacter* in silages treated with organic acids	[58]

acid bacteria in silage, ultimately enhancing animal performance. The majority of microbial additives currently on the market consist of selected strains of uniform lactic acid-producing bacteria, including species such as *Lactobacillus plantarum*, *Pediococcus*, and *Enterococcus* [25]

In general, inoculants are chosen for their effectiveness in quickly reducing the pH of silage by fermenting water-soluble carbohydrates into lactic acids, which helps to inhibit proteolytic activity and preserve nutrients. Currently, efforts are being made to develop functional inoculants that not only enhance silage quality but also have beneficial effects on animal health, production, stress resilience, and improve silage consumption and digestibility. Some research has shown that silages treated with inoculants can lead to higher milk production in cows compared to those without inoculants [79; 84], although the exact mechanisms behind this are still not fully understood. In this section, we will discuss advancements related to several promising functional inoculants for silage. These inoculants enhance silage safety while preserving fermentation quality and lowering pH. Additionally, they boost animal performance, increase feed intake and digestibility, and can even improve the quality of animal products (Fig. 11).

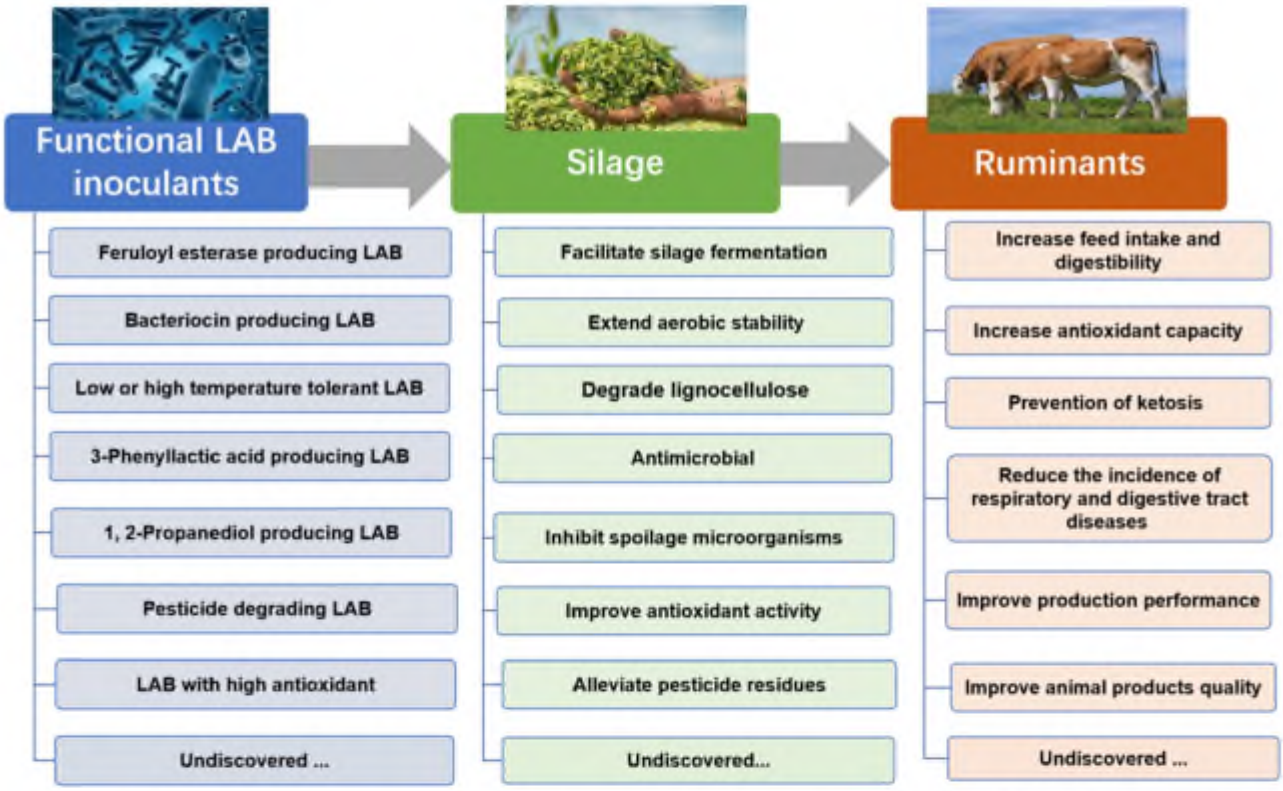

Fig. 11. Functions of lactic acid bacteria and their effects on silage and ruminants [50].

Factors affecting the activity of microbial additives

Temperature

Elevated temperatures are the primary cause of poor-quality silage, as the heat generated during fermentation is positively linked to microbial growth and plant respiration. Initially, there is a rapid increase in temperature, which halts the growth of various bacteria. Under these circumstances, commercial additives that contain lactic acid-producing bacteria typically offer minimal advantages [142]. The water temperature in the inoculation tank for silage plays a crucial role in the survival of bacterial additives, with high temperatures potentially leading to heat shock in the bacteria [7]. In a study by Mulrooney et al. [93], five bacterial additives were cultured and examined at four different temperatures over a period of six hours. The findings indicated that the viability of the bacterial additives remained stable at temperatures between 30 and 40 °C, but temperatures exceeding 40 °C resulted in a decline in their viability. Different additives exhibited varying responses to temperature; *Lactobacillus plantarum* demonstrated the highest tolerance to elevated temperatures, while *Lactobacillus buccaneri* showed moderate tolerance. The other bacterial strains were unable to withstand the higher temperatures, leading to a significant reduction in their numbers.

Teixeria et al. [120] found that Lactobacillus buccaneri is compromised at 62 degrees due to damage to its cell wall, and at temperatures exceeding 65 degrees, ribosomes are affected. Additionally, Spinks [117] noted that certain bacteria exhibit heightened heat sensitivity during starvation when there is a scarcity of fermentable substrates. The findings from these studies indicate that heat sensitivity in lactic acid bacteria can significantly impact the effectiveness of additives in silage, as it not only enhances microbial survival prior to ensiling but also improves their survival rate during the ensiling process when temperatures rise. Ohmomo et al. [101] noted that the poor quality of silage and the ineffectiveness of commercial additives may be due to temperatures exceeding 45 degrees during the early stages of ensiling in the silage stack. Zhang et al. [142] found that among eight strains of epiphytic bacteria tested at 25 degrees, all enhanced fermentation; however, at 45 degrees, only one additive was effective in improving fermentation. Ultimately, it is suggested that to achieve better outcomes, heat-resistant species should be used, along with ice packs and pure, chlorine-free water during inoculation.

Preparation before use

The use of dry and liquid additives can also affect the type of fermentation. In Whiter and Kung's [132] experiment, Lactobacillus plantarum was used in two forms: dry granules and soluble in water on two levels of dry matter. In low dry matter, both additives had equal performance, but with increasing dry matter, silage containing soluble additive showed its effect better. In the study conducted by Merry et al. [85], the use of a fresh bacterial culture combined with fast-fermenting foods in a liquid environment enhanced the fermentation process more effectively than when a freeze-dried bacterial additive was applied for silage inoculation. This improvement is likely due to a reduction in the lag phase and a quicker increase in bacterial population.

In the study by Kizilsimsek et al. [66], two forms of a bacterial additive — one dried through freezing in both high and low doses, and another as a fresh culture — were applied to alfalfa fodder. Within the first 6 and 12 hours after ensiling, the number of lactic acid bacteria and the level of lactic acid rose in the treatment with the fresh culture. After 12 hours, levels of acetic acid and ethanol decreased, and by 24 hours post-ensiling, ammonia nitrogen levels also dropped compared to other treatments. The findings indicated that the fresh culture bacterial additive resulted in a quicker pH reduction and more effective fermentation than the dried additive, which was rehydrated in water. The silage with the fresh culture additive exhibited the most favorable response and a more uniform fermentation process. In their 1998 experiment, Winters et al. [133] demonstrated that introducing fresh cultures of bacteria led to greater dry matter consumption and weight gain compared to the control group. Additionally, the study highlighted that the quantity of dry matter influences the effectiveness of the bacterial additive, particularly in relation to the pasteurization of feed.

The effect of bacterial additives on fermentation characteristics of silage

Up until now, various bacterial additives that generate uniform lactic acid have been applied to alfalfa silage, and their beneficial impacts on fermentation quality have been studied. In a study by Cai et al. [26], two distinct strains of *Lactobacillus plantarum* were utilized at a concentration of 10^5 for three alfalfa plants with 45 % dry matter, along with rye fodder at the flowering stage and sorghum fodder at the milking stage. The use of the bacterial additive notably enhanced the fermentation parameters, leading to increased production of lactic acid and soluble carbohydrates, as well as higher levels of butyric acid, propionic acid, ammonia nitrogen, and dry matter loss. Muck et al. [89] inoculated alfalfa forage with a blend of lactic acid bacteria and found that there was a gradual enhancement in the rate of silage acidification and an increase in the ratio of lactate to acetate. They concluded that lactic acid bacteria should be applied at levels of 10 % or higher than the natural amounts of epiphytic lactic acid bacteria.

Hristov and McAllister [54] inoculated entire barley plants with a combination of *Enterococcus faecium* and *Lactobacillus plantarum*. They found that the quantity of lactic acid bacteria and the total lactic acid produced rose, while the final pH of the silage dropped in the inoculated group. However, they concluded that the bacterial additive was more effective when the dry matter content was low. Koc et al. [68] investigated the effects of two types of bacterial additives and enzymes, finding that the inclusion of bacteria enhanced fermentation parameters. In treatments where both enzymes and bacterial additives were used simultaneously, there was a notable decrease in pH. This reduction in pH was accompanied by a decrease in soluble sugar levels. Treatments with additives showed an increase in lactic acid bacteria and lactic acid production, while acetic acid levels were lower, and butyric acid was not detected in any of the treatments.

In the experiment conducted by Kung et al. [71], the use of a bacterial additive in both normal and double doses in feed with 42 % dry matter resulted in a reduction of pH, with no significant difference observed between the two doses. Initially, the soluble carbohydrates in water were consistent across all treatments, but by the end of the experiment, the control treatment had a higher amount of these carbohydrates, while lactic acid levels exhibited a negative correlation with soluble carbohydrates. The findings indicated that at elevated dry matter levels, pH decreased and lactic acid increased. Ultimately, it was concluded that adding bacteria to alfalfa silage led to an increase in lactic

acid, with this effect being more pronounced at higher dry matter levels.

In a separate study by Filya et al. [43], researchers examined the impact of the bacterial additive *Propionibacterium*, both with and without *Lactobacillus plantarum*, on corn plants. The treatments that included *Lactobacillus plantarum* resulted in higher levels of lactic acid, while *Propionibacterium* did not lead to an increase in propionic acid. Throughout the entire experiment, the levels of soluble carbohydrates, propionic acid, acetic acid, butyric acid, and ethanol remained unchanged due to the bacterial additive. In the experiment conducted by Rizk [113], alfalfa with a high dry matter content was examined with the inclusion of uniform lactic acid bacteria (*Lactobacillus plantarum*). The pH levels in the treatments with bacterial additives dropped quickly, falling below 4.5 within two days, whereas the control treatment only reached this pH after 45 days. By the end of the study, the pH and soluble carbohydrates in the treatment with the bacterial additive were lower than those in the control treatment, but the production of lactic acid was higher in the bacterial additive treatment.

In the study conducted by McAllister et al. [80], two varieties of bacterial additives were utilized, one containing Lactobacillus plantarum and the other containing both *Lactobacillus plantarum* and *Enterococcus faecium*. The quality of all silages was found to be good. The presence of lactic acid bacteria showed a tendency to rise, leading to an increase in lactic acid concentration, while pH levels, butyric acid, and water-soluble carbohydrates decreased in the treatments with bacterial additives. The levels of ammonia nitrogen remained unchanged, but the amount of acetic acid increased by over 50 % in the treatment that included amphoteric acid. In a study by Nadeau et al. [96], alfalfa and orchard grass were ensiled with dry matter contents of 22 % and 32 %, respectively. The researchers utilized bacterial additives such as *Lactobacillus plantarum* and *Pediococcus cerevisiae*. The addition of a bacterial additive containing cellulase to alfalfa resulted in a slight but significant reduction in pH, a change not observed in orchard grass. The presence of bacteria led to an increase in lactic acid levels in both types of fodder. The lactate to acetate ratio rose by 15 % in alfalfa and by 8 % in orchard grass. While the level of acetic acid in orchard grass remained unchanged by the additives, it decreased by 16 % in alfalfa compared to the control group. Additionally, succinic acid levels were higher in wilted fodder than in non-wilted forage for both plant species, likely due to increased proteolysis in the silage, which is partly facilitated by enterobacters that convert glucose into succinate. The study highlighted that varying bacterial additive contents led to different responses, ultimately influencing the quality of the silage in distinct ways.

In the study conducted by Ely [39], researchers examined the impact of adding lactobacilli to fodder plants such as alfalfa, corn, sorghum, and wheat. The treatment involved using 5 grams of dry *Lactobacillus acidophilus* per kilogram of fresh fodder, and the fermentation process was analyzed. The fermentation results showed no significant differences between the inoculated and control groups, indicating that the addition of bacterial additives did not provide any benefits. The lack of success with the bacterial additive in this study may be attributed to several factors. 1) a readily available energy source for microbes is essential for controlled fermentation, and 2) the added microbes must be able to compete with the existing epiphytic population for successful silage fermentation. For high-quality silage from corn and alfalfa fodder, both conditions must be met; otherwise, the bacterial additive will not enhance fermentation quality. In the case of wheat fodder, it appears that both conditions were lacking, and even high doses of the bacterial additive failed to lower the pH or prevent the development of secondary harmful fermentation products. Additionally, Burghardi et al. (1980) demonstrated that bacterial additives can be advantageous when the soluble carbohydrate content in the plant is low.

In the experiment of Adesogan et al. [1], the effect of a bacterial additive containing *Pediococcus pentosens* in the amount of 10^5 and *Lactobacillus buccaneri* in the amount of 10^5 was used on bermuda grass fodder. pH was lower throughout the fermentation period in the treatment containing bacterial additive, and the recovery of dry matter from day 4 and soluble carbohydrates from day 30 to the end of the period was higher in the treatment containing bacterial additive. NDF was not affected, but ADF was reduced in the additive treatment. The amount of acetic acid, propionic acid, lactic

acid, ethanol, lactate to acetate ratio, lactic acid producing bacteria, yeasts and molds were not affected by the additive after 60 days of ensiling. The amount of butyric acid and total acids produced in the bacterial additive treatment decreased and finally it was reported that access to fermentable carbohydrates is more effective on the rate of primary fermentation in tropical plants with low soluble carbohydrate content than desirable bacterial additives. But in general, the effectiveness of the bacterial additive in the final stages of fermentation confirms the usefulness of the bacterial additive to overcome homogeneous or non-homogeneous epiphytic lactic acid producing bacteria.

Recent developments in creating dual-purpose additives for both homogeneous and heterogeneous lactic acid-producing bacteria have been significant and yielded beneficial outcomes in this area. Adesogan and Salawu (2004) found in their study that these dual-purpose bacterial additives enhanced silage fermentation but did not influence aerobic stability. Conversely, Filya et al. [43] demonstrated that these additives improved both aerobic stability and fermentation, differing from the findings of Driehuis et al. [37]. This variation in results is likely due to differences in the type of feed and harmful organisms involved. Previous studies have indicated that aerobic stability is mainly linked to bacteria. McAllister et al. [80] proposed that *Lactobacillus buccaneri* inhibits the growth of aerobic yeasts in the initial phase, but it seems unlikely to impact bacteria that damage silage. In the experiment conducted by Kent et al. [64], researchers examined the impact of a bacterial additive that included *Lactobacillus plantarum* and *Pediococcus* lactic acid on alfalfa silage. The findings indicated that the pH level dropped in the silage with the bacterial additive, while factors such as crude protein, ADF, ADIN, and yeast count remained unchanged. The swift generation of lactic acid in this study likely contributed to the observed reduction in pH in the treated silage.

In a meta-analysis conducted by Kleinschmit and Kung [67], it was found that an additive containing *Lactobacillus buccaneri* at concentrations exceeding 10^5 CFU resulted in an increase in pH, a decrease in lactic acid levels, and a greater loss of nutrients from the surface compared to the control group. Across all treatments, the levels of acetic acid were lower than what is typically observed in corn silage. However, starting from day 56, the levels of this organic acid were higher in the inoculated treatments. Additionally, the ratio of lactate to acetate in this study was greater than 3 in all silages, indicating a consistent fermentation of lactic acid. In the experiment conducted by Sheperd and Combs [114], two types of additives containing *Lactobacillus plantarum* and *Pediococcus cerevisiae*, along with enzymes such as cellulase, amylase, and pectinase, were applied to first harvest alfalfa fodder. Throughout the fermentation and ensiling process, the pH levels were lower in the treatments that included both types of bacterial and enzyme additives. The levels of lactic acid and glucose remained consistent across all treatments during the fermentation period, but by the end of the 177 days, the control treatment had the lowest levels of both. The production of acetate in the treatment with the bacterial additive began to decline from the 8th day of ensiling and continued to decrease until the end of the study. The experiment also demonstrated that the bacterial additive could lower the pH even when the dry matter content limited fermentation. Based on his findings, Jones proposed that incorporating bacteria into alfalfa forage with high dry matter (over 35 %) is more beneficial than adding fermentable substrates [33].

In the experiment conducted by Bolsen [19], a bacterial additive that included *Lactobacillus plantarum*, *Enterococcus faecium*, dextrose, and a combination of the two was tested. The bacterial additive alone did not affect the pH, but the combination of the two significantly lowered both pH and acetic acid levels, while lactic acid levels increased. According to Spoelstra [118], alfalfa silage with bacterial additives and fermentable substrates exhibited higher lactic acid and lower acetic acid levels. The presence of the bacterial additive and dextrose led to a decrease in ethanol levels. The microbiological analysis of the silage revealed that the populations of *lactobacilli*, *pediococcus*, *leuconostoc*, *enterobacter*, yeast, mold, lactate-consuming yeast, and clostridium remained unchanged across treatments. The fermentation process analysis indicated that the bacterial additive in the fodder from the second and fourth harvests resulted in the lowest pH on the first day, but thereafter, a combination of lactic acid and dextrose-producing bacteria maintained the lowest

pH. The lactic acid levels were highest on the first day for both the bacterial additive alone and the combination with dextrose, but from the third day onward, only the treatment with both bacterial additives and dextrose continued to show this trend. Throughout the ensiling period, the treatment with bacterial additives and dextrose had the lowest acetic acid levels from the third day onward. In corn silage, no significant differences were found between the control and inoculated treatments regarding any fermentation indices.

Bolsen [19] found that alfalfa is an inconsistent forage and challenging to ensile. The addition of 2 % dextrose, a bacterial additive, or a combination of both did not lead to successful microbial activity for improved fermentation during the ensiling process. However, these additions significantly enhanced fermentation efficiency, and the researchers ultimately achieved the best fermentation results with a combination of the two additives. Bolsen and his team concluded that the lactic acid-producing epiphytic population on the entire corn plant is substantial and predominantly uniform. As a result, the bacteria from the bacterial additive were unable to dominate during ensiling and did not demonstrate their intended effects. Additionally, the natural properties of the corn plant are conducive to producing high-quality silage without the need for any additives. In the study conducted by Aksu et al. [3], the impact of bacterial additives, molasses, and formic acid was examined. The experiment utilized uniform lactic acid bacteria, and it was found that the level of lactic acid rose, while the levels of butyric acid and acetic acid remained unchanged. In a study conducted by Filya et al. [43], the effects of *Lactobacillus buccaneri*, *Lactobacillus plantarum*, and a combination of both at a concentration of 10^6 CFU were examined on corn and sorghum fodder with low dry matter content. The results showed that after two days of ensiling, the levels of acetic acid were higher in silages treated with *Lactobacillus buccaneri* and the combination of both additives compared to other treatments. This trend continued on days 4, 8, and 15 post-ensiling. During the fermentation process, there was a decrease in pH and water-soluble carbohydrates, while the concentrations of lactic acid, acetic acid, ethanol, and ammonia nitrogen increased. After 90 days of ensiling, silages treated with *Lactobacillus buccaneri* exhibited a higher pH than those inoculated with *Lactobacillus plantarum*, the combination of both, and the control group. Additionally, silages treated with *Lactobacillus plantarum* and the combination of both Lactobacillus strains had higher lactic acid levels compared to the control silages treated solely with *Lactobacillus buchneri*. The ammonia nitrogen levels in silages treated with *Lactobacillus plantarum* and the combination were lower than those in silages treated with *Lactobacillus buchneri* alone. Furthermore, the loss of dry matter in silages treated with *Lactobacillus buchneri* was greater compared to the control and those treated with both Lactobacillus strains. Lastly, the control and silages treated with *Lactobacillus plantarum* retained more residual water-soluble carbohydrates than those treated with *Lactobacillus buchneri* and the combination of both strains.

In the experiment conducted by Ranjit and Kung [110], researchers examined the impact of an additive with *Lactobacillus buchneri* at two different levels, along with two strains of *Lactobacillus plantarum* and a treatment that included a propionic acid buffer. The findings indicated that there were no significant differences in pH across the various treatments. In the treatment with a high dose of *Lactobacillus buchneri*, the lactate levels decreased while acetate levels increased significantly. Additionally, propionate levels rose in the treatment with the propionate buffer. Butyrate levels could not be detected in any of the treatments. The ratio of lactate to acetate decreased in the high-dose *Lactobacillus buchneri* treatment and one of the *Lactobacillus plantarum* treatments, while it increased significantly in the other *Lactobacillus plantarum* treatment. Furthermore, the number of yeasts diminished in the high-dose *Lactobacillus buchneri* treatment. None of the *Lactobacillus plantarum* strains achieved a uniform fermentation in this study, suggesting that these bacteria lacked the strength to outcompete the existing epiphytic microbial population.

In a study by Filya et al. [44], researchers examined the impact of 14 different bacterial additives on alfalfa fodder during its first and second cuts. All additives were applied at a concentration of 10^6 CFU per gram of DM. In the first cut, all additives containing *Enterococcus faecium* lowered the pH compared to the control group, although two of the heterogeneous

additives resulted in a smaller pH reduction than anticipated. The levels of lactate increased by 2.5 % to 106 % in treatments with bacterial additives, while seven additives raised the acetic acid levels by 15 % to 255 %. Consequently, the lactate-to-acetate ratio was lower than in the control group for only three additives. Additionally, two additives with *Lactobacillus buchneri* and *Enterococcus faecium* produced higher ethanol levels than the control. In the second cut, only five additives significantly lowered the pH, with *Lactobacillus buchneri* showing the highest pH. Most additives, except for two, increased lactic acid levels. Silaged alfalfa with *Lactobacillus buchneri* and *Lactobacillus* pentazeus had the highest acetate and ethanol concentrations, and these were the only treatments with a low lactate-to-acetate ratio. After fermentation, the control treatment in the first cut retained more soluble carbohydrates, while the lowest amounts were found in treatments with *Enterococcus faecium*, *Lactobacillus pentozeus*, and two strains of *Lactobacillus buchneri*. In the second cut, the control treatment had intermediate levels, with the lowest values in treatments containing *Lactobacillus buchneri* and the highest in those with *Enterococcus faecium*. Overall, the concentrations of NDF, ADF, and acid-insoluble lignin were greater in both cuts compared to non-ensiled forage, attributed to dry matter loss from fermentation and respiration, which led to sugar loss and an increased NDF ratio. Ultimately, bacterial additives positively influenced silage characteristics by lowering pH and enhancing lactic acid production.

According to Ridwan et al. [112], incorporating lactic acid bacteria (LAB) significantly improves the silage microbiome and quality by changing the diversity of bacteria and the metabolic byproducts of the silage materials, ensuring safe preservation.

In the study conducted by Weinberg et al. [130], researchers examined the impact of 10 different bacterial additives on corn and wheat silage. For ensiled wheat, the pH levels remained consistent across all treatments, while the dry matter content was lower in six of the additives compared to the control, but higher in two additives that included *Lactobacillus buchneri*. The NDF content was elevated in four bacterial additives and decreased in two that contained *Lactobacillus buchneri*. Lactic acid levels were higher in treatments with *Lactobacillus plantarum* and *Enterococcus faecium*, while lower levels were found in treatments with *Lactobacillus plantarum*, with no significant differences in other treatments compared to the control. Ethanol levels increased in six of the bacterial additives, with the highest concentration linked to *Pediococcus pentazeus* and the lowest in two treatments with *Lactobacillus buchneri*. Acetic acid levels were highest in five additives containing *Enterococcus faecium* and *Lactobacillus plantarum*, and lowest in one treatment with *Lactobacillus pentazeus*. Lactic acid-producing bacteria were more prevalent than in the control in eight treatments. In silage corn, five treatments had a higher pH than the control, and none of the additives were able to lower the pH compared to the control. All treatments had lower dry matter than the control, with the lowest amount associated with *Lactobacillus buchneri*. NDF levels were higher in eight additives, with the highest observed in the *Lactobacillus buchneri* treatment. Lactic acid levels were higher in three treatments and lower in three compared to the control, with the highest from *Lactobacillus pentozeus* and the lowest from *Lactobacillus plantarum*. Ethanol levels were greater in all treatments compared to the control, with the highest from *Enterococcus faecium*. Acetic acid was elevated in eight additives, with the lowest in the *Lactobacillus pentazos* treatment. Lactic acid-producing bacteria were more abundant than in the control in all treatments except for *Lactobacillus buchneri*. Guo et al. [50] found that in different types of silages, lactic acid bacteria (LAB) inoculants influence the composition of microbial communities in various ways, depending on the existing microbiota on fresh forage. Essentially, LAB inoculants streamline the relationships between bacterial species to improve fermentation quality.

Na et al. [94] studied the effects of six common commercial LAB additives (*Lactobacillus plantarum*, *L. buchneri*, and *Enterococcus faecalis*; *L. plantarum* and *L. casei*; *L. plantarum* and *L. buchneri*; *L. plantarum*, *L. buchneri*, *L. casei*, and *Pediococcus acidilactici*; *L. plantarum*; and *L. buchneri*, *P. acidilactici*) on the bacterial community and fermentation quality of alfalfa silage. They showed that using commercial LAB additives during the ensiling of alfalfa enhanced fermentation quality, aided in preservation, and changed the bacterial composition

of the final silage. In the Control silage, *Lactobacillus*, *Enterococcens*, and *Pediococcens* were the predominant bacteria. According to Kim et al. [65], LAB inoculants enhance the quality of silage and mitigate DM losses during prolonged storage. LAB represents one of the most effective organic additives for the regulation of undesirable bacterial proliferation in silage. These inoculants facilitate the conversion of water-soluble carbohydrates and complex secondary metabolites present in forage crops into organic and mineral acids, thereby effectively inhibiting pathogenic growth and improving the nutritional quality of silage for livestock

Ling et al. [77] indicated that the incorporation of LB enhanced the relative abundance of Lactobacillus and contributed to the improved quality of alfalfa silage. The research conducted by Jiang et al. [59] demonstrated that the incorporation of a compound comprising LAB and sugar sources significantly enhanced the fermentation quality, nutrient profile, and microbial diversity of high-moisture alfalfa silage

Conclusion

The goal of ensiling is to preserve fresh forage crops or other types of biomass for later use The quality of silage can be enhanced by incorporating different bacterial inoculants, which help during fermentation, storage, and feeding by improving fermentation processes, encouraging beneficial microbial diversity, and inhibiting harmful microorganisms. Alfalfa is the most important forage, and microbial additives can enhance its silage preparation.

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ВИКОРИСТАННЯ МІКРОБНИХ ДОБАВОК ПІД ЧАС ПРИГОТУВАННЯ СИЛОСУ З ЛЮЦЕРНИ (огляд літератури)

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Зелена маса люцерни є доволі проблемною для сінажування через підвищений вміст білка, низьку кількість водорозчинних вуглеводів, низький вміст сухої речовини та високу буферну ємність. У зв'язку з цим нещодавно було запропоновано нові підходи до покращення виробництва сінажу за допомогою добавок, серед яких в останні роки активно використовуються силосні закваски. Бактеріальні добавки використовуються для підвищення якості силосу сільськогосподарських культур, з особливим акцентом на сінаж. Основною метою додавання молочнокислих бактерій у сінаж є пригнічення проліферації небажаних мікроорганізмів, включно з представниками Clostridium і Enterobacteriaceae. Це досягається швидким підвищенням концентрації іонів водню до порогового значення, несприятливого для росту цих небажаних бактерій. Останні дослідження функцій бактеріальних добавок при силосуванні та сінажуванні біомаси сільськогосподарських культур свідчать про значний потенціал для покращення продукту не лише як ферментованого корму, але й для доставки пробіотичних мікроорганізмів, які можуть принести користь здоров'ю тварин. У статті представлено комплексний огляд процесу приготування силосів та критично оцінено низку досліджень щодо їхньої якості, а також впливу бактеріальних добавок на сінаж із люцерни. Якість силосів можна підвищити шляхом додавання різних бактеріальних інокулянтів, які допомагають під час бродіння, зберігання та годівлі, покращуючи процеси бродіння, стимулюючи розвиток різноманітних корисних бактерій та пригнічуючи проліферацію шкідливих мікроорганізмів. Люцерна є одним із найважливіших кормів, і мікробні добавки можуть покращити приготування сінажу економічно ефективним і екологічно прийнятним способом.

Ключові слова: *люцерна, бактеріальна добавка, буферна ємність, сінаж, в'ялення.*

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