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Review

Dry Matter Losses in Silages Resulting from Epiphytic Microbiota Activity—A Comprehensive Study

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Abstract: An overview was made of dry matter (DM) and quality losses that occur during the ensiling process. The aim was to review the current knowledge on the course of the fermentation pathways in various raw materials and the loss of DM accompanying this process. This review discusses the main groups of microorganisms involved in the ensiling process, the accompanying fermentation patterns, and the resulting DM losses. The possibility of reducing DM and quality losses during the ensiling process in practice is presented. The paper concludes with future perspectives and recommended management practices to reduce losses over the whole ensiling process.

Keywords: lactic acid bacteria; acetic acid bacteria; clostridia; bacilli; molds; yeast; silage

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1. Introduction

The preservation of forage via fermentation processes provided by lactic acid bacteria aims to obtain a stable silage with a high recovery of DM, energy, and nutrients [1,2] intended for feed as well as for the production of biogas from renewable raw materials. Silages enable feeding in periods of raw plant absence or shortage but also give an opportunity for uniform feeding with complete daily nutrient doses throughout the year [3–5]. Most often, green fodder from whole-plant maize, grasses, and legumes [6] as well as maize grain is ensiled. Recently, the ensilage of food industry waste, e.g., beet pulp, has become common [7,8]. In some countries (Japan, Israel, China), silages are produced on the basis of total mixed ratios (TMR), which, due to their good quality and simplified animal feeding systems, are increasingly used in farms specializing in the production of dairy cattle [9,10]. The production of silage from TMR also enables the use of various wastes of the agri-food industry, which usually contain high moisture [11–15].

The quality of silage is a result of the microbial community involved during all stages of fermentation as well as their metabolites [16]. The fermentation process determines the quality and quantity of stored feed that will be available at feed-out. Harvesting ensiled forages at the proper moisture and stage of maturity [17,18], rapidly filling, properly packing, and covering silages directly impact the fermentation process.

The achievement of high nutritional value and good hygienic quality of ensiled forage is important in terms of the quality and economy of animal breeding. Good-quality silage determines the profitability of milk and beef production [19] and influences the natural environment by limiting the emission of greenhouse gases. A 50% reduction in CO_2 emissions related to a unit of milk is obtained for cows with a milk yield of 40 kg compared to cows with 4 times lower milk yield [20]. Even greater disproportions in CO_2 emissions can be calculated from the daily weight gain of calves. If they are 1.5 kg, the CO_2 emission related to the unit of weight gain is lower by 70% compared to the gain of 1 kg [20].

The changes taking place in ensiled feed are inextricably linked with the loss of nutrients [21,22]. The losses associated with fermentation in the silo are primarily from CO_2 production. These losses typically are in the range of 2 to 4% [23,24]. The amount of DM (dry matter) loss from fermentation depends on the dominant microbial species and the substrates fermented.

The losses can be expressed as weight loss of forage DM. They concern water-soluble carbohydrates (WSC) as well as proteins and amino acids. Carbohydrates are substrates for microbial fermentation, the product of which is lactic acid. Conversely, the transformations of proteins and amino acids take place under the influence of plant enzymes and microorganisms that usually grow under conditions unfavorable for the development of the desired microflora [25].

Silage quality can also be described by the gross energy in silage, which can better express the relation between fermentation quality and potential animal performance because all fermentation products, including those from less effective microorganisms (clostridia, enterobacteria, fungi, etc.), contribute to the energy value of silage. Fermentation end products (organic acids) can increase the gross energy of silage by up to 10–14% [26]. Therefore, large differences in silage quality and DM losses might not be reflected in a significant variation in gross energy value. Therefore, one of the main aims of ensiling is to reduce losses of dry matter during the preservation process. In recent years, numerous studies have been conducted in many countries to reduce these losses. The basis is the recognition of the interactions between the groups of microorganisms growing in the ensiled material. On that basis, some good silage management practices for preventing or at least minimizing losses in forage were elaborated.

The aim of the study is to review the current knowledge on the course of the fermentation process in various raw materials and the loss of DM accompanying this process. The work will discuss the main groups of microorganisms involved in this phenomenon. Finally, the ways of reducing losses in practice will be presented.

2. Epiphytic Microflora

The course of the fermentation process and, consequently, the quality of the silage are highly dependent on the composition of the epiphytic microflora [27–29], which can significantly differ depending on the ensiled material and environmental conditions [30,31]. Lactic acid bacteria (LAB) play the greatest role in the process of forage ensiling [32]. In addition to lactic acid bacteria, the epiphytic microflora includes butyric acid bacteria, acetic acid bacteria, putrefying bacteria, yeasts, and molds. Most of the microorganisms present in the silage obtain energy through fermentation, producing energy-rich intermediate compounds. The exceptions are molds and yeasts as well as acetic acid bacteria that perform aerobic respiration (Figure 1).

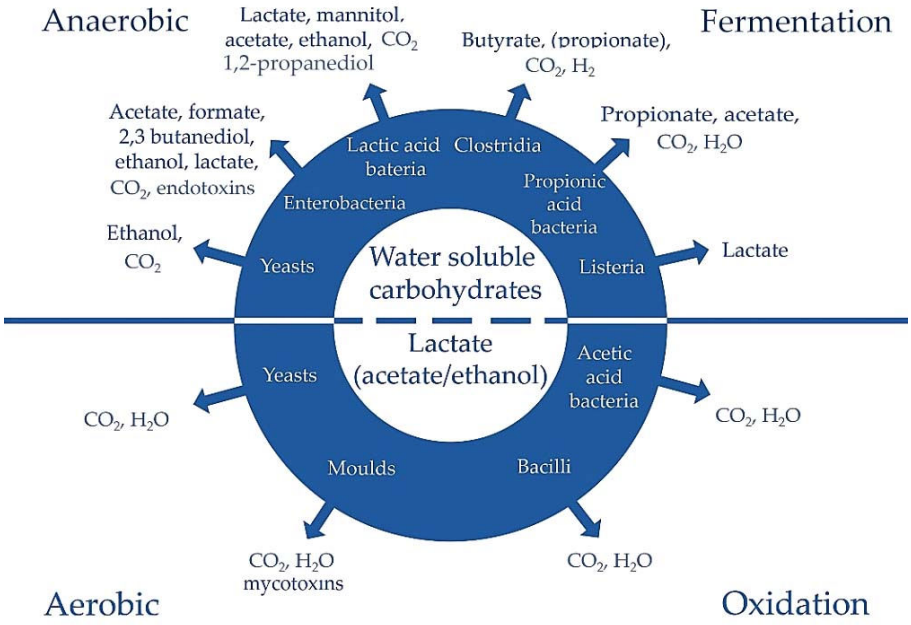


Figure 1. A main group of microorganisms involved in the fermentation process (modified on the basis of [33]).

2.1. Lactic Acid Bacteria

The competitiveness of lactic acid bacteria (LAB) in relation to other microorganisms during the fermentation process is determined by both the physical and chemical properties of the ensiled plant material, including the content of simple sugars, DM content, buffering capacity, and the properties of LAB strains, i.e., osmotolerance, resistance to low pH, and the ability to use specific substrates [34].

The LAB abundance in raw plant material generally does not exceed 1% of the epiphytic bacterial microflora. LABs that are most often found in the material intended for ensilage are of the following genera: *Enterococcus*, *Lactobacillus*, *Lactococcus*, *Pediococcus*, *Leuconostoc*, *Weissella*, and *Streptococcus* [35]. The population of bacteria of the genus *Lactobacillus* (according to the *International Journal of Systematic and Evolutionary Microbiology* classification before 2020) does not exceed 10^3 colony-forming units (CFU) g^{-1}. The number of *Enterococcus* bacteria ranges from 10^2 to 10^5 CFU g^{-1} while the number of *Pediococcus* bacteria does not exceed 10^3 CFU g^{-1}. Bacteria of the genus *Leuconostoc* are also numerous (10^4 to 10^5 CFU g^{-1}) [36–38]. Of these, *Pediococcus*, *Lactococcus*, *Enterococcus*, and *Weissella* grow at the initial stage of fermentation. After acidification of the environment, growth of the genus *Lactobacillus* is observed [39].

The number of epiphytic LAB cells depends on plant species and their developmental phase, air temperature, insolation, and other environmental conditions [39]. On fresh plants intended for ensilage, the number of LABs may vary widely: from 10^1 CFU g^{-1} (lucerne) to 10^8 CFU g^{-1} (maize and sorghum forage). Plants harvested in the middle of the growing season are characterized by the greatest number of these beneficious microorganisms, which means in the case of grasses, the second cut; in the case of alfalfa, the third cut; and in the case of maize, early varieties in the middle of the growing season. Air temperature is an extremely important factor determining the life processes of epiphytic microflora, which affects the rate of its growth. In cool weather, the number of bacteria present on the aerial parts of the plants is usually small [40–43]. Epiphytic LABs react positively to mechanical processes that the green forage is subjected to during harvesting (cutting, moving, crushing, shredding). Damage to the structure of plant tissues causes the release of the cell cytoplasm, which becomes a medium for rapidly multiplying LAB cells. In the literature, this phenomenon is called chopper inoculation [44].

LABs are Gram-positive, catalase-negative microorganisms that tolerate low pH and do not produce endospores. LABs are rod-shaped cells (e.g., *Lactobacillus*) or cocci (*Lactococcus*, *Streptococcus*), and *Bifidobacterium* can take the shape of the letter Y or V. Based on their temperature requirements, LABs can be classified as mesophilic or thermophilic. Depending on the type of ensiled plant material, they are capable of reducing the pH to 4–5 due to the production of organic acids such as lactic, acetic, and propionic acids [45].

LAB belong to obligatory or relative anaerobes, so they lack effective oxygen defense mechanisms, such as catalase activity [46]. These bacteria use two different defense mechanisms that aim to lower the concentration of toxic oxygen free radicals. The first of these are superoxide anion disproportionation reactions catalyzed by superoxide dismutase (SOD). The second method is based on the accumulation of manganese cations inside LAB cells [47].

Due to their high nutritional requirements, LABs are most often found in environments rich in carbohydrates, amino acids, and nucleotide derivatives. Under anaerobic conditions, LABs can use a wide variety of carbohydrates such as fructose, galactose, glucose, lactose, maltose, and mannitol, which are converted via lactic acid fermentation into lactic acid. Most LAB strains have the ability to utilize xylose, which is the main component of hemicelluloses in grasses. Due to the type of sugar transformation, lactic bacteria are classified as obligatorily homofermentative, optionally heterofermentative, and obligatorily heterofermentative. Heterofermentation results in the production of many volatile compounds: acetic acid, acetaldehyde, ethanol, CO_2, propionic acid, formic acid, acetone, esters and acetoin, diacetyl, and butanodiol [39,48].

Lactic homofermentation is a process of glucose conversion in the Embden–Meyerhof–Parnas pathway (EMP), resulting in the production of 2 pyruvate molecules, 2 ATP molecules, and 2 $NADH^+$ molecules from one glucose molecule. The majority of pyruvate molecules are reduced to lactic acid. A small part of pyruvate is decarboxylated, and two-carbon by-metabolites (acetate, ethanol) and CO_2 are formed. The amount of by-products depends on the availability of oxygen. Glucose heterofermentation is the process of converting glucose in the pentose pathway. Glucose, after oxidation to glucose-6-phosphate, is converted into xylulose-5-phosphate with the simultaneous formation of a CO_2 molecule. Xylulose-5-phosphate is cleaved into glyceraldehyde-3-phosphate and acetylphosphate. Glyceraldehyde-3-phosphate, in the same reactions as in the EMP pathway, is converted to pyruvate and further to lactic acid, acetic acid, or ethyl alcohol. Thus, glucose heterofermentation produces 1 mole of lactic acid, 1 mole of ethyl alcohol or acetic acid, and CO_2. Facultatively heterofermentative LABs metabolize hexoses in the EMP pathway and pentoses and other substrates (e.g., gluconate) in the pentose pathway, which is induced by the presence of pentoses. Fermentation products depend on the availability of substrates and environmental conditions, especially pH and temperature [49–51]. It is generally accepted that the ratio of lactic acid to acetic acid is above 3.0, which indicates homofermentation. When the value of this ratio is less than 3.0, it testifies heterofermentation [52].

The LAB strains of epiphytic microflora can be characterized by different carbohydrate metabolisms. For example, of the 11 LAB strains isolated from maize silage [53], two showed homofermentative metabolism, three showed heterofermentative metabolism, and six species showed facultative heterofermentative metabolism. The homofermentative species are: *Pediococcus acidilactici* and *Lactobacillus acidophilus*. Heterofermentative metabolism was demonstrated by *Lentilactobacillus buchneri*, *Lentilactibacillus hilgardii*, *Lentilactibacillus diolivorans*, and optionally heterofermentative: *Lacticaseibacillus rhamnosus*, *Lacticaseibacillus casei*, *Lentilactobacillus parafarraginis*, *Lacticaseibacillus paracasei*, *Lactiplantibacillus plantarum*, and *Lacticaseibacillus zeae*. It is generally recognized that the homofermentative strains, i.e., *Pediococcus* sp., usually multiply first in the first phase of fermentation. Then, they are replaced by heterofermentative strains [34,54–57]. The higher the fermentation temperature, the faster this process proceeds.

2.2. Enterobacteriaceae Family

Unfavorable conditions for the growth of lactic acid bacteria in silage may cause an insufficient reduction of the pH in the plant matrix, and as a consequence, the efficiency of pathogenic microflora inhibition can be limited [58]. After the initial phase of acidification of the plant material, intensive growth of relatively aerobic or anaerobic bacteria takes place, and the process is then called secondary fermentation. The main microorganisms responsible for the secondary fermentation processes in silage are bacteria of the *Enterobacteriaceae* family (enterobacteria), spore-producing bacillus, and Gram-positive cocci of the genera *Bacillus* and *Clostridium* [59].

Gram-negative bacteria of the *Enterobacteriaceae* family are widespread in nature. Enterobacter species constitute the endogenous flora of the digestive tract in up to 80% of the healthy human population. Those opportunistic microorganisms are generally not capable of causing pathogenic changes in humans, animals, or plants. The exception is endotoxin-producing bacteria that cause mastitis [60]. Bacteria of the Enterobacteriaceae family cause decarboxylation and deamination of amino acids and are able to use nitrogen compounds as a source of energy in respiration processes. The optimum pH for its growth ranges from 6.0 to 7.0. Most strains cannot grow at a pH below 4.5 [61]. In properly ensiled feed, they develop only in the first phase of primary fermentation, when the concentration of hydrogen ions is not too high. The research carried out showed that the number of *Enterobacteriaceae* bacteria after three days of ensiling maize was lower than immediately before placing plants in the silo [62].

2.3. Bacteria of Clostridium Genus

Bacteria of the genus *Clostridium* are Gram-positive, anaerobic spore-producing bacilli. Most species are anaerobes, although their oxygen tolerance can vary widely. Among the large group of *Clostridium* sp., there are usually only seven or eight species that are important in the process of forage ensiling. This group of microorganisms also includes bacteria of the species *C. botulinum*, which are rarely found in silages, and their presence in fodder may be dangerous due to the poisonous exotoxins that they produce [63–65].

The optimum pH for clostridia growth is in the range of 7.0–7.4, and the minimum water activity is 0.93 [63,66]. High temperatures in ensiled fodder and its high humidity constitute perfect conditions for the growth of the bacteria [56]. In properly ensiled forage, clostridia develop only in the first phase of primary fermentation, when the concentration of hydrogen ions is not too high. The level of their growth can be inhibited if the pH of the ensiled material drops to a level of 4.5 [34,66]. *Clostridium* bacteria can also be limited when the osmolality of the environment is 0.3 Osm l^{-1}, which corresponds to a level over 11 times higher than the osmolality of the ensiled fodder environment, which limits the development of the most desirable lactic acid bacteria (*L. plantarum*, *L. casei*, *P. acidilactici*, and *P. pentosaceus*) [67].

2.4. Propionic Acid Bacteria

Propionic acid bacteria are Gram-positive, non-spore-forming, anaerobic, or facultatively anaerobic bacteria. These bacteria can utilize a variety of carbon sources, such as carbohydrates, polyols, and organic acids, including lactic acid and gluconic acid. The substrates are oxidized to pyruvate by glycolysis or the pentose phosphate pathway; subsequently, pyruvate is reduced to propionic acid via the Wood–Werkman cycle or oxidized to acetate and CO_2 [16]. The main fermentation products are propionic, acetic, and succinic acids, as well as CO_2 [68]. These bacteria have a low acidity tolerance of not less than 4.8 [69]. There are limited data on the propionic acid bacteria population or on the identification or survival of these bacteria in silages. The only species that has been identified in silages is *Propionibacterium acidipropionici* [70] and then applied in silage inoculants in combination with LABs [71].

2.5. Acetic Acid Bacteria

Acetic acid bacteria are Gram-negative bacteria with a high tolerance to acidic conditions. They cause the incomplete oxidation of alcohols, sugars, and lactic acid. Most are aerobic and have therefore been identified in the aerobic stages of fermentation, mainly in the opening phase, which is associated with aerobic deterioration. The most commonly encountered species is *Acetobacter pasteurianus*, but other genera such as *Gluconobacter* have also been identified. The formation of CO_2 can be observed, thus initiating aerobic deterioration. Those bacteria synthesize acetic acid, known for its antifungal action and positive effect on aerobic stability. On the other hand, the compound may be a factor in reducing silage intake [72].

2.6. Bacillus

Bacteria of the genus *Bacillus* are endospore-forming cells, usually catalase-positive, anaerobic, or relatively anaerobic, which can ferment many carbohydrates. Bacilli synthesize organic acids (e.g., acetic, lactic, butyric), ethanol, 2,3-butanediol, or glycerol [65]. There are also pathogenic species that develop in living organisms (*B. anthracis* and *B. cereus*). *Bacillus* sp. are mostly saprophytes commonly found in soil and dead plant tissues. Species isolated from silage are: *B. cereus*, *B. lentus*, *B. firmus*, *B. sphaericus*, *B. licheniformis*, and *B. polymyxa*. The number of endospores of bacteria of the genus *Bacillus* in silages obtained under production conditions may vary widely. In grass and maize silages, their endospore number ranges from 10^2 to 10^6 CFU/g and is much lower in silages from the deeper silo layers [73].

A rich source of bacilli is manure, e.g., bovine manure, which may contain from 10^4 to 10^6 endospores per gram [59,74]. The results of the research by [74] showed that meadow sward fertilized with manure had almost 100 times more *Bacillus* sp. cells than plants from meadows fertilized with mineral fertilizers. It was also shown that the silage obtained from plants fertilized with manure was of low quality (high pH, high NH_3 content, and presence of butyric acid).

2.7. Yeasts

Yeasts are microscopic fungi with a nucleus separated from the rest of the cytoplasm by a membrane. The typical method of asexual reproduction for them is budding. Yeasts are widely distributed in the environment and survive in a wide variety of temperature, pH, and osmolarity conditions. Yeast is not a homogeneous taxonomic group and among them, both saprophytes and parasites can be distinguished. Due to the phylogenetic relationship and the ability for generative reproduction, yeasts are classified into three classes: Ascomycetes, Basidiomycetes, and Deuteromycetes. More than 30,000 species have been described in each class. The most frequently described yeast in different types of silage is *Issatchenkia orientalis* (synonyms: *Pichia kudriavzevii* and *Candida krusei*) [75–78].

Some factors may favor yeast growth in silage, such as the increased availability of water-soluble carbohydrates, lower temperatures (20 °C), and air ingress during fermentation [79]. Lactic acid produced during fermentation may be a source of energy for yeast cells [65]. Many studies emphasize the high yeast count in maize and grass silages in which the initial fermentation period was carried out with a significant share of air, and in silages from the outer part of the silo, as well as in silages with visible signs of mold growth [80,81].

The results of studies conducted in the USA showed that the average yeast counts in maize silage (whole plants, wet grain) obtained on a production scale differed slightly [82]. The average number of yeasts in wet maize silage (69.2% DM) was 6.3 log CFU g^{-1} and was 0.9 log CFU g^{-1} higher than in whole plant silage (34.7% DM) [29,34,80]. Moreover, these silages differed significantly in yeast species composition. Out of 106 yeast colonies isolated from whole maize plant silage, as many as 23 belonged to the species *Candida ethanolica* and 19 to *S. boulderi*. On the other hand, in the wet maize grain silage, the dominant yeast species were: *Pichia anomala* (32.5%), *Issatchenkia orientalis* (22.4%), *S. cerevisiae* (16.1%), and *P. fermentas* (12.4%). No yeast of the genus *Candida* was found in them. In silages from

other farms, the samples of wet corn silage contained mainly *P. anomala* and *I. orientalis* yeasts. The latter yeast species was isolated from 46% of samples of whole-plant maize silage produced in Brazil [76]. The results of other studies also showed the presence of *P. anomala* in silage: from whole-plant maize [83], grasses [84–86], and sugar cane [87].

The silage environment, which is usually characterized by a lack of oxygen and an acid reaction, is unfavorable for the development of yeast. Short carbon chain fatty acids (propionic, acetic) are known to be inhibitors of yeast growth under low pH conditions, when molecules are undissociated [88]. This fact is explained by the greater diffusion capacity of undissociated molecules into the yeast cells, which results in lowering the pH of their environment as a result of the release of H^+ ions. This process can lead to the rapid death of yeast cells unless sufficiently counteracted by their defense mechanism, which requires energy to remove H^+ ions [89].

2.8. Moulds

Molds may be present in the silage during all stages of fermentation, but they are present in significant amounts in silage only after it has been significantly spoiled by yeast and other aerobic bacteria. Molds hydrolyze carbohydrates, proteins, and organic acids, lowering the acidity of the silage. They tolerate high temperatures and low pH of 2.5–3.0. They are obligatory aerobes [90]. Molds often appear in the upper layers of silage if their cover is not tight. Some molds already multiply in the field when the plants are growing, e.g., *Fusarium graminearum* and *A. flavus*.

3. Silage Fermentation Process

The fermentation process of silage is a dynamic process in which a succession of different groups and species of microorganisms occurs. The fermentation process involves both aerobic (oxygen-needing) and anaerobic (non-oxygen-needing) bacteria and is generally divided into six different phases. Aerobic fermentation occurs when the silo or bag is being filled (phase 1) and at feed out (phase 6). All these phases have different lengths, various biochemical processes with different intensities taking place, and accordingly, different losses can occur. The remainder of the phases (phases 2 through 5) occur under anaerobic conditions. In the first phase (aerobic phase), the temperature of the newly fermenting crop increases due to ongoing cell respiration, where CO_2, water, and heat are produced. The initial growth of aerobic spoilage organisms (yeasts and *Bacillus* species) occurs during this phase. The aerobic phase should be as short as possible to eliminate not only the growth of aerobic microbes but also respiration and enzymatic activity that cause detrimental effects on the silage quality and increase silage losses. During the second fermentation phase, anaerobic (without oxygen) heterofermentation occurs. The primary bacteria during this phase are *Enterobacteria*, which produce both acetic and lactic acid but are usually inefficient at producing these acids relative to nutrient losses in the fermenting crop. During the next phase (phase 3), the homofermentative bacteria (more efficient than the hetero-fermenters) rapidly drop the pH by producing lactic acid as an end-product. As the temperature of the silage mass decreases and the pH continues to drop, the bacteria in this phase become inhibited, and phase 4 lactic acid bacteria increase. Phase 4 is a continuation of phase 3. Homofermentative bacteria convert water-soluble carbohydrates to lactic acid, which is very effective at dropping the pH, which helps preserve silage. Phase 5 lasts through the remainder of storage, where the fermentation process is stable as long as oxygen does not penetrate the silage. Phase 5, the last phase, occurs during feed out and can result in DM losses as oxygen is reintroduced into the fermented crop [91].

Heat production is normal during the ensiling process, and a rise of up to 12 °C in relation to silage temperature at harvesting is common even in a well-managed silo. Ensiling at high temperatures or in wet conditions, especially in tropical areas, is known to increase the rate of DM losses before silo sealing [92].

4. Dry Matter Losses

Losses of DM and gross energy as a result of fermentation processes in forage depend mainly on the type of microorganisms growing in the plant matrix and the availability of individual substrates. Some products of fermentation are characterized by a higher energy value compared to the substrates, which results in significantly greater losses of DM than of gross energy (Table 1) [34]. This is because the energy recovery is higher than the DM recovery during the chemical transformations of the substrate in silages [93].

Table 1. Losses of DM and gross energy related to the substrate being fermented by epiphytic microorganism [34,92].

Organism	Substrate	Products	Losses (%)	
			DM	Gross Energy
LAB (Ho)	Glucose	2 lactate	0	0.7
LAB (He)	3 Fructose	1 lactate, 1 acetate, 2 mannitol, 1 CO_2	4.8	1.0
Enterobacteria	2 Glucose	1 lactate, 1 acetate, 1ethanol, 2 CO_2	17	11.1
LAB (He)	Glucose	1 lactate, 1 ethanol, 1 CO_2	24	1.7
LAB (Ho/He)	2 Citrate	1 lactate, 3 acetate, 3 CO_2	29.7	−1.5
LAB (Ho/He)	Malate	1 lactate, 1 CO_2	32.8	−1.8
Yeasts	Glucose	2 ethanol, 2 CO_2	48.9	0.2
Clostridia	2 Lactate	1 butyrate, 2 CO_2, 2 H_2	51.1	18.4

LAB—lactic acid bacteria, HO—homofermentative, HE—heterofermemtative.

4.1. Losses Caused by Lactic Acid Bacteria

Homofermentative bacteria under anaerobic conditions synthesize mainly lactic acid from glucose or fructose. This process takes place without loss of DM, as there are no gaseous products and the gross energy loss is only 0.7%. Glucose fermentation by heterofermentative lactic acid bacteria causes a DM loss of 24%, and the gross energy loss is only 1.7% (Table 2).

Some obligatorily heterofermentative bacteria (*L. buchneri* and *L. parabuchneri*) have the ability to biotransform lactic acid into acetic acid, 1,2-propanediol, ethanol, and CO_2. 1,2-propanediol is an important component of silages, positively influencing their aerobic stability and quality and possibly affecting the intake and utilization of silage-based diets [94]. The research results showed that the intensity of this transformation depends on the environmental pH and temperature [73]. The optimal temperature for most of the studied strains ranged from 15 °C to 37 °C. If the pH was 5.8, no changes in the content of lactic acid were recorded. The highest intensity of lactic acid degradation was in an acidic environment with a pH of 3.8. However, heterofermentations are accompanied by relatively high losses of dry matter, which amount to 25.4%, because 0.52 moles of CO_2 are produced from one mole of lactic acid. They do not have a large share in the total DMDM losses resulting from the changes taking place in the ensiled fodder, but their importance in improving the stability of the final product is significant [95].

Most recently, combination inoculants have been introduced. These inoculants contain both *L. buchneri* and more traditional homofermentative strains. The goal is to have the homofermentative strains dominate early fermentation to achieve an efficient fermentation and a rapid reduction in silage pH. After active fermentation, *L. buchneri* slowly converts some lactic acid to acetic acid [92].

Data on the impact of inoculants based on *L. buchneri* (together with homo- and obligatory fermentative species) on ensiling of forages (grasses, lucerne, sorghum, whole-plant maize, wet maize grain, sugarcane, alfalfa-grass mixtures, clover-grass mixtures, potatoes, oats) were subjected to meta-analysis. Results showed that their influence on the fermentation profile was favorable in terms of the stability of the silage. Silages with the addition of lactic acid bacteria based on *L. buchneri* contained higher concentrations of acetic acid compared to those obtained by natural fermentation. The largest difference in the mean raw results concerned the silages produced with the addition of: *L. buchneri*,

L. plantarum, and *P. pentosaceus* (LB + LP + PP), and L. *buchneri*, *L. plantarum*, and *Enterococcus faecium* (LB + LP + EF). The addition of starter cultures caused a reduction in the number of molds and yeasts. Silages with the addition of LAB inoculants were on average 234 h more stable in terms of oxygen than those obtained without the inculants. Taking into account the recovery of dry matter, it should be stated that only a positive measure of this effect related to the silages was obtained with the addition of *L. buchneri* and *P. pentosaceus*. The results of the meta-analysis of data on maize silage, grass green forage, and cereal plants showed that the addition of *L. buchneri* slightly increased the loss of DM compared to silage obtained by natural fermentation. Average losses of maize silage DM were only 0.5 percentage points higher than those resulting from silages prepared without the addition of *L. buchneri*. On the other hand, the average DM losses of the remaining silages (grasses, cereals) were about 1.5 percentage points higher when the inoculum was used [96].

The quantitative synthesis of the research results obtained from 97 studies showed a diversified effect of bacterial preparations and fibrolytic enzymes on the fermentation profile and silage DM losses [97]. Silages were divided into two groups. The first one included those obtained from high-protein feeds or with a significant share thereof (the average protein content in the raw material was 20.18% of dry matter). The second group included those obtained from raw material containing on average slightly more protein (8.56% dry weight) than water-soluble carbohydrates (7.56% dry weight). Of the total number of inoculants used, 44.4% contained homofermentative or facultative heterofermenting LAB, and 32.3% contained only the obligatory hetrofermentative species. The remaining inoculants contained three LAB groups. *L. plantarum* constituted a component of 29.8% of inoculants, and *L. buchneri* −14.1%. Silages made from forage with a similar content of crude protein and water-soluble carbohydrates were of very good quality. The addition of homo- and heterofermentative bacteria (MS group) did not improve the quality of silages compared to those obtained by natural fermentation (BZ group). On the other hand, silages prepared from fodder with high protein content, obtained with or without the addition of only enzymes, were of poor quality. Average losses for silages of the high-protein matrix were on average about 3% of dry matter. Much greater losses of DM were demonstrated as a result of ensiled forages belonging to the second group (5.48% on average). This may be the result of more intensive fermentation changes in ensiled plant material, which contained more water and water-soluble carbohydrates. It should be added, however, that the losses of DM were much smaller than the losses that arise during the ensilage of various forages with similar moisture [98–102].

Statistical analysis of the data compared by [97] showed that the highest values of DM loss were 4.67% and concerned the ensilage of whole maize plants. The range of losses of DM ranged from 22.2 to 27.4% [103].

Table 2. Examples of lactic fermentation reactions occurring in ensiled forages along with the balance of DM and gross energy [34,93,104].

Reactions	Type of Bacteria	Losses, %	
		Dry Matter	Gross Energy
glucose + 2 ADP → 2 lactic acid + 2 ATP 180 g 180 g 2815 kJ 2729 kJ 67 kJ	HO	0	0.7
fructose + 2 ADP → 2 lactic acid + 2 ATP 180 g 180 g 2815 kJ 2729 kJ 67 kJ	HO	0	0.7
glucose + ADP → lactic acid + ethanol + CO_2 + ATP 180 g 90 g 46 g 2815 kJ 1364 kJ 1371 kJ 33.5 kJ	HE	24.5	1.7

Table 2. *Cont.*

Reactions	Type of Bacteria	Losses, %	
		Dry Matter	Gross Energy
3 fructose + 2 ADP → lactic acid + acetic acid + 2 mannitol + CO_2 + 2 ATP 540 g 90 g 60 g 364 g 8478 kJ 1364 kJ 876 kJ 6090 kJ 67 kJ	HE	4.8	1.0
2 citric acid + H_2O + ADP → 3 acetic acid + lactic acid + 3 CO_2 + ATP 384 g 18 g 180 g 90 g	HO/HE	32.8	-
malic acid → lactic acid + CO_2 + 2 ATP 134 g 90 g	HO/HE	32.8	-
lactic acid → 0.48 acetic acid + 0.48 1,2- propanediol +0.04 ethanol + 0.52 CO_2 90 g 28.8 g 36.5 g 1.84 g 22.9 g	OHE	25.4	-

HO—homofermentative, HE—heterofermemtative. OHE—obligatory heterofermentative. ADP—adenosine diphosphoric acid, ATP—adenosine triphosphoric acid.

4.2. Losses Caused by Enterobacteria

Enterobacteria compete with lactic acid bacteria for the available sugars and can also break down proteins. Protein degradation not only reduces the nutritional value but also leads to the production of toxic compounds such as biogenic amines. In addition, ammonia formed as a result of proteolysis increases the buffer capacity of the ensiled biomass, which makes it difficult to quickly lower its pH [34,105,106]. Bacteria from the Enterobacteriaceae family are microorganisms responsible for the breakdown of nitrates in the ensiled material. The intermediate products of nitrate decomposition, mainly nitrites, are reduced to nitrous oxide and NH_3. The unstable and highly reactive nitric oxide (II) NO can react with oxygen, and the products of this reaction are highly poisonous and irritating to the respiratory tract of animals. On the other hand, the reduction of nitrites is considered to be positive in terms of the impact on the quality of the silage, as NO_2 and NO are very effective inhibitors of bacteria of the genus *Clostridium* [107–109].

A rapid reduction in the growth of *Enterobacteriaceae* bacteria in silage is desirable. This is mainly due to the reduction in the intensity of fermentation of sugars to acetic acid and other products limiting the decrease in the pH of the ensiled feed environment. The harmful processes of amino acid metabolism, the product of which is, e.g., NH_3, which increases the pH of the preserved material, are also important. As a result of glucose fermentation by *Escherichia coli*, two moles of lactic acid, one mole of acetic acid, and two moles of CO_2 are produced. A less favorable course of glucose fermentation may take place with the participation of bacteria of the genus *Klebsiella*. From two moles of glucose, only one mole of lactic acid is formed, and the weight loss is 1.5 times higher than the losses resulting from glucose fermentation by *E. coli* [104].

4.3. Losses Caused by Clostridium

Bacteria of the genus *Clostridium* utilize organic substances such as carbohydrates, proteins, amino acids, amides, and lactic acid as a source of energy. The products of their fermentation are usually: butyric acid, acetic acid, NH_3, CO_2, H_2, and many other substances that are the effect of the coupled oxidation of two amino acids (Stickland reactions) (Tables 3 and 4).

Table 3. Selected reactions of amino acid deamination by proteolytic bacteria of the genus *Clostridium* [34].

Substrates	Products
$C_6H_{14}N_2O_2 + H_2O$ lysine	$CH_3COOH + CH_3(CH)_2COOH + 2\ NH_3$ acetic acid + butyric acid + ammonia
$C_3H_7NO_3$ serine	$C_3H_4O_3 + NH_3$ pyruvic acid + ammonia
$C_{11}H_{12}N_2O_2 + H_2O$ tryptophan	$C_{11}H_{11}NO_2 + NH_3$ 3-indolepropionic acid + ammonia
$C_6H_{14}N_4O_2 + 2\ H_2O$ arginine	$C_5H_{12}N_2O_2 + 2\ NH_3 + CO_2$ ornithine + ammonia + carbon dioxide
$C_5H_{11}NO_2S + H_2O$ methionine	$C_4H_6O_3 + CH_4S + NH_3$ α-ketobutyric acid + methyl mercaptan + ammonia
$C_5H_9NO_4 + H_2O$ glutamic acid	$CH_3COOH + C_3H_4O_3 + NH_3$ acetic acid + pyruvic acid + ammonia
$C_6H_9N_3O_2 + 3\ H_2O$ histidine	$CH_3NO + C_5H_9NO_4 + NH_3$ formamide + glutamic acid + ammonia

Table 4. Examples of selected Stickland reactions and the related DM losses [34].

AA [1] OX/RE	Reaction Equation [2]	DML [3] %
Ala/Gly	$CH_3CH(NH_2)COOH + 2\ NH_2CH_2COOH + 2H_2O \rightarrow 3\ CH_3COOH + 3\ NH_3 + CO_2$ alanine glycine acetic acid	18.39
Leu/Gly	$(CH_3)_2CHCH_2CH(NH_2)COOH + 2\ NH_2CH_2COOH + 2H_2O \rightarrow CH_3(CH_2)_3COOH + 2CH_3COOH + 3\ NH_3 + CO_2$ leucine glycine isovaleric acid acetic acid	15.64
Ala/Pro	$CH_3CH(NH_2)COOH + 2\ (CH_2)_3NHCHCOOH + 2H_2O \rightarrow CH_3COOH + 2\ NH_2(CH_2)_4COOH + NH_3 + CO_2$ alanine proline acetic acid δ-amino-valeric acid	13.78
Ala/Orn	$CH_3CH(NH_2)COOH + 2\ (NH_2)_2(CH_2)_3CHCOOH + 2H_2O \rightarrow CH_3COOH + 2\ NH_2(CH_2)_4COOH + 3\ NH_3 + CO_2$ alanine ornithine acetic acid δ-amino-valeric acid	12.45
Val/Pro	$(CH_3)_2(CH)_2NH_2COOH + 2(CH_2)_3NHCHCOOH + 2H_2O \rightarrow (CH_3)_2CHCOOH + 2NH_2(CH_2)_4COOH + NH_3 + CO_2$ valine proline isobutyric acid δ-amino-valeric acid	12.66
Leu/Pro	$(CH_3)_2CHCH_2CH(NH_2)COOH + 2\ (CH_2)_3NHCHCOOH + 2H_2O \rightarrow$ $(CH_3)_2CHCH_2COOH + 2NH_2(CH_2)_4COOH + NH_3 + CO_2$ leucine proline isovaleric acid δ-amino-valeric acid	12.17
Ile/Pro	$(CH_3)_2CHCH_2CH(NH_2)COOH + 2\ (CH_2)_3NHCHCOOH + 2H_2O \rightarrow$ $(CH_3)_2CHCH_2COOH + 2\ NH_2(CH_2)_4COOH + NH_3 + CO_2$ isoleucine proline valeric acid δ-amino-valeric acid	12.17

[1] AA—amino acid, OX—oxidized, RE—reduced, Ala—alanine, Leu—leucine, Val—valine, Ile—isoleucine, Gly—glycine, Pro—proline, Orn—ornithine, [2] stoichiometric equations and the DM losses calculations are own elaboration, [3] DML—dry matter losses.

The produced butyric acid is weaker than lactic acid, and its presence in the silage increases its pH. In addition, only one mole of butyric acid is formed from two moles of lactic acid. Thus, the losses of preserved material occur due to the appearance of gaseous fermentation products. They are 51.1% for the fermentation of glucose and lactic acid to butyric acid, CO_2, and hydrogen [33].

Some species of bacteria from the *Clostridium* genus [34] obtain the energy necessary for their growth through oxidative and reductive changes of amino acids, which may be the only source of carbon and nitrogen. The mentioned transformations refer simultaneously to two different amino acids, one of which is a donor and the other is a proton acceptor (Stickland reactions). The oxidation substrates in Stickland double reactions are usually: alanine, histidine, isoleucine, lecithin, phenylalanine, serine, tryptophan, and tyrosine [110].

Among the reducible amino acids, there are mainly arginine, aspartic acid, cysteine, glycine, methionine, ornithine, proline, tryptophan, and tyrosine. The oxidized amino acids are converted into fatty acids, which contain one less carbon atom in their chemical composition compared to the substrate. The reducible amino acids are converted into fatty acids, in which the number of carbon atoms is equal to the number of carbon atoms of the substrate. In all types of transformation mentioned (except for proline), the products also include ammonia and CO_2 (Table 4).

The presence of proteolytic bacteria of the *Clostridium* genus in preserved feed is detrimental due to their ability to ferment proteins and amino acids (Table 5). In the first phase of fodder ensilage, protein hydrolysis takes place, mainly by proteolytic plant enzymes. The products of these transformations are amino acids, which are then metabolized by clostridia cells.

The results of the research conducted under laboratory conditions on grass ensiling (with DM, protein, and sugar contents of approx. 24.5%, 17.5%, and 8.6%, respectively) showed that the addition of *C. tyrobutyricum* in the amount of 10^2 endospores per one gram of ensiled material adversely affected the quality of the final product. The silage was characterized by a high pH (from 5.2 to 5.7) and a high content of butyric acid (from 3.4 to 4.2% in DM) in relation to lactic acid (average of only 4.0% in DM). The number of *C. tyrobutyricum* endospores in the silage was almost 1.7×10^7 g^{-1} and was over 6.3×10^4 higher than in the ensiled material. Weight losses in silages inoculated with clostridium after 20–30 days of ensilage amounted to 60–70 g kg^{-1}. On day 95, the total weight loss from the silage inoculated with clostridia ranged from 74 to 86 g kg^{-1} DM. High weight losses in combination with a high level of butyric acid indicated that these silages could be characterized by an intensive growth of clostridial bacteria [111].

Table 5. Selected reactions of amino acid decaroboxylation by proteolytic bacteria of the genus *Clostridium* with losses of dry matter (DML).

Reaction Equation [1]	DML [2], %	References
$C_6H_{14}N_4O_2 + H_2O \rightarrow CH_4N_2O + C_5H_{12}N_2O_{21}$ arginine urea ornithine	25.3	[34]
$C_5H_{12}N_2O_2 \rightarrow C_4H_{12}N_2 + CO_2$ ornithine putrescine		
$C_4H_7NO_4 \rightarrow C_3H_7NO_2 + CO_2$ aspartic acid alanine	33.1	[112]
$C_5H_9NO_4 \rightarrow C_4H_9NO_2 + CO_2$ glutamic acid γ-aminobutyric acid	29.9	
$C_6H_9N_3O_2 \rightarrow C_5H_9N_3 + CO_2$ histidine histamine	28.4	
$C_6H_{14}N_2O_2 \rightarrow C_5H_{14}N_2 + CO_2$ lysine cadaverine	30.1	
$C_9H_{11}NO_2 \rightarrow C_8H_{11}N + CO_2$ phenylalanine 2-phenylethylamine	26.6	[34]
$C_3H_7NO_3 \rightarrow C_2H_7NO + CO_2$ serine ethanolamine	41.9	
$C_{11}H_{12}N_2O_2 \rightarrow C_{10}H_{12}N_2 + CO_2$ tryptophan tryptamine	21.5	[34,112]
$C_9H_{11}NO_3 \rightarrow C_8H_{11}NO + CO_2$ tyrosine tyramine	24.3	

[1] Reaction equation were developed on the basis of their verbal notation [34]; [2] DML—dry matter losses.

It was confirmed that the clostridial fermentation caused by the cell growth of the *Clostridia* genus is responsible for the anaerobic deterioration (secondary fermentation) in silage [113,114]. Clostridial fermentation not only reduces the nutritional value of silage but also affects the health of humans and animals [64,115]. Moreover, the potential risks to cattle health and milk contamination, mainly caused by *C. botulinum* and *C. tyrobutyricum*, can result in great economic losses to the dairy industry when clostridial-contaminated silage is fed [116].

Since the amount of DM and energy losses related to butyric acid fermentation can be significant, attempts were made to reduce the number of clostridium cells in ensiled forage [24,117–119]. The primary method of inhibiting the growth of *Clostridium* bacteria is to pre-dry the ensiled material. This was evidenced by [120] in silages made of alfalfa fertilized with slurry, which contained more than 15 times more copies of the genome of bacteria of the genus *Clostridium* than forage from non-fertilized plants. On the other hand, the silage obtained from this green forage contained over 100 times more of these copies of the bacterial genome than the silage from unfertilized plants. It should be added, however, that significant differences in the content of genome copies of *Clostridium* cluster bacteria in the ensiled material did not significantly affect the quality of the final product (similar pH value, slight differences in the content of NH_3-N and water-soluble carbohydrates). Therefore, it can be stated that the drying of the ensiled material (from 47.1 to 52.6% of dry mass) and sufficiently strong compaction (from 196 to 210 kg of dry mass μm) made it possible to obtain good-quality silages.

For many years, the main role in reducing the butyric acid content in silages with an increased DM content was attributed only to the reduced water activity. Later studies on silages from wheat and barley showed that low humidity of preserved fodder and a low content of nitrates proved to be factors inhibiting the growth of *Enterobacteriaceae* bacteria in the initial stage of fermentation, which resulted in an insufficient concentration of nitrates in further biochemical processes. As a consequence, it was not possible to effectively limit the growth of bacteria of the genus *Clostridium*. Therefore, a special role in the effective inhibition of the germination of clostridial endospores in the initial phase of fermentation under high pH conditions is assigned to nitrates. The results of [121] also acknowledged the beneficial effect of nitrates on inhibiting the growth of bacteria of the genus *Clostridium*. It was observed that the addition of up to 10^6 *C. tyrobutyricum* endospores to one gram of ensiled material had no negative effect on the quality of the final product. In the resulting silage, lactic acid constituted 80% of the total acids, and the share of butyric acid did not exceed 0.7%. However, ensiled material was characterized by a high content of nitrates (0.01 mol kg^{-1}), which effectively limited the growth of clostridial cells.

The preventive effect against the growth of *Clostridium* is also demonstrated by the use of chemical preparations in the form of a mixture of sodium nitrate and calcium formate as well as sodium formate, sodium acid sulfate, and sodium nitrite [122]. Based on the results of the experiments provided in Germany, regression equations were developed to define the minimum content of nitrate nitrogen in the ensiled material, necessary to obtain a silage from green fodder free of butyric acid [108].

Among methods limiting microbiological contamination of silages, there should be mentioned the biotechnological group of methods relating to bacterial inoculants for ensiling containing strains capable of producing not only lactic acid, but also bacteriocins, bacteriocin-like substances, and exopolysaccharides [57,123–128]. Of particular interest are bacteria producing class IIa metabolites, which show very strong antibacterial activity against *Clostridium* sp. and *Listeria monocytogenes* [127,129].

4.4. Bacteria of the Genus Bacillus

Although bacteria of the *Bacillus* genus are not responsible for the initiation of aerobic decomposition of silages, they may participate in the later stages of this process, which is initiated by yeast [109]. Noteworthy, the presence of many *Bacillus* species is undesirable [16]. This is due not only to the lower efficiency of the production of lactic or acetic acid compared to lactic acid bacteria but also to their destructive effect on the silage under aerobic conditions [130,131]. The threat of *B. cereus* contamination of milk is also significant [58,132].

Meanwhile, some *Bacillus* species, because of their favorable ability to improve animal performance when orally administered [133] and improve fermentation quality and aerobic stability [134], are used as fourth-generation silage inoculants. Refs. [134,135] found that *B. subtilis* used in silage improved fermentation quality and inhibited aerobic spoilage.

Moreover, Ref. [136] reported the improvements in the aerobic stability of corn silage with *B. subtilis* addition, which were attributed to antifungal and antibacterial metabolites produced by *B. subtilis* [137]. According to [138], *B. subtilis* and *B. amyloliquefaciens* inoculants decreased the relative abundances of undesirable bacteria such as *Acetobacter* sp. and *Acinetobacter* sp.

Many studies acknowledge lower losses of DM for silages prepared with the addition of bacterial preparations than those obtained by natural fermentation [139–141]. However, it is not possible to unequivocally assess the effect of inoculants containing *B. subtilis* on the quality of silage and loss of DM [130,142,143]. As reported by [130], ensiling sunflower (*Helianthus annuus* L.) with the addition of a starter culture consisting of *L. buchneri* and *B. subtilis* was associated with high losses of DM (12.42%), greater than the ensiling of this feed by natural fermentation (10.58%). The main cause of these losses was unfavorable fermentation transformations. A much greater loss of DM was demonstrated during the ensiling of sugar cane (*Saccharum officinarum*) [142]. They amounted to 26.4% if the ensiling was carried out by natural fermentation. The addition of *L. buchneri* and *B. subtilis* starter cultures reduced these losses to 20.7%. It is worth emphasizing that the silage obtained only with the addition of *B. subtilis* CP7 was characterized by the highest content of lactic acid (4.42% DM). According to the authors, this was the result of favorable conditions for LAB growth in ensiled feed due to the production of bacilli peptides with antimicrobial activity directed against harmful bacteria such as *E. coli*, *Salmonella* sp., and other pathogens [137].

The results of research on the ensiling of lentils [144] and kidney beans [145] showed the synergistic effect of *B. subtilis* and *Lactobacillus* sp. bacteria. It can therefore be argued that the addition of *B. subtilis* CP7 interacted with lactic acid bacteria, which resulted in a good quality silage with a much lower non-protein nitrogen content than in the naturally fermented silage [134]. The results concerning DM losses and protein metabolism deserve special emphasis. The bacterial additives used significantly reduced the intensity of harmful changes, which resulted in: lower DM losses and lower non-protein nitrogen content.

4.5. Yeast

Based on respiratory activity, yeast can be divided into non-fermenting types with aerobic metabolism, in whom aerobic and anaerobic metabolism is in equilibrium proportions and showing mainly anaerobic metabolism (the share of respiration does not exceed 10–15%). Under anaerobic conditions, many yeast species ferment sugars such as glucose, maltose, and sucrose [82], which products are ethanol and CO_2, and in small amounts, other metabolites are formed, e.g., propanol, 2-butanediol, 2-methylpropanol, pentanol, 3-methylbutanol and volatile fatty acids, e.g., acetic, propionic, butyric. Transformations of glucose and lactic acid into ethanol and CO_2 cause a loss of DM (49%) and an increase in the pH of the ensiled fodder. Moreover, yeast can decompose the silage in the presence of oxygen and oxidizing sugars. Dry matter (DM) losses are then higher [65].

Under aerobic conditions, yeast can obtain energy much more easily through respiration than through fermentation to produce ethanol and CO_2 [34]. The processes of aerobic and anaerobic respiration are inseparable in yeast cells. In the presence of oxygen, the rate of sugar fermentation is reduced or even completely stopped. Most yeasts, however, require some level of oxygen to trigger alcoholic fermentation, and a complete absence of oxygen can inhibit their growth. The exception is the yeast *Saccharomyces cerevisiae*, in whose cells fermentation can take place under strictly aerobic conditions. Still, the synthesis of the cytoplasmic membranes of *S. cerevisiae* yeast cells requires a negligible amount of molecular oxygen [146]. The presence of these microorganisms in the anaerobic environment of maize and sorghum silages was documented by [147]. *S. cerevisiae* are widely used as probiotics for ruminants as well [148].

Yeast can be constituents of probiotics and/or microbial feed additives, e.g., *S. cerevisiae*, *Humicola grisea*, and *C. glabrata*, which are marked with the symbol DFM (direct-fed microbials) [149]. Their use inhibits the growth of harmful microorganisms in forage [150–152] and has a positive effect on the use of nutrients by animals, which consequently increases

their productivity [153]. *Pichia anomala* J121 strain has a strong antifungal effect against *Aspergillus candidus* and *Penicillium roqueforti* and inhibits the growth of some Gram-negative bacteria [150]. Barley grain (18% water content) with the addition of *P. anomala* was characterized by a lower number of undesirable microorganisms than that from the control group, and after five months of storage, it contained only this one yeast species [154].

4.6. Molds

The relationship between DM losses and mold count in spoiling farm silage has been demonstrated [92]. When the mold level in the silage was greater than 5 $\log_{10}$ cfu/g (i.e., mold became visible on the silage), the DM losses were greater than 20%. When mold counts exceeded 6.0 $\log_{10}$ cfu/g of silage, losses could exceed 40% of the original ensiled DM. Furthermore, when the mold count rose to over 5.0 $\log_{10}$ cfu/g of silage, substantial changes in nutritional quality occurred, with starch content beginning to decrease and falling below 10% of DM when the mold count was higher than 7.0 $\log_{10}$ cfu/g of silage.

The decrease in nutritional quality, coupled with the DM losses, results in a dramatic decrease in the potential milk production of the original harvested crop [155]. It was shown that there were reductions in potential milk production when the mold count exceeded 4.0 log cfu/g of silage, and it was almost halved when the mold count was greater than 8.0 log cfu/g of silage [156].

The presence of mold causes not only a loss of nutrients in the forage but also potential contamination of the silage with mycotoxins [157]. Mycotoxins are a group of highly toxic secondary metabolites secreted by fungal organisms, mostly belonging to the genera *Aspergillus*, *Fusarium*, *Alternaria*, and *Penicillium* [158] [Dey et al., 2022]. A 4-year mycotoxin survey in Poland revealed that up to 95% of feedstuffs contained at least one mycotoxin [159]. Mycotoxin production depends on specific unfavorable conditions specific to each mold species' growth such as availability of nutrients, pH, water activity, temperature, or the presence of other microorganisms [157,160]. There are approximately 400 types of mycotoxins that can cause undesirable effects in cattle and be harmful to human health. Some of the mycotoxins harmful to ruminant health include: aflatoxins, ochratoxin, trichothecenes, fumonisins, zearalenone, mycophenolic acid and roquefortine C, ergot alkaloids, and gliotoxin [76,157,161,162]. The same mycotoxin can be produced by different mold species.

The most common genera of potentially toxic fungi in silage produced in temperate climates are *Penicillium*, *Geotrichum*, *Fusarium*, and *Schizophyllum* [90]. In silages produced in tropical climates, the dominant species is *A. fumigatus* [76,90,163]. The silages also contain species from the genera: *Monascus* and *Byssochlamys* [90].

In farm animals, mycotoxin toxicity can cause many different minor chronic illnesses; in rare situations, high mycotoxin concentrations may cause death. Symptoms of mycotoxicosis depend mainly on the type of mycotoxin, age, overall health condition, and dietary status of the affected organisms. Mycotoxins ingested by livestock can cause vomiting, reduced fertility, lameness, impaired resistance to infections, reduced feed intake, and feed refusal [160]. Ruminants (cattle, sheep, and goats) are less sensitive to some mycotoxins since rumen microbiota can effectively degrade them over time [164]. Some mycotoxins and the by-products of their detoxification may accumulate in animal or human tissues but are mostly expelled in feces, urine, or milk. The consumption of aflatoxin-contaminated diets by dairy cows, sheep, or goats results in the transfer of the toxin to milk, resulting in a human health hazard. Aflatoxins contained in cattle feed can be metabolized by cows to aflatoxin M1 [165–167]. The presence of toxic residues in edible cattle products (milk, meat) may have some detrimental effects on human health [160,168].

The occurrence of mycotoxins in maize silage or wheat silage is higher than in grass silage [169–171]. This is probably a result of maize crops' composition, including proteins and polysaccharides [172]. The number of mold fungi in silages is an indicator of their hygienic quality and should not be higher than 4.0 log CFU g^{-1} [163]. However, the low number of mold fungi does not always mean the absence of mycotoxins. [76] observed

that in the majority of the tested silage samples (97.2%), the number of molds was below the recommended level (4.0 log CFU g^{-1}), while the presence of mycotoxins was found in almost all samples (92% of the samples tested).

There are methods that decrease the risk of mycotoxin occurrences: screening plant material for fungal contamination, improved cultivation, harvest, and storage methods, and eliminating mycotoxins from contaminated food [76,90,157,160,173].

5. Strategies to Reduce DM Losses

Silage losses always occur even in a well-managed conserve system used for silage making. Ensiling is a complex of rapidly changing microbiological processes and biochemical transformations, which are uncontrolled in nature and depend on many factors. The level of losses and the quality of the ensiling process can be affected by many factors.

The fermentation process depends on the chemical and microbiological composition of the crop. Crops characterized by a high WSC content and low buffering capacity (BC) such as maize or grasses, are more favorable for LAB fermentation, resulting in less DM loss, while forages such as legumes (alfalfa, red clover) with a low WSC content and high BC are more prone to clostridial fermentation [65,174]. Increasing the concentration of fermentable carbohydrates in the crop is imperative to avoid DM losses due to poor fermentation.

Good silage management practices can help prevent or at least minimize losses in forage DM. All mechanical treatments of forage during the silage-making process affect silage fermentation in terms of microbial activity. An important treatment for forage intended for ensiling is to increase the DM content by wilting. Forage requiring wilting to the correct DM concentration before ensiling should be cut with a mower conditioner and wilted in a wide swath to reduce field wilting time and retain the maximum content of soluble carbohydrates. This procedure is aimed at reducing effluent losses and, above all, eliminating the activity of undesirable microorganisms in the ensiling process, especially clostridia.

Precise chopping of forage results in the release of more available substrate and water from the damaged cells, which ultimately increases the rate of silage fermentation [175].

Packing density is also of great importance in the ensiling process. The forage should be packed to a proper bulk density in the least time possible. The silage porosity as well as the DM content determine the silage quality, which is very important in the feed-out phase of the forage to eliminate aerobic degradation processes and thus DM losses [176].

Slow silo filling and delayed silo sealing also negatively affect silage quality. Fast and proper silo sealing decreases the activity of enterobacteria, and heterolactic fermentation can also be seen in silages where packing or sealing is delayed, which leads to a decrease in acetic acid concentration [79].

Elimination of fermentation losses is not possible, but the use of silage additives may help minimize them [177]. Lactic acid bacteria inoculants are used to reduce DM losses by enhancing homolactic fermentation and minimizing CO_2 losses during the initial ensiling fermentation [178]. Ensiling with the addition of bacterial inoculants containing lactic acid bacteria is usually associated with lower DM losses compared to silages obtained without any additives [149,179–181].

Moreover, using propionic or formic acid as silage additives was reported to reduce the microbial activity of clostridia in wet silages [182].

The combination of sodium benzoate, potassium sorbate, and sodium nitrite was used as an effective silage additive against clostridia spores in low-DM silages and was also successful against yeast in high-DM silages [183]. All these practices contribute to reducing losses to a minimum and obtaining the highest quality of silage.

6. Conclusions

Without the use of silage additives, the fermentation process is a result of the activity of the epiphytic microorganisms in plant material intended for ensiling. The principal fermentative microbial groups include lactic acid bacteria, *enterobacteria*, clostridia, and

yeasts. The numbers of different groups of microorganisms on plants during ensiling are dependent on the plant species, growing conditions, environmental factors during wilting, etc. LAB play a significant role in fermentation, but *Enterobacteria* are often at higher populations than LAB and thus influence early fermentation in the silo. The losses associated with fermentation are primarily from CO_2 production, and the amount of DM loss from fermentation depends on the dominant microbial species and the substrates fermented. DM losses in the form of CO_2 are generally high if non-LAB microorganisms play a significant role in fermentation. The examples are yeasts producing ethanol from glucose or clostridia producing butyrate from lactate or glucose. Elimination of fermentation losses is not possible, but the use of silage additives, particularly LAB strains enhancing homolactic fermentation, may help minimize them. Future investigations should consider lowering DM losses through controlled fermentation with the participation of LAB. Meanwhile, compliance with the principles of good agricultural and production practices is essential. We still don't know everything about the interactions between the groups of microorganisms growing in the ensiled material. The new silage studies should be occupied by research on metagenomic analyses and metabolomic studies.

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