Silage fermentation processes and their manipulation

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1. Introduction.

Fresh forage crops such as maize, grasses, legumes, wheat and lucerne can be preserved by ensiling. In many countries ensiled forages are highly valued as animal feed. In European countries such as The Netherlands, Germany and Denmark more than 90% of the forages locally produced are stored as silage. Even in countries with generally good weather conditions for hay making such as France and Italy approx. 50% of the forages are ensiled (Wilkinson *et al.* 1996). It is essential to have a good microbial fermentation process to produce high quality silage. A good fermentation process is not only dependent on the type and quality of the forage crop, but also on the harvesting and ensiling technique. In this paper our current knowledge on general silage microbiology is reviewed with the aim to aid with the choice of the best ensiling strategy to produce high quality silage.

2. The ensiling process.

Ensiling is a forage preservation method based on a spontaneous lactic acid fermentation under anaerobic conditions. The epiphytic lactic acid bacteria ferment the water-soluble carbohydrates (WSC) in the crop to lactic acid, and to a lesser extent to acetic acid. Due to the production of these acids the pH of the ensiled material decreases and spoilage microorganisms are inhibited. Once the fresh material has been stacked and covered to exclude air, the ensiling process can be divided into 4 stages (Weinberg and Muck 1996; Merry *et al.* 1997),

Phase 1, aerobic phase. This phase normally only takes a few hours in which the atmospheric oxygen present between the plant particles is reduced, due to the respiration of the plant material and aerobic and facultative aerobic microorganisms such as yeasts and enterobacteria. Furthermore, plant enzymes such as proteases and carbohydrases are active during this phase, provided the pH is still within the normal range for fresh forage juice (pH 6.5-6.0).

Phase 2, fermentation phase. This phase starts when the silage becomes anaerobic, and it continues for several days to several weeks, depending on the properties of the ensiled forage crop and the ensiling conditions. If the fermentation proceeds successfully lactic acid bacteria develop, and become the predominant population during this phase. Due to the production of lactic and other acids the pH decreases to 3.8-5.0.

Phase 3, stable phase. As long as air is prevented from entering the silo, relatively little occurs. Most microorganisms of phase 2 slowly decrease in numbers. Some acid tolerant microorganisms survive this period in an almost inactive state, others such as clostridia and bacilli survive as spores. Only some acid tolerant proteases and carbohydrases and some specialized microorganisms, such as *Lactobacillus buchneri* continue to be

active at a low level. The activity of this latter organism will be discussed in more detail further on in this paper.

Phase 4, feed-out phase or aerobic spoilage phase . This phase starts as soon as the silage gets exposed to air. During feed-out this is unavoidable, but it can already start earlier due to damage of the silage covering (e.g. by rodents or birds). The process of spoilage can be divided into two stages. The onset of deterioration is due to the degradation of preserving organic acids by yeasts and occasionally acetic acid bacteria. This will cause a rise in pH, and thus the second spoilage stage is started, which is associated with increasing temperature, and activity of spoilage microorganisms such as bacilli. The last stage also includes the activity of many other (facultative) aerobic microorganisms such as moulds and enterobacteria. Aerobic spoilage occurs in almost all silages that are opened and exposed to air. However the rate of spoilage is highly dependent on the numbers and activity of the spoilage organisms in the silage. Spoilage losses of 1.5-4.5 % dry matter loss/day can be observed in affected areas. These losses are in the same range as losses that can occur in airtight silos during several months of storage (Honig and Woolford 1980).

To avoid failures it is important to control and optimize each phase of the ensiling process. In phase 1 good silo filling techniques will help to minimize the amount of oxygen present between the plant particles in the silo. Good harvesting techniques combined with good silo filling techniques will thus minimize WSC losses through aerobic respiration in the field and in the silo, and in turn will leave more WSC available for lactic acid fermentation in phase 2. During phases 2 and 3 the farmer cannot actively control the ensiling process. Methods to optimize phases 2 and 3 are therefore based on the use of silage additives that are already applied at the time of ensiling as will be discussed in section 4. Phase 4 will start as soon as oxygen is available. To minimize spoilage losses during storage an airtight silo is required, and any

damage to the silo covering should be repaired as soon as possible. During feed-out spoilage by air ingress can be minimized by a sufficiently high feed-out rate. In addition, at the time of ensiling silage additives can be applied that are able to decrease spoilage losses.

3. The silage microflora.

The silage microflora plays a key role in the successful outcome of the conservation process. The flora can basically be divided into two groups namely the desirable and the undesirable microorganisms. The desirable microorganisms are the lactic acid bacteria. The undesirable ones are the organisms that can cause anaerobic spoilage (e.g. clostridia and enterobacteria) or aerobic spoilage (e.g. yeasts, bacilli, listeria and moulds). Many of these spoilage organisms do not only decrease the feed value of the silage, but also have a detrimental effect on animal health and/or milk quality (e.g. listeria, clostridia, moulds and bacilli).

3.1. Desirable microorganisms.

3.1.1. Lactic acid bacteria. **(LAB)**

Lactic acid bacteria belong to the epiphytic microflora of plant material. Often the population of LAB increases substantially between harvesting and ensiling. This is probably mainly due to the resuscitation of dormant and non-culturable cells, and not by inoculation by the harvesting machinery or growth of the indigenous population. Crop characteristics like sugar content, dry matter content, and sugar composition, combined with lactic acid bacterial properties such as acid and osmotolerance, and substrate utilization will decisively influence the competitiveness of the lactic

acid bacterial flora during silage fermentation (Woolford 1984; McDonald *et al.* 1991).

Lactic acid bacteria that are regularly associated with silage are members of the genera *Lactobacillus, Pediococcus, Leuconostoc, Enterococcus, Lactococcus* and *Streptococcus.* The majority of the silage lactic acid bacteria are mesophilic, i.e. they can grow at temperatures between 5 and 50°C, with an optimum between 25 and 40°C. They are able to decrease the silage pH to pH 4-5, depending on the species and the type of forage crop. All lactic acid bacteria are facultative aerobes, but some have a preference for anaerobic conditions (Holzapfel and Schillinger 1992; Hammes *et al.* 1992; Devriese *et al.* 1992; Weiss 1992; Teuber *et al.* 1992). Based on their sugar metabolism lactic acid bacteria can be classified as obligate homofermenters, facultative heterofermenters or obligate heterofermenters. Obligate homofermenters produce more than 85% lactic acid from hexoses (C-6 sugars) such as glucose, but cannot degrade pentoses (C-5 sugars) such as xylose. Facultative heterofermenters also produce mainly lactic acid from hexoses, but in addition they also at least degrade some pentoses to lactic acid, and acetic acid and/or ethanol. Obligate heterofermenters degrade both hexoses and pentoses, but unlike homofermenters they degrade hexoses to equimolar mounts of lactic acid, CO_2, and acetic acid and/or ethanol (Hammes *et al.* 1992; Schleifer and Ludwig 1995). Obligate homofermenters are species such as *Pediococcus damnosus* and *Lactobacillus ruminis.* Facultative heterofermenters are for example *Lactobacillus plantarum, Lactobacillus pentosus, Pediococcus acidilactici, Pediococcus pentosaceus,* and *Enterococcus faecium.* To the obligate heterofermenters belong members of the genus *Leuconostoc,* and some *Lactobacillus* sp. such as *Lactobacillus brevis* and *Lactobacillus buchneri* (Devriese *et al.* 1992; Weiss 1992; Holzapfel and Schillinger 1992; Hammes *et al.* 1992).

3.2. Undesirable microorganisms.

3.2.1. Yeasts.

Yeasts are eucaryotic, facultative anaerobic, heterotrophic microorganisms. In silages anaerobic as well as aerobic yeast activity is considered undesirable. Under anaerobic silage conditions yeasts ferment sugars to ethanol and CO_2 (Schlegel 1987; McDonald *et al.* 1991). This ethanol production in silage does not only decrease the amount of sugar available for lactic acid fermentation, but it can also have a negative effect on milk taste (Randby *et al.* 1998). Under aerobic conditions many yeast species degrade the lactic acid to CO_2 and H_2O. The degradation of lactic acid causes a rise in silage pH, which in turn triggers the growth of many other spoilage organisms (McDonald *et al.* 1991).

Yeast populations can reach up to 10^7 colony forming units per gram during the first weeks of ensiling, prolonged storage will lead to a gradual decrease in yeast numbers (Jonsson and Pahlow 1984; Middelhoven and Van Balen 1988; Driehuis and Van Wikselaar 1996). Factors that affect the survival of yeasts during storage are the degree of anaerobiosis, and the concentrations of organic acids. The presence of oxygen enhances survival and growth of yeasts during storage (Jonsson and Pahlow 1984; Donald *et al.*1995), whereas high levels of formic or acetic acid reduce survival during storage (Driehuis and Van Wikselaar 1996; Oude Elferink *et al.* 1999). Initial yeast activity appears to be enhanced in crops with a low initial pH (< 5), e.g. due to the addition of acid additives, and in crops with a high sugar content, e.g. potatoes, orange peels or sugar beets. These crops often result in silages high in ethanol and low in lactic acid (Henderson *et al.* 1972; Ashbell *et al.* 1987; Weinberg *et al.* 1988; Driehuis and van Wikselaar 1996). Silage additives developed to inhibit yeast activity are described in section 4.3.

3.2.2. Enterobacteria.

Enterobacteria are facultatively anaerobic. Most silage enterobacteria are regarded to be non-pathogenic. Nevertheless, their growth in silage is undesirable because they compete with the lactic acid bacteria for the available sugars, and in addition they can degrade protein. This protein degradation does not only cause a reduction in feeding value, but also leads to the production of toxic compounds such as biogenic amines and branched fatty acids. Biogenic amines are known to have a negative effect on silage palatability (Woolford 1984; McDonald *et al.* 1991; van Os and Dulphy 1996), especially in animals that are not yet accustomed to the taste (van Os *et al.* 1997). Moreover, the ammonia formed through proteolysis increases the buffer capacity of the ensiled crop, thus counteracting a rapid decrease of silage pH. A special characteristic of enterobacteria is their capability to reduce nitrate (NO_3) to nitrite (NO_2) under silage conditions. In silage nitrite can be degraded by enterobacteria to ammonia and nitrous oxide (N_2O), but it can also be chemically degraded to NO and nitrate (Spoelstra 1985; 1987). With air NO is oxidized into a mixture of gaseous, yellow-brown nitrogen oxides (NO_2, N_2O_3, N_2O_4). Gaseous NO and NO_2 have a damaging effect on lung tissue, and can cause a disease with pneumonia-like symptoms known as "silo filler's disease" (Woolford 1984). To prevent animals from getting in contact with gaseous nitrogen oxides they should not be housed in buildings adjoining silos during silo filling or the first week of silage storage (O'Kiely *et al.* 1999). Despite the above mentioned problems, a little nitrite reduction is considered positive for silage quality, because the formed nitrite and NO are very effective inhibitors of clostridia (Woods *et al.* 1981, Spoelstra 1985).

Enterobacteria will not proliferate at low pH. Ensiling methods that induce a rapid and sufficient drop in silage pH will therefore help to decrease enterobacterial growth (McDonald *et al.* 1991).

3.2.3. Clostridia.

Clostridia are endospore-forming anaerobic bacteria. Many clostridia ferment carbohydrates as well as proteins, thus causing problems such as the reduction in feeding value and the production of biogenic amines, similarly as has been described for enterobacteria. Furthermore, clostridia in silage impair milk quality. This is due to the fact that clostridial spores can survive the passage through the alimentary tract of a dairy cow. Clostridial spores present in silage are transferred to milk, via feces and fecal contamination of the udder. The acid tolerant *Clostridium tyrobutyricum* is the most relevant species for the dairy industry. In addition to carbohydrate fermentation *C. tyrobutyricum* can degrade lactic acid to butyric acid, H_2 and CO_2 according to the following overall reaction:

2 lactic acid --> 1 butyric acid + 2 H_2 + 2 CO_2

This butyric acid fermentation does not only counteract the lactic acid fermentation in silage and cheeses, but it also is responsible for a significant gas production, causing a cheese defect called "late blowing" in hard and semi-hard cheeses such as Emmental, Grana, Gouda and Parmesan (Gibson 1965; Goudkov and Sharpe 1965, Klijn *et al.* 1995).

Some clostridia can cause serious health problems. An extremely toxic *Clostridium* sp. is *C. botulinum.* This organism can cause botulism, which can be deadly for cattle. Fortunately, *C. botulinum* has a limited acid tolerance, and does not grow in well-fermented silage. Incidences of animal botulism caused by silage contaminated with *C. botulinum* could nearly always be attributed to the presence of a cadaver (e.g. mouse, bird, etc.) in the silage (Kehler and Scholz 1996).

A typical "clostridial silage" is characterized by a high butyric acid content of more than 5 g/kg dry matter, a high pH (over pH 5

in low dry matter silages), and a high ammonia and amine content (Voss 1966; McPherson and Violante 1966). Ensiling methods that cause a rapid and sufficient drop in silage pH will help to prevent the development of a "clostridial silage", because similar to enterobacteria, clostridia are inhibited at low pH. Furthermore, clostridia are more susceptible to a low availability of water (i.e. a low water activity (a_w)) than lactic acid bacteria (Kleter *et al.* 1982; 1984, Huchet *et al.* 1995). For this reason decreasing the a_w-value of a crop, e.g. by wilting to a higher dry matter content, can be a way of selectively inhibiting clostridia (Wieringa 1958). Finally, clostridia will also be inhibited by nitrite and NO or compounds that are degraded in silage to nitrite and NO (Spoelstra 1983; 1985).

3.2.4. Acetic acid bacteria.

Acetic acid bacteria are obligate aerobic, acid-tolerant bacteria. Thus far all acetic acid bacteria that have been isolated from silage belonging to the genus *Acetobacter* (Spoelstra *et al.* 1988). The activity of *Acetobacter* ssp. in silage is undesirable because they can initiate aerobic deterioration, due to the fact that they are able to oxidize lactate and acetate to carbon dioxide and water. Generally, yeasts are the main initiators of aerobic spoilage, and acetic acid bacteria are absent, or play only a minor role. However, for whole crop corn silages there is evidence that acetic acid bacteria alone can initiate aerobic deterioration (Spoelstra *et al.* 1988). Furthermore, selective inhibition of yeast also can increase proliferation of acetic acid bacteria in silage (Driehuis and van Wikselaar 1996).

3.2.5. Bacilli.

Bacilli are like clostridia endospore-forming rod shaped bacteria. Nevertheless, they can easily be distinguished from clostridia due to the fact that they are (facultative) aerobes, whereas all clostridia are obligate anaerobes (Claus and Berkeley

1986; Cato *et al.* 1986). Facultative aerobic bacilli ferment a wide range of carbohydrates to compounds such as organic acids (e.g., acetate, lactate, and butyrate) or ethanol, 2,3-butanediol, and glycerol (Claus and Berkely 1986). Some specific *Bacillus* sp. are able to produce antifungal substances, and have been used to inhibit aerobic spoilage of silage (Phillip and Fellner 1992; Moran *et al.* 1993). Except for these specific strains, the proliferation of bacilli in silage is generally considered undesirable. Not only are bacilli less efficient lactic and acetic acid producers than lactic acid bacteria (McDonald *et al.* 1991), they can also enhance (later stages of) aerobic deterioration (Lindgren *et al.* 1985; Vreman *et al*, in press). Furthermore, high numbers of *Bacillus* spores in raw milk have been associated with high spore numbers in fresh cow feces (Waes 1987; te Giffel *et al.* 1995). In seems very plausible that bacillus spores are transferred from silage to milk via feces similar to clostridial spores (Vreman *et al*, in press). Psychrotrophic *B. cereus* spores are considered to be the most important spoilage organism of pasteurized milk (te Giffel 1997). High numbers of these (psychrotrophic) *B. cereus* spores have been found in silages (Labots *et al.* 1965; te Giffel *et al.* 1995).

To decrease bacillus growth in silage, storage temperatures should not be too high (Gibson *et al.* 1958) and air ingress should be minimized (Vreman *et al*, in press). In addition, initial contamination of fresh plant material with soil or manure should be prevented (McDonald *et al.* 1991; Rammer *et al.* 1994).

3.2.6. Molds.

Molds are eucaryotic microorganisms. A mold-infested silage is usually easily detected by the large filamentous structures and colored spores that many species produce. Molds develop in parts of the silage were (a trace of) oxygen is present. During storage, this is usually only in the surface layers of the silage, but during aerobic spoilage (phase 4) the whole silage can become moldy.

Mold species that regularly have been isolated from silage belong to the genera *Penicillium, Fusarium, Aspergillus, Mucor, Byssochlamys, Absidia, Arthrinium, Geotrichum, Monascus, Scopulariopsis* and *Trichoderma* (Pelhate 1977; Woolford 1984; Frevel *et al.* 1985; Jonsson *et al.* 1990; Nout *et al.* 1993). Molds do not only cause a reduction of feed value and palatability of the silage, but can also have a negative effect on human and animal health. Mold spores are associated with lung damage and allergenic reactions (May 1993). Other health problems are associated with mycotoxins that can be produced by molds (Oldenburg 1991; Auerbach 1996). Depending on the type and amounts of toxin present in the silage heath problems can range from minor digestive upsets, small fertility problems, and reduced immune function, to serious liver or kidney damage, and abortions (Scudamore and Livesey 1998). Some important mycotoxin producing mold species are *Aspergillus fumigatus, Penicillium roqueforti,* and *Byssochlamys nivea.* Especially *P. roqueforti,* a species which is acid tolerant and can grow at low levels of oxygen and high levels of CO_2, has been detected as the predominant species in different types of silages (Lacey 1989; Nout *et al.* 1993; Auerbach *et al.* 1998; Auerbach 1996). There is still uncertainty under which conditions mycotoxins are formed in silage. A heavily infested silage does not necessarily contain high amounts of mycotoxins, and not all types of mycotoxins a mold species can produce have to be present in one silage (Nout *et al.* 1993; Auerbach 1996). For Aflatoxin B1, a mycotoxin of *Aspergillus flavus,* it is known that in can be transferred from animal feed to milk. However, thus far it is unknown if a similar transfer can occur with mycotoxins from *P. roqueforti,* or *A. fumigatus* (Scudamore and Livesey 1998).

Ensiling methods that minimize air ingress (e.g. good compaction and covering of the silo), and additives that prevent initiation of aerobic spoilage, will help to prevent or limit mold growth.

3.2.7. Listeria.

Members of the genus *Listeria* are aerobic or facultatively anaerobic. Regarding silage quality the most important *Listeria* spp. is the facultative anaerobic *L. monocytogenes*, because this species is a pathogen to various animals and man. Especially animals with a suppressed immune system (e.g. pregnant females and neonates) are susceptible to *L. monocytogenes* infections (Jones and Seeliger 1992). Silage contaminated with *L. monocytogenes* has been associated with fatal cases of listeriosis in sheep and goats (Vazquez-Boland *et al.* 1992; Wiedmann *et al.* 1994). In addition, Sanaa *et al.* (1993) have identified poor quality silage as one of the main sources of contamination of raw milk by *L. monocytogenes*. Growth and survival of *Listeria* in silage are determined by the degree of anaerobiosis, and the silage pH. *L. monocytogenes* can tolerate a low pH of 3.8-4.2 for long periods of time only if (small amounts) of oxygen are present. Under strictly anaerobic conditions it is rapidly killed at low pH (Donald *et al.* 1995). Silages that have a higher chance of aerobic surface spoilage, such as big bale silages seem, to be particular liable to *Listeria* contamination (Fenlon *et al.* 1989). *L. monocytogenes* generally does not develop in well fermented silages with a low pH. Thus far the most effective method to prevent growth of *L. monocytogenes* is to keep the silage anaerobic (McDonald *et al.* 1991).

4. Silage additives.

In the past decade it has become increasingly common to use silage additives to improve the ensiling process. The choice of additives appears to be sheer limitless if one looks at the large number of chemical and biological silage additives that are commercially available. The UKASTA Forage Approval Scheme of the UK for example lists more than 80 products (Rider 1997).

Fortunately, the choice of a suitable additive is less complicated than it seems, because the modes of action of most additives fall within a few categories (Table 1).

Table 1, Categories of silage additives (adapted from McDonald *et al*. 1991).

Additive category	**Selection of Active ingredients**	**Remarks**
Fermentation stimulants	Lactic acid bacteria Sugars (molasses) Enzymes	May impair aerobic stability
Fermentation inhibitors	Formic acid* Lactic acid* Mineral acids Nitrite salts Sulfite salts Sodium chloride	Inhibition of clostridia
Aerobic deterioration inhibitors	Lactic acid bacteria Propionic acid* Benzoic acid* Sorbic acid*	
Nutrients	Urea Ammonia Minerals	Can improve aerobic stability Can improve aerobic stability
Absorbents	Dried sugar beet pulp Straw	

*or corresponding salt

Between products of one category differences exist in product properties such as general effectiveness, suitability for certain crop type, and ease of handling and application. These factors, together with the price and availability, will determine what product will be the most adequate for a specific silage. A drawback of some of the chemical additives is that they can be corrosive to the equipment used, and/or can be dangerous to handle. The biological additives are non-corrosive and safe to

handle, but they can be costly. Furthermore, their effectiveness can be less reliable, since it is based on the activity of living organisms. Proper storage of these biological additives by the manufacturer, retailer and farmer is of vital importance. Despite these disadvantages, in Europe and the USA bacterial inoculants have nowadays become the most commonly used additives for corn, and grasses and legumes that can be wilted to above 300 g DM kg^{-1} (Bolsen and Heidker 1985; Pahlow and Honig 1986; Bolsen *et al.* 1995; Kung 1996; Weinberg and Muck 1996). In the Netherlands the absolute as well as the relative amount of silages treated with bacterial inoculants has increased in the past 4 years. Last year 13.7 % of all grass silages in the Netherlands was ensiled with an additive, of these treated silages 31 % was treated with an inoculant, 37% with molasses and 29% with fermentation inhibitors (Hogenkamp 1999).

4.1. Additives improving silage fermentation.

Assuming good harvesting and ensiling techniques initial silage fermentation (phase 2) can still be sub-optimal. This can be due to a lack of sufficient numbers of suitable lactic acid bacteria or a lack of sufficient amounts of suitable water-soluble carbohydrates, or both.

The amount of water-soluble carbohydrates necessary to obtain sufficient fermentation depends on the dry matter content and the buffer capacity of the crop. Weissbach and Honig (1996) characterized the relation between these factors as follows,

FC = DM (%) + 8 WSC/BC

FC = fermentation coefficient
DM = dry matter content
WSC = water-soluble carbohydrates
BC = buffer capacity.

Forages with insufficient fermentable substrate or too low a dry matter content have a FC < 35. In these forages sufficient

fermentation can only be achieved if the sugar content of the material is increased, either by adding sugars directly (e.g. molasses) or by adding enzymes that release extra sugars from the crop. In forages with a FC of 35 or more sufficient fermentable substrate is available. Also, adding suitable lactic acid bacteria can accelerate and improve the ensiling process. In high dry matter silages with reduced water availability the presence of suitable, osmotolerant lactic acid bacteria could become a limiting factor in the ensiling process. It has been shown that these bacteria represent only a small percentage of the indigenous microflora on forage crops (Pahlow and Weissbach 1996). Forages with a dry matter content above 50% are considered difficult to ensile (Staudacher *et al.* 1999).

The formula of Weissbach and Honig (1996) does not apply for crops with a low nitrate content such as extensively managed grasses and immature whole crop cereals, because these crops are more liable to clostridial fermentations than crops with a moderate nitrate content (Spoelstra 1983; 1985). Inoculants that increase lactic acid fermentation might be useful to inhibit clostridial activity. The minimum number of lactic acid bacteria required to inhibit clostridial activity was found to be at least 100 000 colony forming units per gram of fresh crop (Weissbach and Honig, 1996; Kaiser et al., 1997).

4.2. Additives inhibiting silage fermentation.

Fermentation inhibitors could in theory be used for all types of forages. However, in practice they are generally only used in wet crops with a low water-soluble carbohydrate content and/or high buffer capacity (McDonald 1991). In the Netherlands salts from acids have become the most popular fermentation inhibitors (Hogenkamp 1999). An advantage of these salts is that they are easier and safer to handle than their corresponding acids.

Silage additives inhibiting silage fermentation can reduce clostridial spore counts. In wilted grass silages a decrease in spore counts by a factor 5 to 20 has been observed. A similar decrease in spore counts could be obtained by adding molasses, a fermentation stimulant. To inhibit clostridial growth the most effective fermentation inhibitors appear to be additives based on formic acid, hexamethylene and nitrite (Hengeveld 1983; Corporaal *et al.* 1989; van Schooten *et al.* 1989; Jonsson *et al.* 1990; Lättemäe and Lingvall 1996).

4.3. Additives inhibiting aerobic spoilage.

Is clear that to inhibit aerobic spoilage, spoilage organisms, in particularly the ones causing the onset of deterioration (i.e. yeasts and acetic acid bacteria) have to be inhibited in their activity and growth. Some additives which have proven to be effective in this respect include chemical additives based on volatile fatty acids such as propionic and acetic acid, and biological additives based bacteriocin producing micro-organisms such as lactobacilli and bacilli (Woolford 1975a; McDonald *et al* 1991; Phillip and Fellner 1992; Moran *et al.* 1993; Weinberg and Muck 1996).

Furthermore, it is known that sorbic acid and benzoic acid have a strong antimycotic activity (Woolford 1975b; McDonald *et al*. 1991). Recently, it was discovered that *Lactobacillus buchneri* is a very effective inhibitor of aerobic spoilage. The inhibition of spoilage appears mainly due to the capability of *L. buchneri* to anaerobically degrade lactic acid to acetic acid and 1,2-propanediol, which in turn causes a significant reduction in yeast numbers (Driehuis *et al.* 1997; Oude Elferink *et al.* 1999; Driehuis *et al.*, in press). This reduction in yeast numbers is in agreement with the finding that volatile fatty acids such as propionic acid and acetic acid are much better inhibitors of yeasts than lactic acid is, and that mixtures of lactic acid and propionic and/or acetic acid have a synergistic inhibitory effect (Moon 1983). The results of

Moon (1983) also explain why biological inoculants that promote homofermentative lactic acid fermentation, in most cases do not improve, or even decrease, aerobic stability (Weinberg and Muck 1996; Oude Elferink *et al.* 1997).

Biological additives based on the propionate producing propionibacteria appear to be less suitable for the improvement of silage aerobic stability, due to the fact that these bacteria are only able to proliferate and produce propionate if the silage pH remains relatively high (Weinberg and Muck 1996).

4.4. Additives used as nutrients or absorbents.

Certain crops are deficient in essential dietary components for ruminants. The nutritional quality of these crops can be improved by supplementation with specific additives at the time of ensiling. Additives that have been used in this respect are ammonia and urea to increase the crude and true protein content of the silage, and limestone and $MgSO_4$ to increase the calcium and magnesium contents. The above mentioned additives generally have no beneficial effect on silage fermentation, but urea and ammonia can improve the aerobic stability of silage (Glewen and Young 1982; McDonald *et al.* 1991).

Absorbents are used in crops with a low dry matter content to prevent excessive effluent losses. Good results have been obtained with dried pulps such as sugar beet pulp and citrus pulp. Straw can also be utilized, but has a negative effect on the nutritive value of the silage (McDonald *et al.* 1991).

4.5. Combined additives.

Most commercial additives contain more than one active ingredient in order to have a high efficacy and a broad range of applicability. Very popular are for example combinations of inoculants stimulating homofermentative lactic acid fermentation

together with sugar releasing enzymes, or combinations of fermentation and aerobic deterioration inhibiting chemicals such as formic acid, sulfite salts and propionic acid (Rider 1997; Arbeitsgemeinschaft der norddeutschen Landwirtschafskammer 1999).

New additives are currently being developed that decrease the negative effect of homofermentative lactic acid fermentation on aerobic stability. Promising results have been obtained by combining homofermentative or facultative heterofermentative lactic acid bacteria with chemicals such as ammonium formate and sodium benzoate (Kalzendorf 1992; Bader 1997), or by combining facultative heterofermentative lactic acid bacteria with the obligate heterofermentative *L. buchneri.*

5. Silage fermentation in tropical silages.

Ensiling of forage crops or industry by-products could make an important contribution to the optimization of tropical and sub-tropical animal production systems, but thus far it has not yet been widely applied (Wilkins *et al.* 1999). This is not only due to the low prices for animal products, the low levels of mechanization, and the high costs of silo sealing materials, but also due to the lack of ensiling experience. More research is needed to address the specific problems associated with tropical silages. Tropical grasses and legumes have for example a relatively high concentration of cell wall components and the low level of fermentable carbohydrates compared to temperate forage crops (Catchpoole and Henzell 1971; Jarrige *et al.* 1982). Furthermore, on average storage temperatures in tropical climates are higher than in temperate climates, which might give bacilli a competitive advantage over lactic acid bacteria (Gibson *et al.* 1958). In addition, it has to be taken into account that some silo sealing materials cannot withstand intense sunlight, and thus might impair

the aerobic stability of the silage. Nevertheless, it seems likely that ensiling technologies from temperate climates can be modified for tropical conditions.

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