Possibilities to Improve Silage Conservation

Effects of Crop, Ensiling Technology and Additives

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Abstract

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The objective of this thesis was to investigate factors to improve the silage quality of different types of forage crops. The focus was on the influence of crop maturity, silage additives and laceration on the quality of whole-crop cereals silages.

The ensilability of whole-crop cereals was highly dependent upon the stage of maturity. An important factor related to the stage of maturity and influencing the ensilability of whole-crop cereals seems to be the concentration of WSC in the fresh forage. Ensiling at the milk stage was found to be a more suitable time to harvest whole-crop cereals since there is a sufficient concentration of water soluble carbohydrates (WSC) that provides the conditions for lactic acid bacteria (LAB) to rapidly dominate the silage microflora and produce enough acids to reduce pH thereby giving a better protection of the silage against the spoilage microflora. The low WSC concentration of whole-crop cereals at dough stage seems to restrain good fermentation. However, silages harvested at the milk stage still appear to have a low aerobic stability.

Precision chopping had variable affects on silage fermentation and showed that laceration is not a guarantee of a good silage quality of whole-crop cereals. At the milk stage, precision chopping improved the silage fermentation, whereas the dough-stage silages tended to give clostridial fermentation resulting in poor quality and high DM losses. Precision chopping promoted a higher silage density at the milk stage and resulted in reduced DM losses at the dough stage.

Application of silage additives was the most important factor in improving silage quality. A mixture of sodium benzoate, sodium propionate, hexamine and sodium nitrite seems to be the most suitable additive to secure the silage quality and improve the aerobic stability in whole-crop cereals as well as in moderate and highly -wilted clover-grass forages. No remaining toxic residues of the nitrite concentration were found in the silages. A similar influence on the ensiling process is found when using the combination of sodium benzoate, sodium propionate and propionic acid.

The results of mixtures of formic acid and propionic acid were closely related to the chop length of the forage. These additive combinations seem to improve the silage quality and storage stability in precision-chopped silages at both maturities, while the quality of long-cut treated silages often appeared to be low.

The variable efficiency of inoculation of whole crop cereals with LAB seems to be associated with types and numbers of epiphytic bacteria in the herbage. The inoculation of homofermentative LAB successfully reduced pH of both precision-chopped and long cut-silages but abundant production of lactic acid did not secure the aerobic stability of these

Keywords: additive, benzoate, clostridia, fermentation, formic acid, hexamine, LAB, losses, mould, nitrite, propionic acid, silage, stability, whole-crop cereal, yeast.

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Content

Appendix

This thesis is based on the following four papers, which will be reffered to in the text by their Roman numerals.

I. Knický, M. & Lingvall, P. 2004. Ensiling of high wilted grass-clover mixture by use of different additives to improve the hygienic quality. *Acta Agr. Scandinavica, Section A,* 54 (4), 197-205.

II. Knický, M., Lingvall, P. & Bertilsson, J. 2003. Factors influencing fermentation, hygienic quality and losses of whole-crop barley silages. (Manuscript).

III. Knický, M., Lingvall, P. & Bertilsson, J. 2003. Preserving of whole-crop barley in big round bales. The use of silage additives and effect of wilting on silage quality. (Manuscript).

IV. Knický, M., Lingvall, P. & Bertilsson, J. 2003. Use of additives to improve ensilage quality of round big bale and precision chopped whole-crop barley and wheat forages harvested at three maturities (Manuscript).

Introduction

Forages supply more than 60% of the nutrients for ruminants in Sweden. The growing period, however, is limited because of climatic condition and therefore, a large proportion of forage production must be preserved. Currently, two main conservation methods are used in forage preservation, hay making and silage making. Hay making was the most common method of preserving of forages in earlier times. However, because of the uncertainty of weather conditions often causing high nutritive losses of hay, this method has been replaced by silage making. This shift in conservation methods was evident in Swedish dairy statistics, which reported 70% of hay production and 30% of silage production in 1970. In 1990, this ratio was reversed. Nowadays, silages account for more than 90% of conserved forage in Sweden and their importance in ruminant nutrition is increasing.

The principle of conservation of wet forage as silage has a long history, but the main developments of ensiling have occurred in last fifty years due to mechanization. The ensiling process is a continuous battle between microorganisms under oxygen-free condition competing for available substrate that is absorbed in the forage. Water-soluble carbohydrates (WSC) are the most important nutrients for microorganisms that ferment to organic acids. Together with a pH drop, WSC preserves the forage. The fermentation process is successful if lactic acid is the predominant acid produced. In addition to lactic acid bacteria (LAB), other microorganisms in the fermentation process compete for substrate. The activity of these microorganisms is responsible for processes that degrade the feeding value and increase ensiling losses. Moreover, certain species can be pathogenic such as *Listeria*, or produce toxins (*Cl. botulinum*) that can be toxic to animals, or *Cl. tyrobutyricum* that affect the quality of dairy products such as blow-cheese defects. A successful inhibition of these undesirable microorganisms in silages is a goal for achieving high nutritional and hygienic quality of silages, which will improve the profitability of animal production.

Nowadays, there is a wide range of crops and systems used for silage making. Grasses dominate the forage in Sweden, where the growth of maize is impossible. In addition to grasses, legumes are commonly grown forages and are particularly important on organic farms, where use of chemical fertilizers is forbidden and legumes are used to overcome nitrogen deficiency in the soil. Legumes create an important source of home-grown protein. However, reduced utilization of N in legume-based diets cause increased ammonia emissions, which are a serious source of pollution that must be reduced. Mixing of legumes with grasses brought a partial improvement in N utilization (Bertilsson & Murphy, 2003), but recently, this possibility has been applied to whole-crop cereals. The possibility to apply a bale ensiling technology, commonly used in conservation of traditional forages, on conservation of whole-crop cereals can open the door to a better utilization of this type of crop. Despite the lower digestibility of whole-crop cereals, preliminary observations of whole-crop cereal silages fed in rations with legume silages indicated promising results in terms of milk quality and reduction of N losses (Bertilsson & Knicky, unpublished data). Cereals harvested as whole-crops give

higher nutrient yields than cereals harvested for grain, which might be important in increasing field productivity. The important role of cereals grown for ensiling can be seen in the flexibility in crop rotation. Under certain circumstances (e.g. insufficient forage on the farm as a result of a dry period), the decision can be made at a later stage of the growing season to harvest cereals earlier for ensiling purposes than for a later grain production. Consequently, the earlier harvesting allows the field to be seeded earlier or an optimal time for spreading manure or slurry. Furthermore, the establishment of cereals with under-sowing of perennial grasses gives a possibility of two harvests per year and promotes weed suppression.

Objectives of the thesis

The objective of this thesis was to investigate factors to improve the silage quality of different types of forage crops. The focus was on the influence of crop maturity, silage additives and laceration on quality of whole-crop cereals silages.
The main hypotheses of the thesis were:

- Additives containing nitrite substances could be an efficient way at improving silage quality of high-wilted clover-grass silage.
- Stage of maturity of whole crop cereals has an important influence on silage fermentation.
- Application of silage additives is needed to secure a good fermentation, low losses and acceptable storage stability.
- Precision chopping is an useful possibility to improve the fermentation of whole-crop cereal silages.

Ensiling process

The process of ensiling can be subdivided into four principal periods (Weinberg and Muck, 1996; Merry *et al*., 1997; Pahlow *et al.,* 2003). There is no clear division between the phases and each of the phases has a different length and various biochemical processes with different intensity taking place. In general, the phases are defined as:

Period I - Initial aerobic phase

It is a period immediately after ensiling of forage in the silo. This phase is characterised by continuing respiratory and enzymatic processes in the herbage, of which respiration and proteolysis are the most important (McDonald *et al.*, 1991). The proteolysis is an undesirable protein decomposition process of decomposition resulting in inhibition of acidification and reducing nitrogen utilization of silage crop (Slottner, 2004). Besides the proteolysis, the carbohydrate degradation in plants by carbohydrases can occur to some extent resulting in an increased amount of water-soluble carbohydrates (WSC). In addition to plant enzyme activities, microbial activities are also of importance. The presence of oxygen promotes

facultative and obligate aerobic microorganisms such as moulds, yeasts, enterobacteria and some genera of bacilli to the detriment of anaerobic microorganisms. As plant respiration continues, the amount of oxygen trapped in the silo is gradually depleted and the microbial population in the forage is shifted from aerobic to anaerobic microorganisms. The duration of the first phase is not usually longer than a few hours and depends mainly on the amount of available oxygen. This stage should be as short as possible to suppress the activity of aerobic bacteria which are undesirable in the continued fermentation process.

Period II – Main fermentation phase

In this phase, the anaerobic conditions are established in the silo and an anaerobic microflora dominate the fermentation process. The lactic acid bacteria (LAB) flora have a fundamental role in silage by fermenting available plant nutrients, namely WSC, to organic acids thereby reducing the pH of the ensiled crop. The main product of silage fermentation is lactic acid, which helps LAB in the early stages of the fermentation to take the upper hand over a variety of other facultative and obligate anaerobic microorganisms, such as enterobacteria, yeast, bacilli and clostridia, that all compete for the available WSC. The principle of lactic acid action is based on the different levels of the microorganisms' resistance to the acidity. The LAB flora in general possesses the highest tolerance to acidity and therefore can withstand the lowest pH. As the ensiling process progresses, the LAB population grows producing increasing amounts of lactic acid thereby dominates the remaining microflora in the forage mass. This process continues until the pH drops so low that even growth of LAB is inhibited. Besides the organic acids, gases and effluent are produced during the fermentation process. Although the intensity and extent of fermentation depends on a variety of factors discussed below, this period does not usually exceed one month. This phase should generate a rapid acidification and sufficiently high concentration of lactic acid so that silage pH will decrease to a stable level.

Period III – Stable silage phase

Under optimal conditions, when oxygen has no access into the silo and a high concentration of lactic acid has lowered pH sufficiently, the fermentation process is almost stopped. Only acid hydrolysis of polysaccharides and proteolysis are sustained as a result of activities of acid-tolerant enzymes. The growth of LAB is inhibited as well as activities of other microorganisms such as acid-tolerant yeasts, clostridia and bacilli. The two latter, however, might survive as endospores (Jonsson, 1991). Under these circumstances, the ensiled crop can be stored for a long time, in practice at least until the next harvest season. During this period, a process of deterioration referred to as secondary fermentation, might emerge. The genesis of secondary fermentation is associated either with a deficiency of available substrate or slow production of lactic acid leading to ineffective inhibition of spoilage flora such as clostridia. The growth of clostridia is accompanied with a shift in fermentation pathways resulting in a rise of pH (Jonsson, 1991). The particular causes of this fermentation will be further

discussed. Such silages are designated as anaerobically unstable having in general high dry matter (DM) losses and a low feeding value.

Period IV – Feed-out phase

During this phase, silos are opened and silages, which were previously kept under oxygen-free conditions are exposed to oxygen. The presence of oxygen initiates the activity of undesirable microflora such as yeast, moulds and acetic acid bacteria. These microorganisms grow on residual substrates and fermentation products. The main indications of this deterioration are the production of heat and carbon dioxide due to respiration, and reduction in lactic acid concentration reflected in a pH increase. Silages where deterioration has occurred are designated as aerobically unstable resulting in a low silage quality and high DM losses. Susceptibility of silages to the aerobic deterioration depends on a variety of factors such as DM content of the crop or degree of forage consolidation in the silo. The particular causes of aerobic instability of silages will be discussed in a later section.

Micro-organisms involved in the ensiling process

As mentioned previously, several genera described as lactic acid bacteria play a fundamental role in the ensiling process. Members of this group compete for available nutrients with undesirable in the ensiling process micro-organisms. The representative of these detrimental microorganisms are in particular clostridia, enterobacteria, bacilli and fungi. The activity of the silage microflora during the ensilig process is illustrated in Figure 1. The main difference between the LAB flora and detrimental flora is in the quality of their end-products and way of degradation of valuable nutrients.

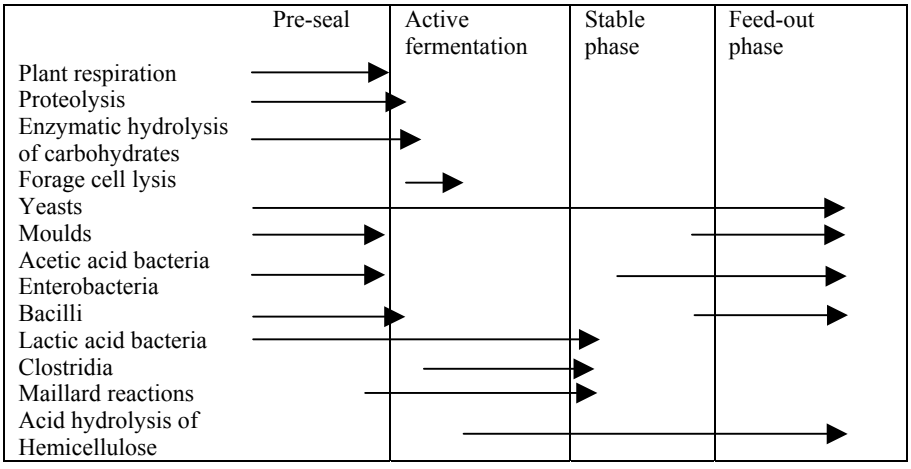

Fig. 1. Major microbial and chemical processes during ensiling phases (Muck, 1993a)

Lactic acid bacteria

Lactic acid bacteria belong to the epiphytic microorganisms which occur on the lower parts of forage plants. The group of lactic acid bacteria can be specified as Gram-positive, catalase-negative, non-sporulating, non-pigment mesophils, rarely motile, obligate fermenters, which include variable shapes from spherical or ovoid cocci to short and long rods (Brookes & Buckle, 1992). Lactic acid bacteria can grow in a temperature range of 5°C to 50°C with optima around 30°C (McDonald *et al.*, 1991). However, the most distinctive feature of the lactic acid bacteria is their high acid tolerance. The optimal pH for their growth is about 5.0 to 6.0, although many of them can grow below pH 4.0, and some as low as pH 3.5. They have variable nutritional requirements across species and strains. The transition of hexoses by lactic acid bacteria to fermentation acids proceeds under anaerobic condition is of particular importance for ensiling. Lactic acid bacteria possess the capability to ferment a wide range of substrates using different pathways. The differences in fermentation of hexoses are used for their classification. In this classification, lactic acid bacteria are subdivided into three groups:

Group I. Obligate homofermentative lactic acid bacteria, which convert hexoses almost exlusively to lactic acid, but are unable to ferment pentoses or gluconate because of the lack of phosphoketolase.

Group II. Facultative heterofermentative lactic acid bacteria also convert hexoses homofermentatively to lactic acid, but some strains under certain conditions they can ferment hexoses heterofermentatively to lactic acid, ethanol (or acetic acid), and carbon dioxide as they possess both aldolase and phosphoketolase.

Group III. Obligate heterofermentative lactic acid bacteria ferment hexoses exclusively heterofermentatively to lactic acid, ethanol, carbon dioxide and optionally to acetic acid. Additionally, they have ability to convert pentoses to lactic acid and acetic acid.

In the ensiling process, homofermentative lactic acid bacteria are preferred due to fast and abundant formation of lactic acid, which has a high acidifying potential. Homolactic fermentation also results in lower DM losses because of higher efficiency in the conversion of WSC to acids compared with heterolactic fermentation (McDonald *et al.*, 1991). However, the type of fermentation used by lactic acid bacteria also depends on the substrate composition, which particularly affects lactic acid bacteria in Group II. Hexoses glucose and fructose and polysaccharide fructans are the most important substrates used. Their ratio and availability in the ensiled forage often affect the type of fermentative pathway used by lactic acid bacteria. It has been reported (Lindgren *et al.*, 1990) that under low substrate availability in the crop, a low ratio glucose/fructose promotes the heterolactic fermentation. Also, it has been shown that when hexoses are limited, certain lactic acid bacteria are capable of utilising lactic acid as substrate and converting it to acetic acid (Lindgren *et al.*, 1990). All these deviations in the substrate utilisation reduce the ensiling efficiency and increase DM losses during the fermentation process.

Enterobacteria

Enterobacteria are defined as Gram-negative, non-sporulating, usually motile, facultative anaerobic rod-shaped bacteria. The entrerobacteria represent a minor part of the epiphytic microflora. Their presence in silage is undesirable as they possess saccharolitic and proteolytic abilities and thereby compete for substrate with lactic acid bacteria. This occurs mainly at the beginning of fermentation before lactic acid bacteria dominate the fermentation process. The enterobacteria use two saccharolitic pathways to ferment carbohydrates. The first mixed acid fermentation is characterized by production of lactic acid, acetic acid, succinic acid and formic acid. This fermentation occurs in silages with pH above 6.3. In silages below pH 6.3 butanediol fermentation takes place resulting in the production of acetoin and 2.3-butanediol (McDonald *et al.*, 1991). However, the enterobacteria are sensitive to a low pH (4.5) and the decline in their activity in silages can be assumed to be a potential measure of good ensiling conditions (Pahlow *et al.*, 2003). This assumption can be applied in the present experiments (**I, II, III, IV**), where in spite of the lack of enterobacterial analyses, concentrations of their fermentation products were found in negligible levels thereby could be expected to have little impact on the fermentation process.

An additional aspect of enterobacterial activity in silages is their ability to degrade nitrate to nitrite and nitric oxide, thereby contributing to a reduction of clostridial growth in silages (Spoelstra, 1987). Because of low enterobacterial activity in the present experiments (**I**, **II**, **III**, **IV**), it can also be assumed that the contribution of enterobacteria to nitrate degradation and hence reduction of clostridial growth during the ensiling process was low.

Clostridia

Clostridia are one of the most detrimental microorganisms involved in the fermentation process. They are identified as Gram-positive, sporulating, usually motile, rod-shaped bacteria, growing under anaerobic conditions. Clostridia in the ensiled crop originate from soil or manure (Jonsson, 1989, Rammer, 1996). This was demonstrated in experiment **IV**, where a higher ash content in the forage indicative of soil contamination, was reflected in increased numbers of clostridia. Clostridia is undesirable because they ferment a variety of substrates and thus reduce substrate availability for lactic acid bacteria. In addition, some clostridia ferment lactic acid and their end-products have a low preservative capability resulting in high ensiling losses and low feeding value of silage. According to substrate requirements, clostridia can be divided into two major physiological groups (McDonald et al, 1991). *Saccharolitic clostridia* ferment predominately carbohydrates and organic acids and possess little proteolytic activity whereas *proteolytic clostridia* ferment mainly amino acids but carbohydrates only to a lesser extent.. However, only a few clostridia species occur regularly in silages. According to Pahlow *et al.* (2003), the most common clostridia found in silages are proteolytic clostridia*, C. butyricum,* and *C. tyrobutyricum*. The group of proteolytic clostridia includes a variety of clostridia with combined proteolytic and saccharolytic properties, such as *C. sporogenes*, *C. spheniodes*, *C. bifermentans*. These preferably ferment amino acids to ammonia, amines, organic acids and

ethanol. *C. butyricum* and *C. tyrobutyricum* mainly use WSC and lactic acid as substrate and convert these to butyric and acetic acid. Thus, an increased concentration of these products, particularly ammonia and butyric acid followed by a slow pH drop of silage give evidence of clostridial fermentation, as was shown in control silages in experiments **II**, **III**, **IV**. Such silages are designated as anaerobically unstable. The clostridial activities described above are the result of their vegetative cells which require anaerobic conditions. In unfavourable conditions (i.e. ingress of air), clostridia survive in the inactive form as endospores. These spores are highly resistant to environmental constraints such as heat or chemicals (Jonsson, 1989).

Clostridial proliferation in silages is generally associated with a slow acidification process. Thus, fast and abundant production of lactic acid resulting in a fast pH drop are one of the most important factors inhibiting clostridial activity. In addition, reduced pH, decreased moisture content and water activity (a_w) of forage also reduces clostridial growth in silages, which was demonstrated in experiment **I**. In addition to these factors, the degradation of nitrate in the ensiled forage also suppresses clostridial growth. The principle of the clostridia inhibition lies in their self-inhibitory effect by formation of nitrite and nitric oxide, which possess antimicrobial properties (Wieringa, 1958; Spoelstra, 1983; Weissbach *et al.*, 1993; Woods *et al.*, 1995). The antimicrobial effects of nitrite and decreased water activity were also demonstrated in experiment **I**. Nitrite and nitric oxide eventually are converted to ammonia, which has no inhibittory effect on clostridia. The inhibiting effect will disappear when all nitrite and nitric oxide is transformed to ammonia. This process proceeds slowly in good, low pH silages and quickly in inferior, high pH silages.

Fungi

As clostridia often are responsible for the anaerobic instability of silages, fungi play a major role in the aerobic instability of silages. Fungi are eukariotic, heterotrophic microorganisms abundant in soil and on vegetation. They represent two major subgroups; moulds which grow mainly as multicellular, filamentous colonies, and yeasts which grow mainly as single cells. The majority of fungi are strict aerobes demanding the presence of oxygen, but some yeast species as *Saccharomyces and Torulopsis* can grow under anaerobic conditions and obtain energy from the fermentation of carbohydrates to CO_2 and ethanol as their main products. Lactate-fermenting species such as *Candida* and *Hansenula* were reported to dominate in silages after air penetrated silos (McDonald *et al.*, 1991). Under these circumstances yeasts are known to withstand a silage pH of 3.5 to 6.0. Because of their high resistance to acidity and capability to assimilate lactic acid under aerobic conditions, yeasts are considered to be the primary causative microorganisms of aerobic spoilage in silage (Beck & Gross, 1964; Pettersson, 1988; Jonsson, 1989; Woolford, 1978b; Woolford, 1990). The growth of yeast is also stimulated in wilted silages, where their undesirable effects can be pronounced (Jonsson & Pahlow, 1984).

Under the anaerobic condition in the silo, moulds are generally restrained and their growth is restricted only to the surface area of silages. This was demonstrated

in all the present experiments. Nevertheless, moulds can occur in insufficiently sealed silages, particularly in high DM silages where ingress of oxygen, a low level of acidification combined with a high concentration of CO_2, can maintain their growth (Lacey, 1989). The undesirable effects of moulds mainly occur during the feed-out phase in the ensiling process when silage is exposed to air. Their role in aerobic deterioration of silages is usually associated with yeast growth. Moulds degrade a variety of substrates that can lead to complete decomposition of plant tissues which is highly undesirable in silage making. Furthermore, many species of moulds such as *Aspergilus, Fusarium* and *Penicilium* produce mycototoxins that are harmful to animals and humans (Oldenburg, 1991).

Bacilli

Bacilli are Gram-positive, spore-forming microorganisms, but their ability to grow under aerobic conditions differs from that of clostridia. Facultative anaerobic bacilli can ferment a variety of carbohydrates and produce ethanol, glycerol, 2.3-butandiol and organic acids including lactate (McDonald *et al.*, 1991). However, lactate production by bacilli is not as efficient as that of lactic acid bacteria which is the reason why the proliferation of bacilli in silages should be avoided. Bacilli seems to be unable to initiate aerobic deterioration of silages, but their can play a secondary role in aerobic deterioration after initiation by yeasts (Woolford *et al.*, 1978b; Jonsson, 1989).

Factors affecting the ensiling process

The ensiling of forage is a process where many variables influence the course and outcome of silage fermentation. Two overriding features of any silage which will affect fementation can be distinguished (Woolford, 1984). The first is the nature of the raw material which is determined by the chemical and microbial composition of the crop. The second feature is related to ensiling conditions imposed by the silage-maker, and include processes such as wilting, mechanical treatment of forage and use of additives.

Crops

Different crops possess a variable capability to complete a successful ensiling process. These capabilities express the ensilability of the crop. Weissbach *et al.* (1974) indicated that the effects of WSC concentration, DM content and buffering capacity of the crop could predict the ensilability of forage crops. The key is to estimate the correct amount of WSC needed to ensure a proper ensiling process in the crop at a given DM content and buffering capacity. In addition to these, variability in concentration of nitrate (Weissbach, 1996) and epiphytic microflora count (Muck, 1993b; Lindgren *et al.*, 1985) in the crop have been seen to influence the ensiling process.

Water soluble carbohydrates in crop

One of the main factors influencing the ensilability of crops is the concentration of WSC in the crop. In temperate forages, the WSC comprise mainly the monosaccharides glucose and fructose, the disaccharide sucrose, and the polysaccharide fructan. Glucose and fructose are the most important water soluble carbohydrates. Since these occur as free sugars, they provide substrate for lactic acid bacteria at the earliest stages of ensiling process. Both sucrose and fructans are available for lactic acid bacteria later on, since they must go through acid hydrolysis to become available as monosaccharides. Sucrose consists of glucose and fructose whereas fructans are composed of mainly fructose residues branched by 2,6- and 2,1-linkages. The extent of branching in fructans determines the degree of their availability for lactic acid bacteria.

The concentration of WSC in temperate forages often depends on the forage species and the stage of maturity. Among grasses grown in temperate zones, ryegrasses (*Lolium spp.*) have the highest WSC content, on average 175 g kg^{-1} DM. The second most commonly grown grass, timothy (*Phleum spp.*), contains about 110 g WSC kg^{-1} DM, and cocksfoot (*Dactylis glomerata*) that contains only 79 g WSC kg^{-1} DM. The concentration of WSC in these grasses appears to increase during early development as the ratio of stem to leaves increases. The enhancement of WSC is related to the increase of the polysaccharide fructan in the stem tissues. Fructans are the most abundant WSC fraction in temperate grasses.

On the other hand, legumes contain relatively low concentrations of WSC. The main WSC in legumes are glucose, fructose and sucrose, but unlike grasses, their main reserve polysaccharide is starch, which is not available as substrate for most lactic acid bacteria. Whole-crop cereals represent the group of forage where the relation between the stage of maturity and concentration of WSC seems to have the most pronounced impact on their ensilability. At early stages of growth, glucose and fructose dominate the WSC fraction. As maturity progresses, sucrose and fructans prevail in the WSC fraction reaching the maxima between the milk and early dough stage of growth. From this point on, the concentration of WSC steadily declines to the detriment of increasing starch concentration (Filia, 2003; Khorasani *et al.,* 1997; **II**, **III**, **IV**). This coincides with an increasing proportion of ears in the forage mass during maturation. However, WSC develompent in forage plants might constrain the ensilability of whole-crop cereals since the dough stage of growth is considered as the most desirable stage at which to harvest cereals for silage (McDonald *et al.*, 1991). Ashbell & Weinberg (1989), Ohlsson (1994) also suggested the optimal harvesting time of whole-crop cereals considering the yield, feeding value and ensilibility to be between early and late dough stage. In this regard, the present studies indicated that reduced WSC concentration at dough stage contributed to the high susceptibility to clostridia activity during the fermentation process. In particular, it is assumed that the decrease in glucose and fructose concentrations at dough stage were responsible for a low substrate supply for lactic acid bacteria fermentation at the beginning of the ensiling process resulting in a slow pH drop, which promoted activity of undesirable microflora, particularly clostridia.

Crop dry matter

The crop DM content can vary considerably depending on the weather. This variation was clearly seen in experiments **III** and **IV**, when whole-crop cereal forage was ensiled directly after the cutting. The DM content is an important factor influencing the microbial population and activity in the ensiled crop (McDonald *et al.*, 1991). But, water content and availability in the crop are essential for microbial activity during the ensiling process. The availability of water for microbial growth is expressed by water activity (a_w) and DM conctent can used as indirect indicator of the water availability. The relationship between DM content and water activity demonstrated decreasing water activity when DM concentration in the crop was increased (Vétra, 1996). The different water requirements for silage microflora (Table 1) can be used to suppress the growth of undesirable silage microorganisms, particularly clostridia.

Clostridia are generally more sensitive to reduced water availability than lactic acid bacteria. This can be tested by wilting of forage, which reduces the DM concentration and hence the water availability in the crop. The effect of wilting on reduction of clostridial activity in grass/clover silages was demonstrated in experiment **I**, whereas in whole-crop barley silages (**III**), the effect of wilting was not pronounced. These results offered evidence of the variable efficiency of wilting (Lättemäe, 1997). The variability in wilting efficiency can be associated with differences in the epiphytic population of forage. Besides its effect on clostridia, a high degree of wilting also restricts most of the LAB and only a small population of osmotolerant LAB is active (Pahlow & Weissbach, 1996) resulting in delayed and more restricted silage fermentation. That was particularly evident in experiment **I** where the crop ensiled at 300 g kg^{-1} DM showed a higher fermentation rate as the result of increased microbial activity than the crop at 600 g kg^{-1} DM. Wilting often results in an increased number of yeast (Jonsson & Pahlow, 1984) which can negatively affect the aerobic stability of silages.

Table 1. Water activity and growth of microorganisms in forage (Lindgren, 1991)

Range (a_W)	Organisms with a lower limit of growth within the range
1.00 - 0.95	Gram-negative bacteria, Bacillus, Clostridia spores
0.95 - 0.91	Clostridia, LAB
0.91 - 0.87	Many yeasts
0.87 - 0.80	Field fungi, Mycotoxicogenic penicillia
0.80 - 0.75	Mycotoxicogenic Aspergillus
0.75 - 0.70	Most storage fungi

Buffering capacity of crop

The buffering capacity of plants determines the ability to resist a pH drop down to 4.0. Most of the buffering properties of forage are attributed to the anions (organic acid salts, nitrate, sulfate). Besides these, some organic acids also possess a buffering ability such as malic and citric acids. Buffering capacity can be increased during the ensiling process due to the production of various organic acids. The concentration of the buffering compounds in the plant is usually reduced with advancing stage of growth. Legumes possess high a buffering capacity whereas grasses and cereals have relatively low capacities. Nitrogen

fertilization increases the buffering capacity of the crop (Tommila *et al.*, 1996). However, this was not evident in the present studies when whole-crop cereals were fertilized with 80 kg nitrogen per ha.

Nitrate poses the most contradictory effect in buffering capacity. The concentration of nitrate is increased by nitrogen fertilization and excess nitrate in forage can have toxic consequences to animals (Kemp *et al.*, 1977). On the other hand, an appropriate amount of nitrate in forage has an inhibitory effect on clostridial growth (Weissbach, 1996).

Mechanical treatments of forage

The level of laceration affects the silage fermentation in terms of bacterial activity in a crop. Increasing laceration of ensiled forage by e.g. precision chopping causes a more available substrate and water released from damaged forage cells, resulting in an increased fermentation rate of silages (Honig, 1978; Pauly & Lingvall, 1999). Typical for such silages is a high quantity of lactic acid and low pH (Lindell *et al.*, 1972; **II**, **III**, **IV**). However, under circumstances of low WSC concentration, the risk of spoilage microflora overwhelming lactic acid bacteria can increase (Murdoch *et al.,* 1955; Pahlow *et al.*, 2003). This effect was pronounced in precision chopped whole-crop cereals silages in experiments **II**, **III**, & **IV**. Silages in those experiments harvested at dough stage resulted in butyric acid fermentation associated with clostridial activity. A similar susceptibility of whole-crop cereals to butyric acid fermentation was obtained by Weissbach & Haacker (1988) and Woolford *et al.* (1982). A low resistance to clostridial fermentation of precision chopped whole-crop cereals silages ensiled at dough stage is attributed to the low concentration of WSC in the crop. Chopping of forage usually increases the numbers of LAB (McDonald *et al.*, 1991). However, in trials **III** & **IV**, the count of LAB in precision chopped forages was lower than in long-cut forage. It is assumed that this contributed to an increased susceptibility of precision chopped whole-crop cereal silages harvested at dough stage to clostridial fermentation.

Another aspect of reduction of particle length of forage is seen in relation to the degree of forage compaction. A more finely-chopped forage is easier to consolidate the forage in the silo as compared to plant material with a longer-chop length. This feature was particularly pronounced in whole-crop barley silages at milk stage (**II**), where the silages density of 250 g DM kg^{-1} could not be reached with the longer particle length.

Density

The degree of consolidation of forage in the silo determined by silage density influences silage quality. Pauly (1999) reported an increased lactic acid formation and reduced pH of silages when forage was ensiled at higher densities but did not find any particular effect on hygienic quality. In addition, reduced DM losses in silages compacted to the higher density are in agreement with Honig (1987). All these effects were obtained also in experiment **II** with whole-crop cereal silages at dough stage. It is assumed that the principle of these improvements in silage fermentation lies in the reduced amount of trapped air in the forage, which reduces

respiratory and enzymatic processes and accelerates the acidification process in silage (Pahlow *et al.*, 2003). Experiment **II** also showed that when silage additives were applied, the impact of density on silage fermentation was negligible.

Silage additives

The application of silage additives has become the conventional implement to control the ensiling process. Although the main objective in using silage additives is to ensure the fermentation process to produce well-preserved silages, attention is also paid to methods of reducing ensiling losses and improving aerobic stability of silages during the feed-out period. Silage additives can be classified into two main categories, stimulants and inhibitors. Silage stimulants are employed to encourage the lactic acid fermentation by promoting the growth of lactic acid bacteria through the provision of acids or fermentable substrate whereas silage inhibitors partially or completely restrict microbial growth. Each additive can be used separately or in mixtures to cover a large spectrum of beneficial effects.

Fermentation stimulants

In order to reduce dependance of the ensiling process on epiphytic LAB, inoculum of selected strains of LAB is applied. The main demands for the selection of LAB inoculants for silage were defined by Whittenbury (1961) and include characteristics for competitive and vigorous growth in the silage, homofermentativity with maximal production of lactic acid in a short time, acid tolerance, and ability to grow in materials with a high DM content, and temperatures extending to 50°C. Among the genera of lactic acid bacteria, the species of *Lactobacillus plantarum* meet most of Whittenbury's criteria and these are therefore the main component of bacterial inoculants used. However, results with these genera were not always satisfactory and thus the combination with other species was necessary (Weinberg & Muck, 1996). In the present work, the mixture of two strains of *L. plantarum* and two strains of *Propionic bacterium* (**II**) and a combination of two strains of *L. plantarum* with *L. lactis* (**IV)** were tested on whole-crop cereal forages. In addition to the type of inoculant, the efficiency of the inoculant is affected by two main factors-the availability of WSC and the quantity of epiphytic lactic acid bacteria in the crop (Muck, 1993b). A high amount of natural lactic acid bacteria in the crop, which competed with lactic acid bacteria in the inoculant for silage deterioration, was probably the main reason of low effectiveness of the mixture of *L. plantarum* and *Propionic bacterium* (**II**) in improving silage fermentation of silages at milk maturity. The low effect of this inoculant (**II**) on silage fermentation was probably emphasised by the insufficient concentration of WSC at dough-matured crop. Presumably, as a consequence of a depletion of WSC and low glucose availability in silages, the homolactic fermentation was changed to heterofermentative resulting in a high concentration of acetic acid (Lindgren *et al.*, 1990). A high concentration of acetic acid suppressed the growth of yeast and moulds and aerobically stabilised these silages (Weinberg *et al.*, 1993). Silages in experiment **IV** responded differently to inoculation. A significant improvement of silage fermentation, reduced DM losses and spoilage microflora in all inoculated silages indicated a suitable bacterial composition and application rate of both bacterial mixtures. However, as a result

of homolactic lactic acid fermentation, these silages contained a low quantity of acetic acid which gave them little protection against aerobic deterioration, particularly in barley silages (Filia *et al.,* 2000; Weinberg *et al.*, 1993, 2002; **IV**). Improved aerobic stability of wheat silages can also be attributed to a lower contamination of wheat forage by natural spoilage microflora.

One of the approaches to improve aerobic stability of inoculated silages was to apply an additional 600 g ton^{-1} FM of sodium benzoate to the mixed inoculum of *L. plantarum* and *Propionic bacterium* (**II**) and 200 g ton^{-1} FM of sodium benzoate in the mixture of *L. plantarum, P. acidilactici, L. lactis* (**III**, **IV**). The results in experiment **II** show no particular improvement compared with silages without sodium benzoate. On the other hand, silages inoculated with of *L. plantarum, P. acidilactici, L. lactis* (**III**, **IV**) and 200 g ton^{-1} FM of sodium benzoate improved the silage fermentation in both barley and wheat silages, but a beneficial impact on aerobic stability was obtained only in study **IV** (Honig *et al.*, 1996). It is assumed that the variation in aerobic stability can be attributed to the difference in natural spoilage microflora contamination of forages. A beneficial impact on aerobic stability was also obtained with the application rate of inoculates with increased dilutions in water. These results are attributed the enhanced production of acetic acid, which possesses protective properties against yeast and mould growth (Weinberg *et al.*, 1993).

Fermentation inhibitors

The investigation by Lättemäe (1997) showed that the silage additives based on a combination of salts of organic acids, hexamine and sodium nitrite had a beneficial effect on grass-clover silage quality. In experiments **I**, **II**, **III**, & **IV,** this additive showed positive results regardless of the type of crop, DM content or degree of laceration. The application of this additive showed considerable reduction of clostridial growth and butyric acid concentration in both grass-clover and whole-crop cereal silages. Lättemäe (1997not in ref list) attributed this effect to the synergistic influence of hexamine and sodium nitrite. In the present work, it was not possible to clearly attribute the effects to particular compounds. Silages treated with the silage additive based on a combination of salts of organic acids, hexamine and sodium nitrite were found to be less effective in reduction of silage pH, which is assumed to be a result of formaldehyde and ammonia (Kung *et al.*, 2000, McDonald *et al.*, 1991) originating from the degradation of hexamine. The role of sodium nitrite in silage fermentation is to break down natural nitrate in the fresh forage to nitrite and further to nitric oxide and nitrous oxide, which are toxic to enterobacteria and clostridia (Spoelstra, 1985; Spoelstra, 1987; Spoelstra, 1991). Because of the volatility of nitric oxide and nitrous oxide, the efficiency of this additive composition was very pronounced in long-cut forages (**I**, **II**, **III**, **IV**). The presence of sodium nitrite in the additive could pose a potential risk of nitrite poisoning to animals (Kemp *et al.*, 1977). The investigation of sodium nitrite applied at three different doses (**I**) showed little risk for nitrite poisoning due to its presence in this additive as the concentration of nitrite in silages at the highest dose was below the threshold level of 1.2 g/kg DM for feeding of forage (Spörndly, 2003).

Besides the positive impact of this additive mixture on reduction of clostridia and butyric acid concentration, a significant reduction of yeast activity in these silages was obtained (**I**, **II**, **III**, **IV**). This result is thought to be due to the presence of sodium benzoate in the additive (Lättemäe, 1997 not in ref list), which inhibits yeast and moulds (Woolford, 1978 a or b check in ref list). The antimycotic effect of sodium propionate (Woolford, 1978 check in ref list) is also assumed to contribute to low amounts of yeasts and moulds in these silages. The benefit of combining sodium benzoate and sodium propionate could be derived from the additive mixture of 672 g sodium benzoate, 425 g sodium propionate and 1890 g propionic acid per ton^{-1} FM applied to grass-clover silages (**I**) and whole-crop barley silages (**II**). This additive composition improved the silage fermentation, reduced DM losses, and decreased clostridia, yeast and mould counts. As a consequence of the remarkable suppression of spoilage microflora, particularly yeasts (Woolford, 1978b), silages treated with additives containing a combination of benzoate and propionate or additional propionic acid had improved aerobic stability.

In contrast to previous additive compositions, the impact of combinations of formic acid with propionic acid and ammonia on quality of whole-crop cereal silages was not that clear. The results in the present work indicate that the efficiency of this additive mixture was related to forage particle length and the forage growth stage. The application of propionic acid, formic acid and ammonia at the rate of 4 l ton^{-1} FM improved the silage quality in precision-chopped forage in terms of reduced DM losses, yeast and clostridia counts and increasing aerobic stability regardless of the stage of forage maturity (**II**, **III**, **IV**). Long-cut silages treated with this additive ensiled in laboratory silos (**II**) showed low efficiency in reduction of yeast growth and improvement of aerobic stability at milk stage, but at dough stage, long-cut silages were well preserved. However, when this additive was applied to baled forage at dough stage (**III**, **IV**), the silage quality deteriorated. The primary reason for the low effectiveness of this additive mixture in baled silages is assumed to be a low application rate (Woolford, 1978a; Jonsson *et al.*, 1990; Pursiainen *et al.*, 2001). Formic acid is the silage additive that has a strong antibacterial effect, which affects also lactic acid bacteria, whereas propionic acid possesses antimycotic properties. As a consequence of the formic acid application, lactic acid bacteria were inhibited and pH of silages was reduced by formic acid alone. However, because of the low application rate, the pH reduction was not sufficiently low and propionic acid was afterwards unable to protect silages against spoilage microflora. The efficiency of this additive was not improved when the proportions of acids were changed from 45% formic acid and 20% propionic acid to 22% formic acid and 41% propionic acid. Insufficient distribution of additive in baled forage could also contribute to a low effectiveness of these additives. Dilution of both additives with water to double the application rate for improved distribution of silage additives in the forage mass (**IV**) improved the efficiency of silage additives. Results offered evidence that the low application rate was the main reason for restricted efficiency of these additive combinations since silages treated with diluted doses were obtained with no improvement of silage fermentation.

Determination of silage quality

Forage and sampling

The main challenge when comparing different silage treatments is to obtain a sample which most accurately represents the composition in a particular silage treatment. However, silage is a heterogeneous product where chemical and microbial compositions can differ within the silo. The heterogeneity of silage originates from the variation in the microbial, chemical, and botanical composition of the fresh herbage. The method of harvesting, further processing of forage such as chopping, tedding, wilting, sealing as well as type of silo affect the silage heterogenity (Pauly, 1999). To eliminate the effect of silage heterogeneity in my results, the treatments were performed in replicates. Also the method of sampling was adjusted to the type of silo used to receive the most representative sample. In this work, both laboratory silos and bale silages were used. When laboratory silos were used, all silage was transferred from the silo into a plastic bag, thoroughly mixed and then samples were taken. To follow a similar sampling procedure with bale silages was impossible. Thus, sampling was performed with a stainless steel corer (Ø 4.0 cm). Nine horizontal cores at a 40-cm depth were extracted from each bale (see Fig. 2). Generally, the conditions in the laboratory are better for ensiling than in full-scale silos (bales) because of more efficient sealing. To reduce the discrepancy between results from lab and bale silages, the bales in my studies were wrapped with 8 (**III**) and 12 layers (**IV**) of stretch film with a width of 750 mm and thickness of 0.025 mm and time between baling and wrapping did not exceed 15 minutes (**III**). In experiment **IV**, the bales were wrapped immediately after baling.

A mixture of grass-clover (timothy-red clover) (**I**), whole-crop barley (**II**, **III**, **IV**) and whole-crop wheat (**IV**) were used in my studies to evaluate the factors influencing the silage quality. Untreated silages were used as a negative control in evaluation of efficiency of silage additives. In experiment **II**, the efficiency of silage additives and other factors to stop spoilage of silages was tested under unfavourable ensiling conditions (Kwella *et al.*, 1993). In order to simulate difficult ensiling conditions, *Clostridium tyrobutyricum* was added to the silage crop as one representative of frequently occurring spoilage microorganisms in silage (McDonald *et al.*, 1991).

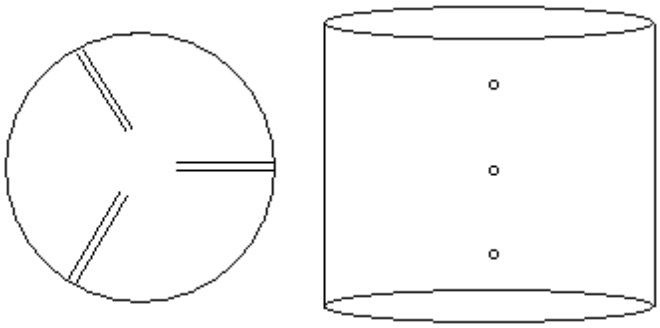

Fig. 2. Design of sampling of silage bales with corer

Assessment of silage quality

Analyses

Prior to the determination of silage quality, fresh herbage was analysed to estimate its ensilability. The fresh crop is usually subjected to analyses such as dry matter of the crop, concentrations of WSC and buffering capacity of the crop. Additionally, the microbial composition of herbage is of interest, particularly numbers of lactic acid bacteria, clostridia, yeast and moulds.

The analyses performed on silages can be divided into those that indicate the fermentation quality of silages and to those that determine the general hygienic condition of silages. Weissbach (1996) suggested pH, ammonia and butyric acid concentrations as measures to determine the fermentation quality of silage. In addition to these, the most common silage parameters are concentration of WSC, fatty acids, and ethanol. All these chemical parameters are usually expressed on a DM basis and are used as one indicator for assessing hygienic quality of silages. Another indicator of hygienic quality of silages is revealed by analysing the microbial composition of silages. Particular interest is directed to the presence of spoilage microflora such as clostridia spores, yeast and moulds since these are primarily responsible for silage deterioration s. The recommended criteria used in the assessment of feed quality in Sweden are presented in Table 2.

Table 3. Assessment of feed quality in Sweden (Spörndly, 2003)

Silage		
Ammonium	<80 g NH_4 kg^{-1} total N	good quality
	80-120 g NH_4 kg^{-1} total N	intermediate quality
	>120 g NH_4 kg^{-1} total N	poor quality
pH value	<(0.0257*DM%)+3.71	
Butyric acid	<1 g kg^{-1} fresh herbage	good quality
	1-3 g kg^{-1} fresh herbage	intermediate quality
	>3 g kg^{-1} fresh herbage	poor quality
Lactic acid	Direct-cut with formic acid	
	60-100 g kg^{-1} DM	normal
	Direct-cut without formic acid	
	80-120 g kg^{-1} DM	normal
	Wilted over 30% DM	
	30-70 g kg^{-1} DM	normal
Acetic acid	10-300 g kg^{-1} DM	normal
Lactic acid bacteria	$>10^4$ g kg^{-1} fresh herbage	desirable
Gram-negative bacteria	10^3-10^4 g kg^{-1} fresh herbage	maximum
Total bacteria count	10^9 g kg^{-1} fresh herbage	
Forage		
Nitrate	<1.2 g kg^{-1} DM	acceptable
	1.3-2.4 g kg^{-1} DM	acceptable
	2.5-4.4 g kg^{-1} DM	max. 50% of feed ratio
	4.5-4 g kg^{-1} DM	unacceptable
	>9.0 g kg^{-1} DM	unacceptable
Aerobic bacteria	10^3 g kg^{-1} fresh herbage	maximum
Spore-forming bacteria:		
Clostridia spp.	10^3 g kg^{-1} fresh herbage	maximum
Bacillus spp.	10^3 g kg^{-1} fresh herbage	maximum
Yeasts	10^5 g kg^{-1} fresh herbage	maximum
Moulds	10^5 g kg^{-1} fresh herbage	maximum

Aerobic stability test

A successfully-preserved forage is only the first step to feeding high-quality silages to animals. The next critical step is the opening and of the silo and removal of the silage for feeding. At this point, the anaerobic silo conditions are changed to an aerobic, and microorganisms that were dormant in the aerobic environment multiply, resulting in silage deterioration (Woolford, 1990). As a result of the proliferation of a facultative and obligate aerobic microorganisms, the nutritive value and hygienic quality of the silage is reduced. To avoid the dangers in this step, the present experiments were subjected to an aerobic stability test in which silages were continuously ventilated for seven days with 22.6 litres of moisturized air per kg dry matter and hour at a temperature of 23–24°C. The amount of silage in the ventilated tube was equivalent to a density of 4 litres of pore volume per kg of silage DM which would give a similar resistance of air flow irrespective of the DM content of the silage. The deterioration process in the silage samples was identified by monitoring the carbon dioxide concentration in the gas flow from the tubes. Carbon dioxide concentration was measured with a non-dispersive infrared photometer (Binos® 100, Leybold AG, Hanau, Germany). The measurement of

carbon dioxide concentration is based on the assumption that carbon dioxide is one of the main products from the respiration of aerobic microorganisms (McDonald *et al.* 1991).

The previous investigations showed that yeasts are the most important microorganisms that initiate aerobic deterioration of silages (Beck & Gross, 1964; Ohyama & Hara, 1975; Woolford, 1978b; Jonsson, 1989). Yeasts possess a high ability to survive low oxygen levels (Jonsson & Pahlow, 1984) and some yeasts have lactate-assimilating capabilities, and, in the presence of oxygen are very tolerant to high concentrations of lactic acid and low silage pH. The present experiments confirmed that low aerobic stability of silages was attributable to the activity of yeasts. It was shown that silages with suppressed yeast growth were more aerobically stable (**I**, **II**, **III**). However, some silages in experiments **II**, **IV**, indicated a low aerobic stability despite a low yeast count. A possible explanation could be that this instability was caused by other aerobic spoilage microorganisms, presumably by acetic acid bacteria (Spoelstra *et al.*, 1988).

Moulds are considered to have a secondary role in aerobic deterioration as their growth often follows the growth of yeasts (McDonald et al, 1991; Pahlow *et al.* 2003). Mould growth usually occurs on the peripheral parts of the silo because the most likely air ingress is through the silo covering. In this regard, bale silages pose a higher risk for mould growth since there is a greater surface area and danger for damage to the stretch film (Jonsson *et al.*, 1990).

The main factors affecting growth of microorganisms during aerobic deterioration are the concentration of oxygen, temperature, water activity, pH, concentrations of organic acids and use of additives. The measures such as fast wilting and filling, proper consolidation, direct sealing or silo tightness reduce the sensitivity of silages to aerobic deterioration (Petersson, 1988). In the present experiments, the effect of silage additives was most pronounced in improving the aerobic stability of silages. Particularly the mixture of sodium benzoate, sodium propionate with hexamine and sodium nitrite and combination of sodium benzoate, sodium propionate and propionic acid showed satisfactory increases in aerobic stability of silages. The effect of these mixtures is attributed to the presence of compounds with antifungal properties such as sodium benzoate or propionic acid and its salts (Woolford, 1978a). On the other hand, the high resistance of yeast and moulds to formic acid (Handerson *et al.* 1972, Jonsson *et al.* 1990) could be a reason for the weak effect of additives containing formic acid (**III**, **IV**). A contribution of acetic acid in improvement of aerobic stability of silages was observed, particularly in lactic acid bacteria/inoculated silages (**II**, **IV**). This phenomenon of homofermentative production of lactic acid and lack of protective acetic acid, which impaired the aerobic stability of inoculated silages is quite common and was reported earlier (**III**, **IV**, Weinberg & Muck, 1996). Another production of fermentation, butyric acid, also showed stabilizing effect on silages. However, silages containing butyric acid are undesirable because they are poorly fermented and indicate a high clostridial activity.

Ensiling losses investigation

Losses during the ensiling process are used as an indirect measure of the efficiency of the conservation system. The sum of losses covers the period from harvesting of forage to feeding of silage, and therefore, losses can be classified as field losses, fermentation losses, and aerobic deterioration losses. In the present work, the main attention was focused on fermentation losses, but some observations were made on field losses.

Field losses

Field losses are usually caused by mechanical treatments during harvest, herbage respiration and leaching during wilting. The variation in field losses in ensiling of whole-crop cereal forage in bales was demonstrated in study **IV**. The differences showed dependence on stage of maturity and type of baler used. There were no DM losses of forage baled with a variable-chamber baler at the heading stage, but at milk stage, losses were about 35 g kg^{-1} DM and at dough stage 60 g kg^{-1} DM. When a fixed-chamber baler was used in forage at dough stage, DM losses decreased down to 10 g kg^{-1} DM. Because forage was ensiled immediately, field losses caused by herbage respiration were assumed to be negligible.

Fermentation losses

Fermentation losses include losses that originate directly from the fermentation process and also from residual respiration during the initial stage of fermentation. The fermentation losses are frequently expressed as DM losses, but they are not as relevant for determining feeding value of silage as energy losses. DM losses arise from the microbial activities during fermentation and the magnitude is thus related to the microbial composition and prevailing biochemical processes occurring in silages. In well-preserved silages where homofermentative lactic acid bacteria dominates, DM losses are low and can be about 2 to 4%, whereas in silages with a high activity of undesirable microorganisms such as clostridia, DM losses can be much greater (**II**, **III**, **IV**, McDonald *et al.*, 1991). These variations are attributed to the different efficiency of fermentation pathways and type of substrate used by particular microorganisms. Therefore, to reduce DM losses, methods must be used that suppress the growth of undesirable microflora in silages and encourage a proper fermentation process. In this regard, the application of silage additives had most pronounced impact on the reduction of DM losses. Lactic acid bacteria inoculants reduced DM losses by promoting oefficient homolactic fermentation (Weinberg & Muck, 1996). Lättemäe & Lingvall (1996) reported reduced DM losses in silages treated with the mixture of hexamine, sodium nitrite with salts of organic acids that resulted in reduced growth of clostridia in silages. The application of formic acid and propionic acid was reported also to reduce the DM losses during the fermentation process (Pursiainen *et al.*, 2001).

Besides the effect of additives, the extent of DM losses is considered to be influenced by the amount of moisture present in forage, which affects the activity of silage microflora and intensity of fermentation process (McDonald *et al.*, 1991). Honig (1987) demonstrated in his investigation that DM losses were reduced when dry matter content of grass forage was increased (**I**). A similar effect was obtained

in study **III**, in which the whole-crop barley forage was wilted. The dry matter concentration of forage is also the main factor affecting the amount of effluent produced during ensiling (McDonald *et al.*, 1991). Avoiding high levels of effluent losses is of particular importance, since high concentrations of soluble substances in the effluent such as carbohydrates, organic acids, minerals and nitrogenous compounds, create a considerable loss of valuable nutrients. In addition, these constitute a potential risk for environmental pollution. Forage with a dry matter of about 250 g kg^{-1} is reported to have little effluent (Zimmer & Wilkins, 1984). The results from the present studies are in agreement with this finding. The risk of effluent losses was seen in whole-crop wheat bales ensiled at milk stage containing 203 g DM kg^{-1} (**IV**).

Residual herbage respiration and enzyme activities in the initial phase of ensiling process contribute to the DM loss. Since these processes depend on the presence of oxygen, measures such as a greater consolidation of forage in the silo will reduce the amount of air in the silo thereby reduce respiration loss.

Aerobic deterioration losses

Aerobic deterioration losses occur during the feeding stage of the ensiling process when the silo is opened and the silage is exposed to oxygen. These losses are attributed to the activities of facultative and obligate aerobic microorganisms such as yeast, mould, acetic acid bacteria, which are responsible for aerobic deterioration and reduction of silage stability as described in the previous section. Therefore, measures to improve the silage aerobic stability are considered to reduce the aerobic deterioration losses.

Summary of presented investigation

Additives and wilted clover-grass silages

The application of silage additives containing nitrite substance on moderate and high wilted clover-grass forage seems to secure a good silage quality and improve aerobic stability without having a high concentration of toxic residues of nitrite in silages (**I**). This result gives a possibility to prevent occurrence of undesirable microflora in unevenly wilted forage.

Ensiling of whole crop cereals

Stage of maturity

In studies **II**, **III**, **IV**, the main focus was on ensiling of whole-crop cereals. The investigated factors appeared to be interrelated; therefore, it was difficult to separately determine the influence of each factor. Nevertheless, the results from ensiling whole-crop barley depended greatly upon the stage of maturity (**II**, **III**, **IV**). The important factor related to stage of maturity and influencing the ensilibility of whole-crop cereals was the concentration of WSC in the fresh

forage. The WSC content in fresh whole-crop cereal forage reached its maxima at milk stage and showed a considerable decrease at dough stage. The drop in WSC concentration at dough stage seems to be a limiting factor for the ensiling process. The insufficiency of WSC seems to be pronounced in the initial phases of fermentation, which depressed the activity of LAB. This is reflected in poor lactic acid production and a slow pH decrease giving little protection in the silage against the spoilage microflora. In this regard, the milk stage indicates a more suitable time to harvest whole-crop cereals for ensiling. There is a sufficient concentration of WSC providing the LAB to rapidly dominate the silage microflora and produce an adequate quantity of acids to reduce pH. The better ensilability of whole-crop cereals at milk stage also can be related to the generally higher numbers of epiphytic LAB and a more stable number than at the dough stage forage. However, silages at milk stage of maturity still appear to have a low aerobic stability.

Another factor related to the stage of maturity was the forage DM content. DM increased as whole-crop cereals matured. However, DM concentration seems to have a secondary impact on the quality of ensiling process. It was seen in studies **II**, **III** where a relatively small DM variation between milk and dough stages still resulted in a better ensiling quality at milk stage. This is attributed to a higher concentration of WSC at milk stage, which probably accelerated the fermentation process by promoting the LAB. In this regard, a reduced concentration of WSC and numbers of LAB in wilted whole-crop cereal forage could be a reason of diminished effect of wilting on the silage quality. A different situation was seen in grass-clover silages (**I**), where extensive wilting considerably reduced the activity of the spoilage microflora and improved silage quality without giving a large reduction of WSC and epiphytic LAB in fresh forage.

Cutting versus chopping

The reduction in chop length had a variable effect and showed that this is not a guarantee of a good silage quality of whole-crop cereals. At milk stage, precision chopping improved the silage fermentation (**II**), whereas dough-stage silages tended to give clostridial fermentation resulting in poor quality and high DM losses (**II**, **III**, **IV**). A positive aspect of precision chopping of the forage was obtained in relation to the silage density. Precision chopping promoted high silage density at the milk stage and a reduction of DM losses at the dough stage. The main benefit of precision chopping, however, was in improvement in efficient application of the silage additives at dough stage, which gave a better distribution of additives in the forage mass.

Application of silage additives

The use of silage additives seems to be the most important factor to improve aerobic stability of whole-crop cereal silages at milk stage and to improve silage fermentation at dough stage. The mixture of sodium benzoate, sodium propionate, hexamine and sodium nitrite seems to be the most suitable to secure the silage quality and improve aerobic stability in both precision-chopped and long-cut silages harvested at milk and dough stages because of the greater inhibition of

spoilage microflora. A similar assumption is made concerning the combination of sodium benzoate, sodium propionate and propionic acid.

The use of formic acid in combination with propionic acid gave the most variable results in relation to the chop length of forage. This additive combination seems to improve the silage quality and storage stability in precision-chopped silages at both maturities, whereas the quality of long-cut silages treated with this additive often appeared to be low. It seems to be a consequence of low application rates in combination with the insufficient distribution in long-cut forages.

The variable efficiency of inoculation of whole crop cereals with LAB seems to be associated with differences in type and number of epiphytic bacteria in the herbage. A homofermentative LAB in the inoculants successfully reduced pH of both precision-chopped and long-cut silages but abundant production of lactic acid did not secure the aerobic stability of these silages.

Conclusions

- The application of additive containing nitrite substances is secure and improves silage quality in moderate and high-wilted clover-grass forages.
- Stage of maturity considerably influences the ensilability of whole-crop cereal forages. The main factor affecting a successful ensiling process is the concentration of WSC, which makes the milk stage of growth more suitable for ensiling. The DM concentration seems to posses an additional role.
- The application of silage additives is an important measure to improve aerobic stability of whole-crop silages harvested at the milk stage of growth and essential to secure the silage fermentation of silages at the dough stage. The use of silage additives gives a better possibility to achieve low silage losses and a good silage quality in both bale silage systems and conventional tower and bunker silos.
- Precision chopping of whole-crop forage alone is not a guarantee of a good silage quality.

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