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Jäst i grönmassan och ensilagets lagringsstabilitet vid 15 svenska mjölkgårdar

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Abstract

This study investigates the role of yeast in green crop and its impact on the aerobic stability of silage. Fresh crop was collected from 15 farms in southern and middle parts of Sweden during the summer 2014; samples from the primary harvest was collected from eight farms and samples from the first regrowth harvest was collected from seven farms. The grass was ensiled in 1.7 l glass silos. After three months of ensiling, silos were opened and silages were stored aerobically for 10 days. Samples from both harvests were ensiled in completely air-tight silos, but samples from the second harvest were also ensiled in slightly ventilated silos. Chemical analyses and yeast counts were performed for fresh crop and silage. During the ensiling period, weight loss was registered for all silos and during the aerobic stability test, the temperature in the silage was measured every second hour throughout a ten day period. Yeast species present in the fresh crop were identified with DNA sequencing. Data of management applied in silage making was collected from the 15 farms and processed in relation to silage quality.

Dry matter (DM) content of the fresh crops ranged from 22.2 to 52.8 %, metabolizable energy content from 9.2 to 11.6 MJ/kg DM, crude protein (CP) content from 110 to 190 g/kg DM and water soluble carbohydrate content (WSC) ranged from 38 to 118 g/kg DM. Yeast counts in the fresh crop averaged log 5.4 colony forming units/g (min log 4.6; max log 5.9) with no difference between first and second harvest. No correlation was found between fresh crop and silage yeast counts. Among the yeast isolates, 16 different yeast species were identified with *Rhodosporidium babjeave* and *Rhodotorula glutenis* being the two most common. In the air tight silos, yeast count was higher in the first harvest than in the second harvest. Among management factors a tendency was observed for higher yeast counts in the fresh crop when manure was used instead of inorganic fertilizer. Silage from the second harvest had shorter aerobic storage stability compared to silage from the first harvest. Also silages with high DM content had shorter aerobic stability than silages with low DM content.

Comparing the air-tight and slightly ventilated silos, the number of yeasts were higher in ventilated than in air- tight silos after the ensiling period. Weight loss was 3.1% of the initial DM in air-tight silos and 3.4% in ventilated silos during the ensiling period. Content of WSC decreased during ensiling by 81% of the initial WSC content in both air-tight and ventilated silos (s.d. 12.59% air-tight; s.d. 13.97% ventilated). After aerobic storage the weight loss was 15.3% of the initial DM (s.d. 8.86%) in the air-tight silos and 28.9% of the initial DM (s.d. 14.99%) in the ventilated silos. Loss of WSC content after the aerobic stability test was 32.6% (s.d. 33.11%) in air-tight silos compared to WSC content at the opening of the silo and 51.6% (s.d. 36.44%) in ventilated silos.

The results showed that the greatest weight loss occurred during the first week of ensiling. The decrease in WSC content, yeast counts and weight loss were lower in air-tight silos compared to ventilated silos and silage in air-tight silos also had longer aerobic stability than silage in ventilated silos. Higher DM content also resulted in shorter aerobic stability. High numbers of yeast in the silage at silo opening was highly correlated to shorter aerobic stability.

Sammanfattning

I den här studien undersöktes jästs roll i grönmassa och dess inverkan på den aeroba stabiliteten i ensilage. Grönmassa samlades från 15 gårdar i södra och mellersta delarna av Sverige under sommaren 2014 och ensilerades i 1,7 liters silor. Åtta av proverna var från första skörden och sju var från andra skörden. Efter en ensileringsperiod på tre månader lagrades ensilaget aerobt i 10 dagar. Proverna från de båda skördarna ensilerades helt anaerobt, men prover från andra skörden ensilerades även i silor som ventilerades med jämna mellanrum. Analys av kemisk sammansättning och jästantal gjordes på grönmassan och ensilaget. Under ensileringen registrerades viktförlusten. Temperaturen registrerades varannan timme under den aeroba lagringen. Jästarterna från grönmassan identifierades genom DNA-sekvensering. Information om ensilageskörden samlades in från varje gård.

Torrsubstanshalten (ts) i grönmassan varierade från 22.2% till 52.8%, mängden omsättbar energi från 9,2 till 11,6 MJ/kg ts, råproteinhalten från 110 till 190 g/kg ts och sockerinnehållet (lättlösliga kolhydrater, WSC) varierade från 38 till 118 g/kg ts. Antalet jäst i grönmassan var i medel log 5,4 (min 4,6; max 5,9) och det var ingen skillnad mellan första och andra skörden. I grönmassan identifierades 16 olika jästarter och *Rhodosporidium babjeave* och *Rhodotorula glutenis* var de arter som var mest vanliga. Jästantalet i ensilaget från lufttäta silor var högre i andra jämfört med förstaskörden. Ingen korrelation fanns mellan jästantalet i grönmassan och i ensilaget. Bland de variabler som rörde handhavandet av ensileringen fanns det en tendens till ökat jästantal ökade i grönmassan när stallgödsel användes jämfört med konstgödsel. Ensilaget från andraskörden hade kortare aerob lagringsstabilitet än ensilaget från förstaskörden. Även ensilage med högre ts-halter hade kortare lagringsstabilitet.

Vid jämförelse av lufttäta och ventilerade silor hade ensilaget från de ventilerade silorna högre antal jäst efter ensileringen. Under ensileringsperioden var viktminskningen 3,1 % av ursprunglig ts i de lufttäta silorna och 3,4 % i de ventilerade silorna. Minskningen i halten lättlösliga kolhydrater under ensileringen var 81 % i både lufttäta och ventilerade silor (sd 12.59 % i lufttät silo; sd 13.97 % i ventilerad silo). Under den efterföljande aeroba lagringen var viktminskningen 15,3% av ursprunglig ts (sd 8,86 %) i de lufttäta silorna och 28,9% (sd 14,99 %) i de ventilerade silorna. Minskningen av WSC-halten efter det aeroba stabilitetstestet var 32.6 % (sd 33.11 %) jämfört med värdet vid öppning av silorna i de lufttäta och 51.6% (sd 36.44%) i de ventilerade silorna.

Resultaten visade att den största viktminskningen skedde under den första veckan av ensileringen. I de lufttäta silorna var minskningen av halten lättlösliga kolhydrater och jästantalet samt viktminskningen under ensileringen lägre än i de ventilerade silorna. Ensilaget i de lufttäta silorna hade också en längre aerob lagringsstabilitet än ensilaget från de ventilerade silorna. Höga ts-halter gav också kortare aerob lagringsstabilitet. Högt antal jäst i ensilaget vid siloöppningen var starkt korrelerat med kortare aerob lagringsstabilitet.

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Introduction

The production of silage is important in countries with harsh winter time where animals cannot obtain the amount of energy or nutrients they require all year round from grazing, and in countries with a moist climate where it is difficult to make and store hay (McGechan, 1990; Pahlow *et al.*, 2003). To produce silage that can be stored aerobically without being deteriorated it is important to understand the ongoing processes that have an impact on the fermentation and storage properties. Microorganisms like yeast, moulds and lactic acid bacteria (LAB) occurs naturally on ensiling crops such as grass and legume leys and maize. Yeasts and moulds are responsible for the aerobic deterioration of silage (Ohyama *et al.*, 1975; Tabacco *et al.*, 2011). During aerobic conditions, pH rises and yeasts become able to proliferate and utilize organic acids and water soluble carbohydrates (WSC), as their main sources of energy (McDonald *et al.*, 1991). If yeasts have access to oxygen and energy substrates, they have a chance to develop and will then cause spoilage of silage (Honig, 1991). Spoilage of silage is undesirable due to the risk of proliferation of detrimental microorganisms, loss of nutrients, production of potentially toxic substances that may be harmful for livestock, and reduced digestibility of the feed through Maillard reactions (Ruxton and Gibson, 1993).

The ensilability of crops depends on the concentration of epiphytic lactic acid bacteria (LAB) availability of WSC (Guo *et al.*, 2014), and water availability for LAB (McDonald *et al.*, 1991). If the crops do not have sufficient content of WSC for a successful ensiling, they can be wilted to raise the WSC concentration (Guo *et al.*, 2014). Wilting the crops can however entail a risk of increased yeast numbers as a result of soil contamination during swathing (McDonald *et al.*, 1991). Yeast growth is encouraged in silages with a high DM content, but they still need a moist surface to be able to grow (McDonald *et al.*, 1991). During the first week of ensiling, yeasts counts may reach log 7 colony forming units/g (Pahlow *et al.*, 2003). Inlet of oxygen during fermentation enhances growth and survival of yeasts. This leads to a shorter aerobic stability when the silo is opened, mainly due to a high number of competitive deteriorating microorganisms (Pahlow *et al.*, 2003). Microorganisms also produce heat through the consumption of nutrients from the silage and this result in temperature increases in the silage (Liu *et al.*, 2014).

The aim of this study was to screen the occurrence of yeast and type yeast species in fresh grass intended for silage at Swedish farms, and to examine the impact of yeast in the fresh crop on the aerobic stability of silage.

Literature review

Microbial flora on fresh crop

The population of microorganisms on forage crops in the field or freshly harvested differs from the microorganisms present during fermentation and in silage. In the field, microorganisms are often present at the lower parts of the plants to prevent themselves from drying and being exposed to the ultraviolet radiation (Pahlow *et al.*, 2003).

Many microbial species on the crops are either obligate or facultatively aerobic. These are for example species of yeast, mould, acetic and propionic acid bacteria and enterobacteria. These are not involved in a positive way in the fermentation process of silage. The growth of this group of microorganisms is inhibited shortly after sealing of the silo (Pahlow *et al.*, 2003).

Some species of yeasts are facultatively aerobic and some are obligate aerobic. They are heterotrophic, eukaryotic microorganisms that normally multiply by budding. Examples of obligate aerobic yeasts are the pink coloured *Rhodotorula graminis* and *Rhodotorula glutinis*

(Kurtzman *et al.*, 2011; Hernández-Almanza *et al.*, 2014). Yeasts are not inhibited by low pH and they are able to grow and multiply within the pH range of 3 to 8 (McDonald *et al.*, 1991). Ethanol and volatile fatty acids (VFA) are formed from glucose by yeasts in an anaerobic environment (Woolford, 1976). During aerobic conditions yeasts produce their energy from glucose through glycolysis and the tricarboxylic acid cycle (TCA) and complete oxidation results in water and carbon dioxide as end products (McDonald *et al.*, 1991). Studies have shown an increase in numbers of yeast, LAB and enterobacteria on the crop if the harvest date is delayed (Müller, 2009; Schenck and Müller, 2014).

Epiphytic lactic acid bacteria (LAB) are essential for spontaneous silage fermentation. There is a big variety in numbers of LAB on the crops, from the lower detection limit of 10^1 colony forming units (cfu)/g on alfalfa up to 10^7 cfu/g on sorghum and maize (Pahlow *et al.*, 2003). Cool weather lowers the numbers of LAB and high amounts of LAB occurs therefore often at second and third harvest when the temperature is at highest in temperate climates like northern Europe for grasses and alfalfa and for early cultivars of maize (Pahlow *et al.*, 2003).

Fermentation process

To make silage of good quality, there must be a rapid removal of air. This is important to prevent aerobic growth of yeast, moulds and bacteria in the silage (Pahlow *et al.*, 2003; Liu *et al.*, 2014). Anaerobic conditions enables lactic acid bacteria (LAB), which are naturally present on the plant surface, to produce lactic acid which result in a drop in pH (McDonald *et al.*, 1991). The quality of natural fermentation in silage depends on the concentration of WSC and the composition of epiphytic LAB (Guo *et al.*, 2014).

The main substrate for LAB is WSC, and the major end product is lactic acid. Therefore it is important that the content of WSC is high when the crops are harvested (Piltz and Kaiser, 2004). If the WSC content in the plants is higher than 2.5% of the fresh forage weight, the forage has good ensiling properties. Forages with lower WSC content can be wilted to raise the concentration of WSC. The WSC content in temperate forages peaks just before head emergence for grasses and just before flowering for legumes (Pettersson, 1988). There are other factors that influence the WSC content. Different cultivars have different WSC content (Pettersson, 1988; Piltz and Kaiser, 2004). Cloudy weather and rainfall during the growth period can lower the WSC content due to reduced photosynthesis (Pettersson, 1988; Piltz and Kaiser, 2004). Grass mowed at mid-afternoon usually has the highest concentration of WSC due to the ongoing photosynthesis during the light part of the day. Concentration of WSC will reduce during night and will be at its lowest in the morning (Pettersson, 1988; Piltz and Kaiser, 2004; Guo *et al.*, 2014).

Assuming water availability is sufficient, the ensilability of crops depends on WSC concentration and buffering capacity of the forage. Legumes generally have a low WSC content and high buffering capacity and are more difficult to ensile compared to many grass species (Buxton and O'Kiely, 2003). The higher buffering capacity in legumes compared to grasses leads to a greater pH drop in grass silages (Buxton and O'Kiely, 2003). Maize has low buffering capacity and high WSC concentration, which make the preconditions for successful ensiling good (Buxton and O'Kiely, 2003).

Exclusion of air during the storage period and limited exposure of oxygen at the feed-out phase is important to prevent silage spoilage (Pahlow *et al.*, 2003; Liu *et al.*, 2014). All fermentation by LAB, other bacteria and yeast lead to losses to some extent (McDonald *et al.*, 1991). The LAB is divided into three distinct groups based on the pathway for fermentation of hexose sugars; 1) obligate heterofermenters which are lacking aldolase; 2) obligate homofermenters lacking dehydrogenases, and; 3) facultative homofermenters which generally

metabolize glucose along the glycolytic pathway. The obligate homofermenters ferment hexoses to nearly solely lactic acid. Facultative homofermenters almost exclusively ferment hexoses to lactic acid but they are also able to ferment pentoses to both lactic and acetic acid. Obligate heterofermenters ferment hexoses to lactic acid, acetic acid or ethanol and carbon dioxide (Buyze *et al.*, 1957). When homofermentative LAB ferment glucose to lactate, no DM is lost, and only 0.7% of the energy disappears from the forage. This is because homofermentative LAB lowers the pH rapidly (McDonald *et al*., 1991). However, heterofermentative LAB fermentation of glucose gives 24% loss in DM and 1.7% of the energy is lost (McDonald *et al*., 1991). When yeast ferment glucose to ethanol the DM loss is calculated to 48.9% and the energy loss only 0.2% (McDonald *et al*., 1991). This could be compared to the considerable loss of DM and energy when clostridia ferment lactate to butyrate, 51.1% and 18.4% respectively (McDonald *et al.*, 1991).

When the silo is opened, oxygen will be available for aerobic microorganisms. When these microorganisms start to grow, they produce heat when they consume fermentation products such as lactic acid from the silage, and they will multiply in numbers leading to spoilage of silage (Pahlow *et al.*, 2003; Liu *et al.*, 2014). Silages with high DM content, 300-500g DM/kg, have been reported to exhibit faster temperature escalation than silages with lower DM content, 150-300g DM/kg, as more energy is required to rise the temperature in wetter materials (Crawshaw and Woolford, 1979; McDonald *et al.*, 1991). The temperature in silage can reach 50-60˚C even when the ambient temperature is slightly above zero (Crawshaw and Woolford, 1979).

To improve the ensiling, four main management factors can be helpful; 1) Wilting the grass on the field to reduce water content and increase sugar concentration in the DM; 2) Chopping the grass increases the availability of the substrate; 3) Adding silage additives, for example acetic acid to prevent fungal growth (yeasts and moulds); and 4) Adding LAB-inoculants to help the naturally occurring LAB population in the crop (Honig, 1991; Conaghan *et al.*, 2012).

Lactic acid bacteria

To prevent or minimize silage deterioration and prolong aerobic stability, biological silage additives can be inoculated into the fresh grass at harvest (Henderson, 1993; Tabacco *et al.*, 2011; Conaghan *et al.*, 2012; Canibe *et al.*, 2014). The intended inoculation of LAB in silage making is to guarantee a rapid fermentation process with a fast accumulation of lactic acid, which results in a fast drop in pH at an early stage of the conservation (Weinberg *et al.*, 1993). Advantages have been reported with inoculating LAB (Rust *et al.*, 1989; Tabacco *et al.*, 2011), but also disadvantages (Ohyama *et al.*, 1975). In a study by Weinberg *et al.* (1993), they concluded that if silage contained low levels of volatile fatty acids (VFA) and high levels of WSC together with a high lactic acid concentration, the treated silage was associated with spoilage due to aerobic deterioration. This was because VFA inhibits yeast and moulds but both WSC and lactic acid are substrates for these microorganisms (Moon, 1983; Canibe *et al.*, 2014). This could be the situation in sugar-rich silages, if homofermentative LAB are used since the homolactic fermentation is more efficient than heterolactic fermentation leading to higher content of residual sugars and lactic acid in the silage (Weinberg *et al.*, 1993). If there are low levels of oxygen in the silage, the fermentation will shift from homolactic to heterolactic (McDonald *et al.*, 1991) and production of acetic and butyric acids, which will inhibit the development of moulds and yeasts (Weinberg *et al.*, 1993).

Tabacco *et al.*, (2011) stated that the aerobic stability was enhanced when two inoculants with different strains of *Lactobacilli buchneri* alone or in combination with *L. casei* were used. The concentration of *L. buchneri* in the inoculant was 1.0×10^5 cfu per gram of herbage.

Lactobacilli buchneri performed heterolactic fermentation in a second phase in the ensiling process and produced acetic acid. The acetic acid restricted growth of yeasts, and the reduced number of yeasts led to a more stable silage when it was exposed to oxygen at opening of the silo (Tabacco *et al.*, 2011).

Aerobic stability

The concentration of moulds and yeasts is the major factor starting the deterioration of silage when it is exposed to air (Moon, 1983; Woolford, 1990). Aerobic deterioration of silage leads to a lower nutritional value due to low water soluble carbohydrate (WSC) content and the risk of proliferating deteriorating microorganisms (Ohyama *et al.*, 1975). Wilkinson and Davies (2013) compiled factors that may increase the risk of yeast and moulds being present on the crops at harvest; 1) dead plant material on the bottom of the leys, 2) damaged crops from rain and/or wind during the last days of growth, 3) crops in an advanced stage of maturity, 4) crops that have begun senescence just before harvest, and 5) swaths that have been wilted for more than two days especially under poor weather conditions (Wilkinson and Davies, 2013).

The stability of silage in aerobic conditions can be defined as the amount of hours passed before the temperature in the silage rise more than 2˚C above the ambient temperature (O'Kiely, 1993).

Yeast growth on crops starts during wilting and continues during the storage period if air infiltrates the silage (Henderson, 1993). If the population of yeast, using lactate as substrate, in the silage exceed 10^5 cfu/g DM it is more likely to become deteriorated (Daniel *et al.*, 1970; Jonsson and Pahlow, 1984). Deterioration of silage is initiated by different species of yeast and depends on whether the environment during ensiling is anaerobic or aerobic. During aerobic ensiling conditions, the genera *Candida* and later during the fermentation *Wickerhamomyces anomalus* (also named *Hansenula anomala* and *Pichia anomalus*), were reported to predominate the fermentation. These genera lactate utilizing species. In anaerobic silages *Saccaromyces sp.* was present which ferment galactose and glucose instead of utilizing lactate (Jonsson and Pahlow, 1984).

There are some differences between crops in aerobic stability. Lucerne is more stable than maize (O'Kiely and Muck, 1992) and legume silages have been reported to be more stable to air exposure than grass silages (Pahlow *et al.*, 2001). O'Kiely and Muck (1992) found that lucerne ensiled in laboratory silos was stable in an aerobic environment for more than 7 days, and did not react on yeast from deteriorated silages which was inoculated into the silage. In the most stable silages, the inoculated yeast counts decreased rapidly from more than 10^6 cfu/g to 10^2 cfu/g within 48 hours after opening the silo. These findings indicate that the most stable silages in aerobic environment either lacked some essential nutrient for yeast growth or there were compounds present that inhibited yeast growth. The authors also find that the probable inhibitory effect was not present in the fresh forage, only after fermentation (O'Kiely and Muck, 1992).

The undissociated acids lactic acid, acetic acid and propionic acid are known to inhibit growth of yeast (Moon, 1983; O'Kiely and Muck, 1992). At low pH, a higher number of undissociated molecules of lactic and acetic acids pass into the yeast cell by passive diffusion and H^+ ions are released inside the yeast cell. To avoid being killed, the cell has to remove the H^+ ions. Under aerobic conditions the yeast cell is capable of removing H^+ ions by active transport. Under anaerobic conditions yeast cells does not get sufficient energy from fermentation of sugars to be able to remove H^+ ions from themselves (Cassio *et al.*, 1987). Therefore the presence of acids at low pH under anaerobic conditions will kill the yeast.

Yeast

The yeast flora in silage depends on access to air, type of crop that is ensiled and silage additives etc. If anaerobic conditions are reached and maintained in the silage, *Saccharomyces cervisiae* survives and utilizes for example hexose sugars, pentoses and organic acids (citric, acetic and lactic acids) (Jonsson and Pahlow, 1984). If air enters the silage during storage the dominating yeast genera are the lactate-assimilating *Candida* and *Pichia* (Jonsson and Pahlow, 1984). The crop can also affect the yeast flora in the silage. Crops that contain menthol or mustard oils *e.g*. leek, turnip and spearmint leads to a predominance of non-fermentative yeasts, for example *Debaromyces hansenii* and *Trichosporon cutaneum* (Middelhoven *et al.,* 1990). The yeast flora in silage can be stimulated by addition of formic acid (Henderson *et al.*, 1972). Silages treated with formic acid sometimes contain reduced number of LAB and acetic acid content but high numbers of yeast. Yeast has a higher tolerance to formic acid than LAB in such treated silages, and probably out-compete LAB (Henderson *et al.,* 1972).

Different yeast species occur in silage depending on when in the ensiling process sampling occurs and which crops are ensiled (Jonsson and Pahlow, 1984). In one study, the non-fermentative genera *Cryptococcus, Sporobolomyces, Rhodotorula, Torulopsis* and *Aurebasidium* were found on the fresh crop prior to ensiling (Jonsson and Pahlow, 1984). Three days after sealing these species were replaced by a storage flora of yeast depending on the ensiling technique. If the ensiling was anaerobic, the dominant species belonged to the genus *Saccharomyces spp*. These yeasts are not able to utilize lactate but ferment galactose and glucose. In aerobic silages, the composition of the yeast flora was dominated by *Candida lambica* and colonies of *Wickerhamomyces anomalus* and *C. krusei* were present from day 49 to the end of the ensiling period. During an aerobic stability test of silage the yeast counts rose from log 7 to log 9 and pH value from 5 during fermentation to above 7 within three days of aerobic storage (Jonsson and Pahlow, 1984).

Middelhoven and van Baalen (1988) performed a study on the development of yeast on whole crop maize. They found *C. ingeniosa, Cryptococcus laurentii, Sporobolomyces roseus, Sporidiobolus salmonicolor* and *R. rubra* on the fresh maize. After two days of ensiling all of these species were replaced by fermentative species such as *C. holmii, C. mileri, C. krusei, C. lambica, C. famata, Geotrichum candidum* and *W. anomalus* at different times during the ensiling period. Total yeast count was highest (log 7 cfu/g) between two and twelve days after the start of the ensiling and gradually decreased to log 5 cfu/g. When the silage was exposed to air, yeast counts increased exponentially to log 8.5 – 9.5 cfu/g by the action of *C. holmii, C. mileri* and *C. lambica,* and pH rose to 7.5 from initial values of around 3.5 (Middelhoven and van Baalen, 1988).

The results of these studies shows that anaerobic fermentation is important to keep the number of yeasts and pH low, and that it is important to maintain the anaerobic conditions through the whole fermentation period (Jonsson and Pahlow, 1984; Middelhoven and van Baalen, 1988).

Materials and Methods

Sampling

Samples of 4-7 kg fresh crop were collected from 15 different farms in Sweden in 2014, eight samples from first harvest (May 28 to June 9) and seven samples from the second harvest (July 15 and July 19). The participating farms were located in the southern and middle parts of Sweden (Table 1). The samples were taken outside the silos when the transport wagons or choppers arrived from the field and unloaded the fresh crop. The farmers were interviewed at

the time of sampling about management factors such as weather conditions, fertilization of the crop and the machinery equipment around the harvest etc. (Appendix 1).

Transportation and storage of samples

The samples were placed in plastic bags and stored in insulated boxes together with ice packs during transport to the laboratory. Samples were kept in the cooled boxes overnight, except samples from farm number 8, 12 and 15 which were collected and transported to the laboratory the same day. Storage and transportation times varied between one hour and 30 hours. If the ice packs melted during this time they were replaced with new ones resulting in an approximate storing temperature of 0 to 5°C.

Ensiling procedure

Samples of the fresh crop were taken for chemical analysis (frozen at -18°C) and for enumeration and identification of yeast. The crop was then packed in laboratory scale silos of 1640 ml volume. Both silos and lids were sprayed with ethanol (70%) and dried upside down on paper towels before being filled. The density of the crop packed in the silos was adjusted after the DM content of the crop, estimated by a quick microwave oven method where 100 g of fresh crop was dried in intervals until stable weight and weighed before air equilibration. Density was 144 kg DM/ kg^3 at DM content of 20%, and 245 kg DM/ m^3 at a DM content of 52%. The silos with air inlet had a density of 80 % of the density in the air tight silos (DLG, 2013). The silos were sealed with lids with water-filled gas locks and were weighed on day 0, 3, 7, 14, 28, 56, 78, 90 and on the day of opening to monitor weight loss during ensiling. The ensiling of the first harvest continued for 108±12 days, and the ensiling of the second harvest continued for 99±2 days.

Samples from the eight farms where first harvest crop was collected were ensiled in triplicates. Samples from second harvest crops (seven farms) were ensiled in four replicates, of which two were made in identical silos as from the first harvest and two were made in silos adapted with two 6 mm ventilation holes, one in the lid and one in the silo wall. These holes were plugged with rubber stoppers, and once a week during the ensiling period the stoppers were removed for two hours to simulate air leakage. After opening the silos, samples were taken for chemical analysis and for enumeration of yeasts.

Cultivation and enumeration of yeast

For cultivation and enumeration of yeast, 30 g fresh crop was placed in a stomacher bag and 270 ml Ringer (Merck, Darmstadt, Germany) solution ¼ -strength, (autoclaved for 15 minutes at 121 °C) was added. For silage samples taken after ensiling, 30 g silage was placed in a stomacher bag and stored at +4°C for two days and after that 270 ml autoclaved Ringer solution was added. The sample was then put in laboratory blender (Stomacher 3500, Seward Ltd, Worthing, West Sussex, UK) for 60 seconds in two rounds at the setting “normal speed”. The solution was diluted in tenfold steps, resulting in dilution series of 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5} for the fresh crop. Dilution series for silage samples were 10^{-2} and 10^{-3} and for silage from ventilated silos 10^{-2}, 10^{-3}, and 10^{-4}. From each dilution, 0.1 ml was spread with an inoculation spreader on malt extract agar (MEA) plates (Merck Darmstadt, Germany). The substrate was autoclaved at 121 ˚C for 10 minutes. After autoclaving and cooling to about 50˚C, 6 ml of a sterile stock solution of streptomycin sulphate and 6 ml of a sterile stock solution of penicillin G was added to the solution reaching a final concentration of 30 mg streptomycin sulphate and 30 mg penicillin G/litre substrate.

Samples from the first harvest were cultivated in two replicates per dilution. The dilutions from the second harvest were spread on eight plates each, four of the plates were the same as for the first harvest and four were MEA-plates that contained chloramphenicol instead of

penicillin and streptomycin. This was performed to evaluate if the number of bacterial colonies were reduced by use of a different antibiotic. After three days in a heating cabinet (Termaks, Bergen, Norway) at 25 °C, the colonies were counted. After counting, plates were placed in a fridge to prevent further growth until all samples from each harvest were analysed.

An estimation of the number of microbial colonies on the plates was done by calculating the sum of the colonies of all plates within one sample divided by the sum of the dilutions of each plate within one sample (Niemelä, 1983). Plates without visible colonies and overgrown plates were not included in the calculation.

Cultivation of yeast isolates and identification of yeast species

Colonies from the fresh crop identified as yeast by microscopy were streaked on MEA-plates to ensure pure yeast colonies. A total of 16 colonies from the first harvest and 40 from the second harvest were inoculated with an inoculation needle into test tubes with 3 ml Yeast Extract Peptone Dextrose (YPD)-medium (Becton Dickinson Company, Sparks, Maryland, USA) and incubated on a shaking table for 48 hours in a heating cabinet at 25°C. The YPD-medium had a concentration of 10 g yeast extract/l, 20 g bacteriological peptone/l and 20 g glucose/l. After incubation, 600 µl of cells from each test tube were pipetted to cryo tubes (2 ml) and mixed together with approximately 600 µl glycerol and put in the freezer at -70°C. One ml of cells was thereafter pipetted to Eppendorf tubes and stored at -20°C until DNA-extraction. Yeast species were not identified in silage samples.

The extraction of DNA, running of PCR, gel electrophoresis, purification of PCR-product and preparation for sequencing were done by the Department of Microbiology at Swedish University of Agricultural Sciences in Uppsala (Blomqvist, 2006). Sequencing of yeast was carried out by Macrogen (Amsterdam, Netherlands).

Yeast sequences were compared with sequences in NCBI's (National Center for Biotechnology Information, Bethseda, Maryland, USA) database using BLAST® (Basic Local Alignment Search Tool; www.ncbi.nlm.nih.gov/BLAST) (Altschul *et al.*, 1990).

Chemical analyses

Samples of frozen fresh crop from the first harvest were thawed overnight in room temperature with ice packs to prevent them from heating. Samples from the second harvest were thawed for half a day in room temperature.

The samples were homogenised manually and 130-300 g fresh weight were spread on aluminium trays. The rest of the samples were put back in the freezer as backup. The samples were pre-dried in a heating cabinet for about 18 hours at 60˚C. After drying, the samples were stabilized in open air for approximately four hours and were then weighed again and ground with a hammer mill (KAMAS Slagy 200, Malmö, Sweden) with a 1 mm particle size screen, then put in plastic jars of 100 ml. The hammer mill was vacuum cleaned between every sample, to prevent mixing particles of the different samples. The fresh crop was analysed for DM, ash, crude protein (CP), digestible organic matter using rumen fluid, (VOS, Swedish method of *in vitro* organic matter digestibility (IVOMD)) and water soluble carbohydrate (WSC) contents. Silage samples were analysed for DM, ash and WSC content.

Dry matter content was determined by drying the milled samples at 103 °C for 16 hours and weighing after cooling in a desiccator. Ash content was determined by incineration in a muffle furnace at 550°C for three hours. Concentration of CP was determined by using 2020 digestor and 2400 Kjeltec Analyser unit (FOSS Analytical A/S Hilleröd, Denmark) (Nordic Committee on Food Analysis, 1976). Estimation of organic matter digestibility content of forages was determined by the VOS method (Eriksson, 2007) and estimation of metabolizable

energy content was done from these values. Content of WSC was determined by an enzymatic-spectrophotometric method (Larsson and Bengtsson, 1983).

Aerobic stability

The silos from the first harvest were opened 109 days after the ensiling period started for the last silos, and the silos from the second harvest were opened 97 days after the ensiling period started for the last silos. After sampling, aerobic stability was measured by rise in temperature when the silage was exposed to air. The silage was taken out of the silo with a metal hook and placed in plastic bags, and was thoroughly mixed to ensure homogeneity. The hook was sterilized by flaming with ethanol (99%) between every silo to prevent contamination between the samples. A defined amount of silage (DLG filling weight list, 2013) was loosely filled in polyvinyl chloride pipes (PVC-pipes) of 1320 ml volume which had a piece of geo textile (a material which air can easily pass) attached to the bottom with a rubber band. The PVC-pipes were sterilized by ethanol (70%) and the geo textile was autoclaved for 20 minutes in 121 ˚C and 10 minutes drying time. The PVC-pipes were placed in a block of Styrofoam with holes and thermocouples were placed in the middle of the silage in the middle of the pipe. The temperature was recorded by a computer every second hour. The measurements lasted twelve days for the samples from the first harvest and ten days for samples from the second harvest. The pipes were placed in a climate controlled room (20˚C and relative air humidity 80 %) to prevent the silage from drying out. The silos were covered with Styrofoam with small holes in it to let air through the samples.

Statistical analysis

Results from the questionnaire were analysed in SAS (SAS 9.3, SAS Institute Inc., Cary, N.C., USA). Responses that were similar from all farms were excluded from the analysis. Pearson correlation coefficients were calculated between chemical variables in fresh crop and silage, yeast counts in fresh crop and silage, ensiling losses, aerobic stability and temperature increase during aerobic phase post opening as well as correlations between these variables and the management factors listed in Appendix 1, using the procedure CORR in SAS. The effect of harvest number and DM content on aerobic stability was tested using variance analysis and procedure General Linear Models (GLM) in SAS, and calculating least square means (LSM). Means were considered statistically different if $P<0.05$, and if P was <0.1 but >0.05, trends to differences were accepted.

Table 1. Farm number, sampling date, number of harvest, type of conservation and use of additives at the participating farms in the study

Farm number and location	**Date for sampling**	**Number of harvest**	**Type of conservation**	**Use of additives?**
1 Grillby	May 28	1st	Bunker silo	Not in the sample, but the farm used lactic acid bacteria
2 Töreboda	May 28	1st	Bunker silo	Not in the sample but the farm used Promyr™ [1]
4 Mariestad	June 1	1st	Bunker silo	No
5 Bollnäs	June 6	1st	Bunker silo	No
6 Bollnäs	June 6	1st	Bunker silo	Not in the sample but the farm used Safesil [2]
7 Bollnäs	June 6	1st	Bagged silo	No
8 Töreboda	June 9	1st	Bunker silo	No
9 Knutby	July 15	2nd	Bagged silo	No
10 Töreboda	July 16	2nd	Bunker silo	Yes, lactic acid bacteria
11 Töreboda	July 16	2nd	Bunker silo	Not in the sample but the farm used Promyr™ [1]
12 Lugnås	July 17	2nd	Bunker silo	No
13 Hova	July 17	2nd	Heap	Yes, lactic acid bacteria
14 Uppsala	July 17	2nd	Bunker silo	Yes, lactic acid bacteria
15 Bälinge	July 19	2nd	Bunker silo	Yes, lactic acid bacteria

1) Silage additive, based on propionic and formic acids and salts thereof.
2) Silage additive, based on sodium benzoate, potassium sorbate and sodium nitrite.

Results

Silage management – results from the questionnaire

All farms used a mixture of perennial grasses and red and white clover. In total, 13 out of 15 fields were fertilized with liquid manure (six farms), commercial fertilizer (four farms) or a combination of them (three farms). The amount of liquid manure spread ranged from 20 to 30 tonnes per hectare. The fields were fertilized before each harvest with one exception as one farm fertilized the ley only before the first harvest. The age of the swards varied between one and four years. None of the farms used herbicides.

The weather was sunny at mowing at ten farms, while three farms had cloudy weather and two farms got rain. The weather during wilting was sunny at eight farms, cloudy or sunny/cloudy at four farms and rainy or rainy and cloudy weather at two farms. The wilting time was mostly one day, except from one farm which wilted the crop for three days because of rainy weather conditions. All farms used mower conditioners. Eleven practised pre-wilting in swaths and four practiced wilting of wide spread crop after the mower. The working width of the mowers varied between 3.7 meters and 12 meters. Swath width varied from 1.2 to 2.5 meters. Stubble height was 7 to 10 cm.

Eight of the farms used a self-propelled forage harvester to chop the fresh crop, and six farms had a chopper mounted on the forage wagon. Two farms used self-loading wagons where the crop was cut at loading. The chop length of the crop was between three and 15 cm. Most of the farms stored their silage in bunker silos (twelve farms), one farm had a heap silo and three of the farms used bag silos. One of the farms had a little amount of rain during silo filling. In the bunker and heap silos the weight of the tractor packing the crop varied between 5.5 and 12.5 tonnes among the farms, but in some silos there were two tractors and the total weight was then up to 25 tonnes.

All farms used white silage film and eight farms used silage film together with micro foil. Farms that covered the silo with micro foil and silage film had three layers in total, farms that only had the silage film had two layers mostly, and one farm had three layers with silage film. Farms with bagged silo had only one film layer. One of the farms protected the bag with gravel on the endings, and another farm put out plastic snakes to protect the silo from bird attacks. Green nets and bags with sand or tires were the most frequently used covering technique on bunker silos. Two farms used sand and tires, one farm covered the film with wood chips and one farm covered the silo with straw and sand. Removal of the silage was done by bucket or block cutter.

Chemical composition and yeast counts in fresh crop

The fresh crop was analysed for DM, ash, IVDOM, CP and WSC. Results are presented in Table 2.

Table 2. Content of dry matter, ash, water soluble carbohydrates (WSC), in-vitro digestibility of organic matter (IVDOM), crude protein (CP) and number of microbial colonies in fresh crop from 15 farms. The first eight farms were sampled during the first harvest and farm 9-15 during the second harvest

Farm	DM (%)	Ash (g/kg DM)	WSC (g/kg DM)	IVDOM (g/kg DM)	CP (g/kg DM)	Number of microbial colonies (log cfu/g)
1	25.0	103	83	873	187	4.6
2	25.5	87	109	886	191	4.7
3	23.7	75	109	885	121	5.6
4	33.2	95	108	866	155	5.6
5	22.2	78	81	878	151	5.9
6	23.3	72	70	892	156	5.9
7	33.7	57	149	890	154	5.5
8	24.2	86	68	824	121	5.0
9	40.2	96	75	877	190	5.1
10	21.5	94	57	801	158	5.4
11	18.8	95	35	862	190	5.6
12	52.8	62	108	732	110	5.8
13	35.9	83	102	832	132	5.6
14	30.1	98	106	834	131	5.6
15	28.1	101	107	848	141	5.0

The number of colonies (yeast, mould and bacteria) in the fresh crop ranged from log 4.6 to log 5.9 cfu/g (Table 2). At the time of counting, all visible colonies were counted but when a closer microscopy examination was performed, most colonies from the fresh crop were moulds and bacteria. Penicillin and streptomycin added to the substrate did not prevent the growth of moulds and bacteria effectively when samples from fresh crop were cultivated. The use of chloramphenicol in the substrate used for cultivation of samples from the second harvest had no effect on the growth of moulds and bacteria. Yeast colonies on the plates were white, light yellow or pink.

The fresh crop from the two harvests contained 16 different species of yeast when using the BLAST ®-programme with an identity match at > 99%. Figure 1 shows the number of farms where the different yeast species were found. *Rhodosporidium babjeave* and *Rhodotorula glutenis* were most common on farms in this study. They were represented at six and five farms respectively.

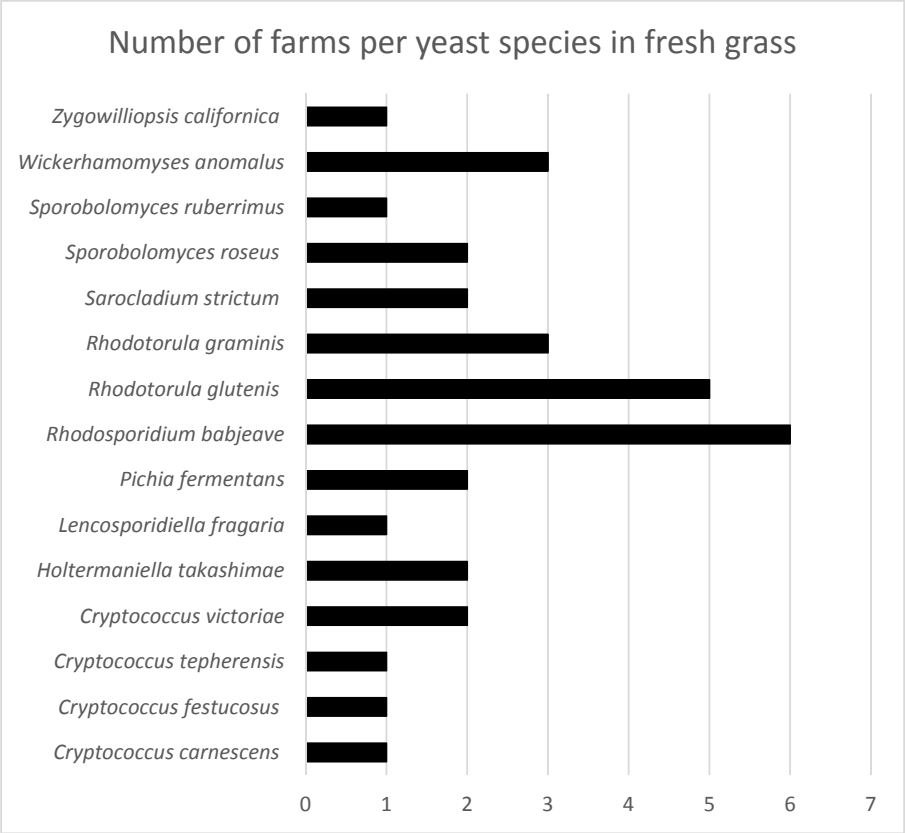

Figure 1. Number of farms per yeast species present in fresh crop from 1st and 2nd harvest at a total of 15 farms.

Silage losses

The greatest weight loss occurred during the first week of the ensiling period, and was 1.1%-3.6% of initial DM. The silos continued to lose weight during the whole period. Mean values and standard deviations of the weight loss in per cent of initial DM for the first harvest is presented in Table 3, and for the second harvest in Table 4.

Table 3. Mean values and standard deviations (s.d.) of weight loss (% of initial DM) in silos from the first harvest (N=8)

Weighing day	**0**	**3**	**7**	**14**	**28**	**56**	**78**	**90**	**108±12**
Weight loss, % of initial dry matter	0.0	1.7	2.3	2.6	3.1	3.9	4.2	4.3	4.6
s.d. (%)	0.00	0.65	0.95	1.01	1.06	1.62	1.76	1.82	1.96

Table 4: Mean values and standard deviations (s.d.) of the weight loss (% of initial DM) in silos for the second harvest (N=7)

Weighing day	**0**	**3**	**7**	**14**	**28**	**56**	**78**	**90**	**99±2**
Weight loss, % on initial dry matter	0.0	1.3	1.8	2.0	2.2	2.6	2.9	2.9	3.1
s.d. (%)	0.00	0.43	0.54	0.60	0.75	1.10	1.38	1.50	1.58

Chemical composition and yeast counts in silage

Silage was analysed for content of DM, ash, WSC and for yeast counts. Results are reported in Table 5. The yeast content in the first harvest ranged from below lower detection limit (log 1.7) to log 3.2 cfu/g. In the second harvest, yeast growth was present from only one farm and was log 3.7 cfu/g. In contrast to the fresh crop, where growth of yeast, mould and bacteria was present, the growth on plates from silage was almost only yeast, even though the plates had the same concentration of penicillin and streptomycin. Cultivation of yeast from silage samples did not result in any pink yeast colonies, all were white or light yellow.

A selection of investigated correlations between chemical variables and yeast count and aerobic stability are presented in Table 6. Dry matter loss during ensiling was negatively correlated with DM content and WSC content in silage. However, DM content was not correlated with rise in temperature or length of aerobic stability during the aerobic phase post opening. Increased ash content was correlated to higher yeast counts in the fresh crop, higher temperature increase and shorter aerobic stability during the aerobic phase after silo opening. There was however a negative correlation between yeast counts in the fresh crop and in the silage ($R=-0.61$; $P<0.016$).

Silage samples where temperature did not rise with at least 2˚C during ten days of exposure to air after opening of the silos were considered stable. During the aerobic storage, six of 38 silos rose in temperature with at least 2˚C above ambient temperature. The maximum and minimum temperature in the silos was 48.6 and 23.8˚C respectively. The maximum number of days until maximum temperature was 9.8 days and minimum number of days was 0.52. The mean values of time (h) until temperature reached +2˚C above the ambient temperature during the aerobic stability test is reported in Table 5. The mean value of the maximum temperature rise for the silos was +2.7˚C.

Table 5. Content of dry matter, ash and water soluble carbohydrates, weight loss during ca 90 days of ensiling, aerobic stability and yeast counts in silage from air tight silos containing silage from first and second harvest from a total of 15 farms

Farm number	DM (%)	Ash (g/kg DM)	WSC (g/kg DM)	Weight loss day 90 (% of DM)	Time (h) until temperature +2˚C above ambient temperature	Number of yeast (log cfu/g)
1	23.2	110	0.3	4.7	213.4	3.2
2	24.5	93	1	5.8	181.6	3.0
3	22.9	81	4.3	6.0	>288	<1.7
4	31.9	98	13.7	2.8	>288	2.05
5	22.5	80	1.3	3.4	>288	<1.7
6	22.4	77	0	7.1	>288	1.7
7	31.3	61	18.8	2.2	>288	2.4
8	23.4	88	0	2.6	>288	<1.7
9	39.3	100	5	2.7	>240	<1.7
10	22.0	97	0.8	6.2	>240	<1.7
11	18.1	99	1.8	3.1	205.9	<1.7
12	53.4	69	43.9	1.6	>240	<1.7
13	35.5	88	18.2	2.5	>240	<1.7
14	31.2	105	26.6	2.3	>240	<1.7
15	25.8	112	17.9	2.1	87.9	3.7

Table 6. Pearson correlation coefficients between chemical variables in the silages and yeast count in fresh crop; ensiling losses; silage yeast count; aerobic stability (time (h) until temperature +2˚C above ambient temperature); and temperature increase during the aerobic phase post opening

Chemical variables in silage	Yeast in fresh crop	Ensiling losses	Yeast count in silage	Aerobic stability	Temperature increase during aerobic storage
DM	0.20 NS	-0.57 P<0.03	0.20 NS	0.12 NS	-0.33 NS
WSC in DM	0.33 NS	-0.65 P<0.009	-0.07 NS	-0.14 NS	-0.10 NS
Ash in DM	0.55 P<0.0347	-0.02 NS	0.29 NS	-0.56 P<0.03	0.49 P<0.06

High yeast counts in silage after opening of silos was moderately correlated to temperature increase and shorter aerobic stability. However, high yeast counts in the fresh crop did not result in shorter aerobic stability or in a higher temperature increase after opening of the silo. Instead, the aerobic stability tended to be longer when yeast counts was high in the fresh crop (Table 7).

Table 7. Pearson correlation coefficients between yeast counts in fresh crop or yeast counts in silage, and ensiling losses; aerobic stability (time (h) until temperature +2˚C above ambient temperature); and temperature increase during the aerobic phase

	Ensiling losses	**Aerobic stability**	**Temperature increase**
Yeast in fresh crop	-0.07	0.47	-0.32
	NS	P<0.07	NS
Yeast in silage	0.08	-0.61	0.43
	NS	P<0.02	P<0.11

Effect of ventilated silos during ensiling

For samples from the second harvest, four replicates from each farm were divided in two air-tight and two ventilated silos. Weight loss during ensiling, WSC content, yeast counts and aerobic stability for air-tight and ventilated silos are presented in Table 8. Silage from the ventilated silos had higher yeast counts and much shorter aerobic stability than silage from air-tight silos. In ventilated silos, silage that deteriorated first had a temperature rise of 2˚C above the ambient temperature after 12.5 h, and the last silo that deteriorated before the test was ended did it after 234.1 hours. Among the air-tight silos, there were two silos where the silage deteriorated; after 87 and 205 h.

During ensiling, air-tight silos had a mean weight loss of 3.1% of DM and ventilated silos had a mean weight loss of 3.4% (Figure 2a). During aerobic storage, silage from air-tight silos had a mean weight loss of 15.3% and silage from ventilated silos had a mean weight loss of 28.9% (Figure 2b). During the ensiling period, WSC content decreased with 81.0% for both air-tight and ventilated silos compared to initial content in fresh crop (s.d. 12.59 % in air tight: s.d. 13.97 % in ventilated silo). During aerobic storage, WSC content decreased with 32.6% (s.d. 33.11%) in air-tight and 51.6% (s.d. 36.44%) in ventilated silos compared to WSC content at opening of the silo. On average, the temperature rose with 2.7˚C in air-tight silos and with 13.5 ˚C in the ventilated silos. The differences in WSC content, weight and temperature are reported in Table 9.

Table 8. Water soluble carbohydrate content, weight loss, number of hours until silage reached temperature 2 ˚C above ambient temperature during aerobic storage, and the number of yeasts in silage from air-tight and ventilated silos ensiled for 90 days

	WSC, % of DM in silage		**Weight loss day 90, % of initial DM**		**Yeast count in silage, log cfu/g**		**Time to 2°C temperature rise, h**	
Farm number	**Air tight**	**Ventilated**	**Air tight**	**Ventilated**	**Air tight**	**Ventilated**	**Air tight**	**Ventilated**
9	0.5	0.5	2.7	3.1	<1.7	5.9	>240	63
10	0.1	0.1	6.2	4.6	<1.7	2.0	>240	234
11	0.2	0.0	3.1	3.9	<1.7	3.2	205	98
12	4.4	4.3	1.6	2.2	<1.7	5.9	>240	226
13	1.8	1.9	2.5	2.9	<1.7	5.9	>240	36
14	2.7	3.4	2.3	2.7	<1.7	5.6	>240	64
15	1.8	1.2	2.1	3.4	3.7	5.6	87	12

Table 9. Changes in content of water soluble carbohydrates and in temperature during ensiling and aerobic storage stability test

	Changes during:			
	Ensiling		**Aerobic storage**	
	Air tight silo	**Ventilated silo**	**Air tight silo**	**Ventilated silo**
WSC loss in % of initial content in fresh crop and in silage	81.0%	81.0%	32.6%	51.6%
Temperature, ˚C *)	n.d.	n.d.	+2.7 ˚C	+13.5 ˚C

*) Compared to ambient temperature 20.2 ˚C; n.d, not determined

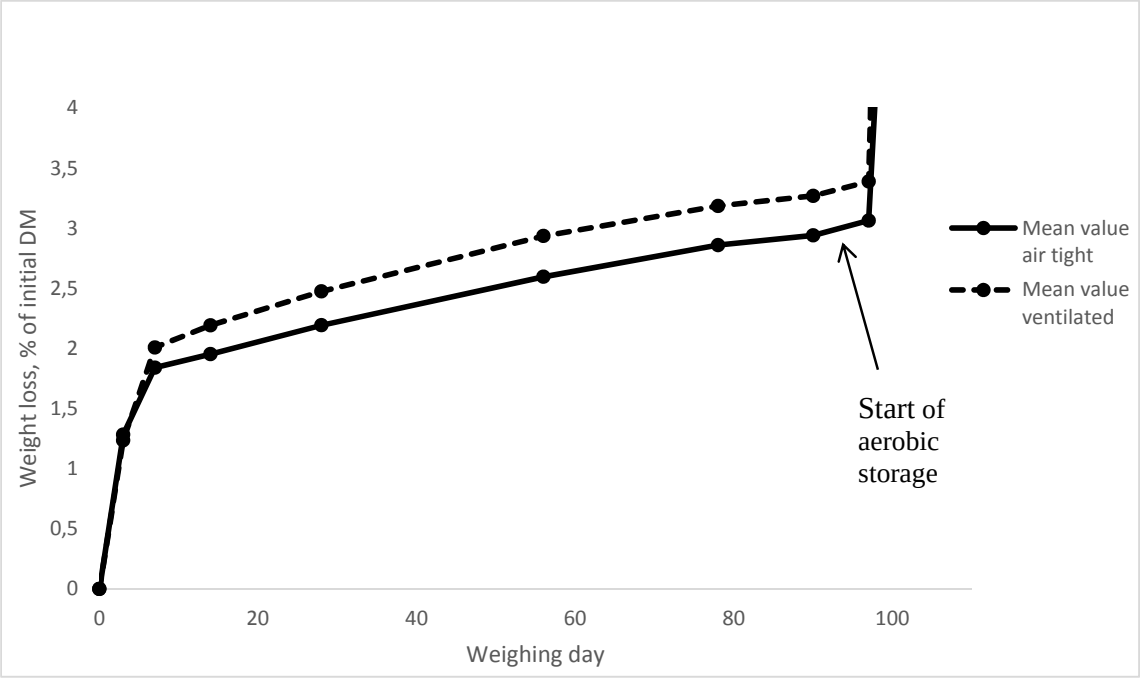

Figure 2a. Comparison of weight loss between air-tight and ventilated silos during ensiling, loss in percentage of initial dry matter weight.

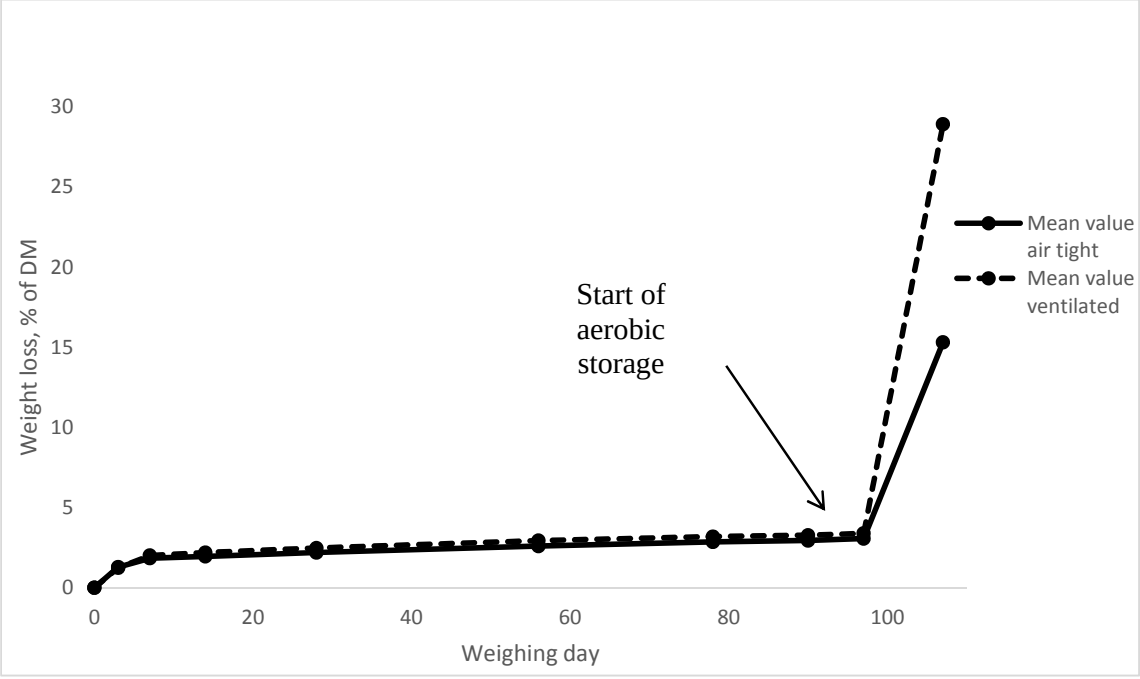

Figure 2b. Comparison of weight loss between air-tight and ventilated silos during aerobic storage stability test, loss in percentage of dry matter at silo opening.

Influence of chemical composition, yeast counts and silage management on silage losses and aerobic stability

There was no correlation between yeast counts in fresh crop and yeast counts in silage ($r=0.11$; $P<0.02$).

The statistical analysis showed that few of the management variables were correlated with the outcome in terms of ensiling loss and aerobic stability. Use of manure as fertilizer tended to result in a higher yeast count in the fresh crop ($P<0.080$).

Harvest number (1st or 2nd harvest) and DM content tended to influence aerobic storage stability when tested with the GLM method using harvest, DM content and heated silage last year (yes or no) as factors in the model. Silages from the first harvest were stable (not exceeding 2˚C above ambient temperature) for 11.3 days while silages from the second harvest were stable for 8.6 days ($P<0.06$). The temperature of the silages from the first harvest only rose on average 0.14 degrees while the temperature of the silages from the second harvest rose on average 3.3˚C during the aerobic phase after opening the silos ($P<0.03$).

Discussion

Silage management

Some of the responses in the survey were identical or nearly identical among farms, and these variables were therefore excluded from the statistical analysis. These variables were use of herbicides, botanical composition, remaining parts of plants from year before, soil contamination, use of the ley last year, thickness of silage film and complete cover of silage film. This means that these variables might have an impact on the ensiling result compared to other ways of doing it, but it could not be demonstrated in the present study.

Chemical composition and yeast counts in fresh crop

Content of DM and ash in the fresh crop from the first harvest were lower compared to in the second harvest, while content of WSC, IVOMD and CP were higher in the first compared to in the second harvest. This means that it was not possible to clearly separate the influence of DM content or harvest number on aerobic storage stability.

Stolt (2013) found a tendency that fresh crop from second harvest contained more yeast compared to fresh crop from the first harvest. Yeast count on crops is affected by different factors e.g. temperature and water availability. Higher air temperature favours growth of yeast and low water availability inhibits yeast growth (Pahlow *et al.*, 2003). In the present study, there was no difference between yeast counts from first and second harvest. This could be due to lower temperatures and DM contents in the fresh crop during the first harvest compared to the second harvest, where the temperatures and DM content in the fresh grass were higher.

The microbial count from the fresh crop was a mix of yeast, moulds and bacteria which made it hard to know the true yeast number. Prior to the inoculation to YPD the colonies were examined in microscope to ensure it was yeast colonies. Sixteen colonies from the first harvest and 40 colonies from the second harvest were inoculated in YPD. This can be an indication of a more realistic number of yeast cfu, and if so, the number of yeast from the second harvest was higher than from the first harvest and in line with previous findings (Kroulik *et al.*, 1955; Lin *et al.*, 1992). In the present study, yeast count in fresh crop was negatively correlated with yeast count in silage, indicating the difficulties of judging ensilability of a fresh crop from its yeast count.

Yeast was cultivated on MEA-plates that contained penicillin and streptomycin. Maybe these antibiotics should be replaced by something more effective against bacteria and moulds.

Before the cultivation of yeast from the second harvest silages, chloramphenicol was used in the growth substrate but without any difference in number of bacteria and moulds compared to growth on plates that contained streptomycin and penicillin. The method of yeast cultivation used in this study was described by Jonsson and Pahlow (1984) for use on silage, but is also frequently used for fresh crops. However, it can be suggested from this study that it is not suitable for yeast counts in fresh crop. Also Stolt (2013) noticed significant growth of bacteria and mould when the method was used for fresh crops.

The yeast isolates from the fresh crop were run in the programme BLAST ® from NCBI's database. The total number of yeast species in the fresh crop was 75, but the number of isolates was 56. The reason why the number of species did not correspond to the number of isolates was difficulties with the BLAST ®-programme. The programme gives identity matches, if the DNA sequence of an isolate matches a previous reported DNA sequence with over 99%, it is reported as the same species. The programme however identified some sequences as more than one species and it was hard to know which species was the correct one. Often the programme had difficulties to separate *Rhodosporidium babjeave, Rhodotorula graminis* and *Rhodotorula glutinis* from each other. Other methods are required to identify which species occurred in the fresh grass and therefore the number of yeast species reported is a bit misleading.

In the present study the following yeast genera made up the main part of the yeast flora in the fresh crop; *Rhodotorula, Rhodosporidium, Cryptococcus, Sporobolomyces* and *Wickerhamomyces.* Jonsson and Pahlow (1984) found the genera *Cryptococcus, Rhodotorula, Sporobolomyces, Aureobasidium* and *Torulopsis w*hich partly correspond to the findings in this thesis. Middelhoven and van Baalen (1988) investigated yeast species in fresh chopped maize and found genera of *Candida, Cryptococcus, Sporobolomyces, Sporidiobolus* and *Rhodotorula,* which were gone after two days of anaerobic ensiling.

Silage losses in laboratory silos

In the present study the greatest weight loss occurred during the first week of ensiling with losses of 1.1-3.6 % of initial DM weight. During this time LAB in the silage starts to produce lactic acid which lowers the pH, but yeasts are still able to compete for substrates (McDonald *et al.*, 1991). The rapid weight loss during the first week shows that the anaerobic environment established during the first week is important to keep the deteriorative microorganisms at a low level (McDonald *et al.*, 1991).

Chemical composition and yeast count in silage

The DM content after ensiling was the same as in the fresh crop. Content of WSC in silage decreased with 81% of initial WSC content in the fresh crop. This result is in line with the findings of Jonsson and Pahlow (1984), who found that nearly 80 % of the total water soluble carbohydrates were metabolized during the first week of ensiling (Jonsson and Pahlow, 1984). Also Pettersson (1988) found that the WSC content was reduced during the initial phase of ensiling, as more than 50% of the WSC were lost after 24 hours of ensiling (Pettersson, 1988).

In the present study, number of yeasts decreased in silage for both harvests compared to the fresh crops. In the second harvest, only one farm had silage that showed yeast growth. Reduction of yeast counts during ensiling has been reported previously; yeast counts at day 91 were below log 2 cfu/g compared to an initial value of log 4.7 cfu/g (Wu-tai *et al.*, 2002).

Yeast species that are aerobic are not likely to survive the ensiling process. There were some yeast colonies from the fresh crop that were pink coloured, but these were not present after ensiling. Pink yeast species found in this study were *Rhodotorula graminis, R. glutinis* and *Sporobolomyces roseus.* An ocular examination of the agar plates where silage samples were

inoculated showed no pink colonies; all colonies had a shade of white. These pink species are strictly aerobic species and are not able to survive the ensiling process (Visser *et al.*, 1990; Kurtzman *et al.*, 2011). This correspond to the results from Middelhoven and van Baalen (1988) who found that strains of *Candida, Cryptococcus, Sporobolomyces, Sporidiobolus* and *Rhodotorula* had vanished after two days of ensiling. Also, Jonsson and Pahlow (1984) found that aerobic *Cryptococcus, Rhodotorula, Sporobolomyces, Aureobasidium* and *Torulopsis* had vanished after three days of ensiling in lab scale silos (Jonsson and Pahlow, 1984; Middelhoven and van Baalen, 1988).

High yeast counts in silage makes the silage more prone to impaired aerobic stability (Daniel *et al.*, 1970 cited by McDonald *et al.*, 1991; Jonsson and Pahlow, 1984). Since some of the species in the fresh crop are aerobic and cannot survive in anaerobic environments, maybe this number is not so interesting. The analysis should instead focus on the species of yeasts that are able to survive with low levels of oxygen, and if they affect the aerobic stability of silages. Species which can survive low levels of oxygen, for example *Candida lambica*, are at a competitive advantage when they get access to oxygen as they can then grow very rapidly (Jonsson and Pahlow, 1984). This was shown in the ventilated silos in the present study, where weight loss was higher, yeast counts higher and aerobic stability shorter compared to the air-tight silos.

Ash content was positively correlated to total microbial counts in the fresh crop. Soil residues in harvested crops enhance the risk of microbial contamination of the crop (Gismervik *et al.*, 2015). Elevated ash contents in fresh crop could be a result of soil contamination during harvest and filling of the silo (Hoffman, 2005). Hence, higher soil contamination leads to higher risk of increased microbial count in the silage. High yeast counts in silage were negatively correlated to aerobic stability after the ensiling period which means that high number of yeast after ensiling lead to a decreased aerobic stability. In the present study, no identification of yeast species after ensiling was performed. Anyhow, high yeast counts and decreased aerobic stability is in agreement with results from previous studies by Middelhoven and van Baalen (1988), who found that yeast counts had an exponential increase during aerobic conditions. They found that it was mainly the genera *Candida* which was responsible for the aerobic deterioration (Middelhoven and van Baalen, 1988).

At opening of the silos from the first harvest, many of them smelled of butyric acid and two of the silos contained mould growth after the ensiling. According to the results of Moon (1983) and Weinberg (1993), silages that contain higher levels of VFA are not likely to deteriorate in aerobic environments. Ohyama *et al.* (1975) reported that if silages contain VFAs higher than C_4 and/or butyric acid, the silage may not deteriorate. This is because VFA possess anti-mycotic activity and will inhibit yeast and moulds during aerobic storage of silage (Moon, 1983; Canibe *et al.*, 2014). Silages that contain butyric acid are however not of good fermentative quality and are not desirable (Ohyama *et al.*, 1975).Therefore it had been interesting to analyse VFA in the silages after the ensiling. Maybe even higher VFA could be used as silage additives?
Six out of 38 air-tight silos had an increase in temperature to 2˚C above ambient temperature during the aerobic stability test. The first silo deteriorated after 83.6 h, and the last after 217.8 h, nearly four and nine days respectively. The silo that deteriorated after approximately four days had a yeast count of 3.7 cfu/g, while all the other silos had a yeast count of < 1.7 cfu/g, and this could have had an impact on the aerobic stability.

Yeast counts in the silage from the first harvest were below log 5 cfu/g and this silage showed no rise in temperature during the aerobic stability test. This correspond to findings from Daniel *et al.* (1970) and Jonsson and Pahlow (1984), who showed that silages that contained yeast counts above log 5 cfu/g were more prone to deteriorate when exposed to air than those with lower yeast counts.

The effect of ventilated silos during ensiling

During ensiling of the second harvest crop, two silos from each farm were ventilated once a week for two hours during the whole ensiling period to mimic the environment in many farm silos, which are difficult to keep absolutely air tight. Visible growth of yeast was present in the laboratory silos, particularly around the holes in the silo wall and -lid. However, this supply of oxygen was probably not enough to make the aerobic yeast species *Rhodotorula graminis, R. glutinis* and *Sporobolomyces roseus* survive, since they were not visible on the plates after cultivation.

Silage from ventilated silos contained higher yeast counts compared to silage from air-tight silos. After ensiling, yeast growth was present in samples from only one farm and from a silo that had been air-tight. In ventilated silos yeast growth was present in all silos. This shows that air inlet in silos contributed to growth of yeast in silage.

Weight loss during ensiling was on average 3.1 % of initial DM weight in the air-tight silos and 3.4 % in the ventilated, which was not a large difference. When the silage was stored aerobically, silage from air-tight silos had a weight loss of 15.3% while silage from ventilated silos had a weight loss of 28.9%. This shows that an anaerobic environment during ensiling is very important to prevent large losses during the aerobic phase at feed-out.

On average, WSC content during the ensiling period was reduced by 81.0% for both air-tight and ventilated silos. This corresponds to the findings of Jonsson and Pahlow (1984) who found that nearly 80% of the available WSC were metabolized during the first week in anaerobic environment, and if the silos were supplied with air, another 5-10% of the WSC were metabolized. In the present thesis one cannot say when the decrease occurred, but the main part of it probably occurred in the beginning of the ensiling period because the weight decreased the most at the beginning. After the aerobic storage, the silage that have been stored anaerobically during the ensiling period had a higher amount of WSC compared to the silages that have been ventilated during the ensiling, which correspond to results from Jonsson and Pahlow (1984).

The aerobic stability test showed that silage temperature rose 2˚C above the ambient temperature in more than half of all silos from the second harvest. All ventilated silos except one reached temperatures of over two degrees above the ambient temperature. The silos with the higher yeast count had shorter aerobic stability compared to the silos with yeast counts below 1.7 cfu/g. Yeast continues to develop if oxygen infiltrates the silo (Henderson, 1993). This shows that silos should be as air-tight as possible to prohibit yeast growth and enhance the aerobic stability of the silage.

Influence of chemical composition, yeast counts and silage management on silage losses and aerobic stability

Dry matter contents in the silages from the first and second harvest were on average 25.2 % and 32.2 % respectively. At the aerobic stability test, the second harvest silage had a shorter time until deterioration and the temperatures were higher. This is in agreement with findings of Crawshaw and Woolford (1979) and McDonald *et al.* (1991), who found that silages with DM contents between 300-500g DM/kg had a greater temperature escalation during aerobic storage compared to silages with lower DM contents. This was explained by the lower energy requirement to rise the temperature in materials containing less water.

The harvest number had an impact on aerobic stability of the silage, as silage from the first harvest was stable for 11.3 days and silage from the second harvest was stable for 8.6 days. As previously mentioned, yeasts are known to contribute to decreased aerobic stability. Crops that has been wilted for more than two days has higher risk of an elevated population of yeast. One of the factors that could favour growth of yeast is dead plant material from the previous harvest. This could be part of the explanation to shorter aerobic stability from the second harvest in the present study. The temperature for the silage from the first harvest rose 0.14 ˚C and from the second harvest 3.3 ˚C during the ten days of aerobic storage.

Conclusions

In conclusion, there were no differences between the harvests regarding yeast counts in the fresh crop. However, the method used for cultivating yeast from the fresh crop was not reliable due to growth of many other microorganisms. The most frequently occurring genera of yeast in the fresh crop were *Rhodosporidium* and *Rhodotorula*. Yeast counts were reduced by ensiling and therefore lower in silage than in fresh crops. Silage from the first harvest had higher yeast counts compared to silage from the second harvest. High yeast counts in silage at opening of silos were correlated to a shorter aerobic stability. Silage in ventilated silos contained higher yeast counts compared to silage in air-tight silos. Ventilated silos also lost more weight during ensiling compared to the air-tight silos, and had shorter aerobic storage stability.

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References

Altschul, S. F., Gish, W., Miller, W., Myers, E. W., Lipman, D. J. (1990). Basic local alignment search tool. *Journal of Molecular Biology*, 215(3), pp 403–410.

Blomqvist, J. (2006). Yeast and lactic acid bacteria populations in an industrial ethanol fermentation process. *Master thesis* (2006:7) Swedish University of Agricultural Sciences.

Buxton, D. R., O'Kiely, P. (2003). Preharvest plant factors affecting ensiling. In: Buxton, D. R., Muck, R. E., Harrison, J. H. (Eds) *Silage science and technology*. pp 199–250. American Society of Agronomy Inc, Madison, Wisconsin, USA.

Buyze, G., Van den Hamer, C. J. A., De Haan, P. G. (1957). Correlation between hexose-monophosphate shunt, glycolytic system and fermentation-type in lactobacilli. *Antonie van Leeuwenhoek*, 23, pp 345–350.

Canibe, N., Kristensen, N. B., Jensen, B. B., Vils, E. (2014). Impact of silage additives on aerobic stability and characteristics of high-moisture maize during exposure to air, and on fermented liquid feed. *Journal of Applied Microbiology*, 116(4), pp 747–760.

Cassio, F., Leao, C., van Uden, N. (1987). Transport of lactate and other short-chain monocarboxylates in the yeast Saccharomyces cerevisiae. *Applied and Environmental Microbiology*, 53(3), pp 509–513.

Conaghan, P., O'Kiely, P., O'Mara, F. P. (2012). Possibilities of increasing the residual water-soluble carbohydrate concentration and aerobic stability of low dry-matter perennial ryegrass silage through additive and cultivar use. *Grass and Forage Science*, 67(2), pp 177–198.

Crawshaw, R., Woolford, M. K. (1979). Aerobic deterioration of silage in and out of the silo. 34, pp 167–178.

Daniel, P., Honig, H., Weise, F., Zimmer, E. (1970). Wirkung von propionsaure bei der Grunfuttersilierung. *Wirtschaftseigene Futter*, 16, pp 239–252. Cited in: McDonald, P., Henderson, N., Heron, S. (1991). *The biochemistry of silage*. Marlow, UK: Chalombe publications.

Gismervik, K., Randby, Å. T., Rørvik, L. M., Bruheim, T., Andersen, A., Hernandez, M., Skaar, I. (2015). Effect of invasive slug populations (Arion vulgaris) on grass silage. II: Microbiological quality and feed safety. *Animal Feed Science and Technology*, 199, pp 20–28.

Guo, G., Yu, C., Wang, Q., Xin, P., Shimojo, M., Shao, T. (2014). Silage Fermentation Characteristics of Italian Ryegrass (Lam.) Harvested at Various Times on a Sunny Day. *Crop Science*, 54(2), pp 851–858.

Henderson, A. R., McDonald, P., Woolford, M. K. (1972). Chemical changes and losses during the ensilage of wilted grass treated with formic acid. *Journal of the Science of Food and Agriculture*, 23(9), pp 1079–1087.

Henderson, N. (1993). Silage additives. *Animal Feed Science and Technology*, 45, pp 35–56.

Hernández-Almanza, A., Cesar Montanez, J., Aguilar-González, M. A., Martínez-Ávila, C., Rodríguez-Herrera, R., Aguilar, C. N. (2014). Rhodotorula glutinis as source of pigments and metabolites for food industry. *Food Bioscience*, 5, pp 64–72.

Honig, H. (1991). Reducing losses during storage and unloading of silage. In: Pahlow, G., Honig, H. (Eds) *Proceedings of European grassland federarion*, Braunschweig, 1991. pp 116–128. Braunschweig.

Jonsson, A., Pahlow, G. (1984). Systematic classification and biochemical characterization of yeasts growing in grass silage inoculated with Lactobacillus cultures. *Animal research and development*, 20, pp 7–22.

Kroulik, J. T., Burkey, L. A., Wiseman, H. G. (1955). The microbial populations of the green plant and of the cut forage prior to ensiling. *Journal of Dairy Science*, 38, pp 256–262.

Larsson, K., Bengtsson, S. (1983). Bestämning av lättillgängliga kolhydrater i växtmaterial. *SLL metod 22*.

Lin, C., Bolsen, K. K., Brent, B. E., Hart, R. A., Dickerson, J. T. (1992). Epiphytic Microflora an Alfalfa and Whole-Plant Corn. *Journal of Dairy Science*, 75(9), pp 2484–2493.

Liu, Q. h., Yang, F. y., Zhang, J. g., Shao, T. (2014). Characteristics of Lactobacillus parafarraginis ZH1 and its role in improving the aerobic stability of silages. *Journal of Applied Microbiology*, 117(2), pp 405–416.

McDonald, P., Henderson, N., Heron, S. (1991). *The biochemistry of silage*. Marlow, UK: Chalombe publications.

McGechan, M. B. (1990). Operational Reasearch study of forage conservation systems for cool, humid upland climates. Part 2: Comparison of hay and silage systems. *Journal of engineering research*, (46), pp 129–145.

Middelhoven, W. J., van Baalen, A. H. M. (1988). Development of the yeast flora of whole-crop maize during ensiling and during subsequent aerobiosis. *Journal of the Science of Food and Agriculture*, 42(3), pp 199–207.

Middelhoven, W. J., Ilona M. de Jong, Marleen de Winter (1990). Yeasts and fungi occurring in ensiled whole-crop maize and other ensiled vegetable crops. *Antonie van Leeuwenhoek*, 57, pp 153–158.

Moon, N. J. (1983). Inhibition of the growth of acid tolerant yeasts by acetate, lactate and propionate and their synergistic mixtures. *Journal of Applied Bacteriology*, 55(3), pp 453–460.

Müller, C. E. (2009). Influence of harvest date of primary growth on microbial flora of grass herbages and haylage, and on fermentation and aerobic stability of haylage conserved in laboratory silos. *Grass and Forage Science*, 64(3), pp 328–338.

Niemelä, S. (1983). Statistical evaluation of results from quantitative microbiological examinations. *Nordisk Metodik-Kommitté för Livsmedel (NMKL)*, 1.

Nordic Committee on Food Analysis (1976). Nitrogen. *Determination in food and feed according to Kjeldahl*, 6 (Third Edition).

O'Kiely, P. (1993). Influence of a partially neutralised blend of aliphatic organic acids on fermentation, effluent production and aerobic stability of autumn grass silage. *Irish Journal of Agricultural and Food Research*, 32, pp 13–26. Cited in: Cherney, J. H. and Cherney, D. J. R. (2003). Assessing silage quality. In: D.R. Buxton, Muck, R. E., and Harrison, J. H. (Eds) *Silage science and technology*. pp 141–198. Amercian Society of Agronomy Inc., Madison, Wisconsin, USA.

O'Kiely, P., Muck, R. E. (1992). Aerobic deterioration of lucerne (Medicago sativa) and Maize (zea mais) Silages—Effects of Yeasts. *Journal of the Science of Food and Agriculture*, 59(2), pp 139–144.

Ohyama, Y., Masaki, S., Hara, S.-I. (1975). Factors influencing aerobic deterioration of silages and changes in chemical composition after opening silos. *Journal of the Science of Food and Agriculture*, 26(8), pp 1137–1147.

Pahlow, G., Muck, R. E., Driehuis, F., Elferink, S. J. W. H. O., Spoelstra, S. F. (2003). Microbiology of ensiling. In: Buxton, D. R., Muck, R. E., Harrison, J. H. (Eds) *Silage science and technology*. American Society of American Inc., Madison, Wisconsin, USA.

Pahlow, G., Rammer, C., Slottner, D., Tuori, M. (2001). Ensiling of legumes. In: Wilkins, R. J., Paul, C. (Eds) *Proceedings of Legume silages for animal production: LEGSIL*, Braunschweig, 2001. pp 27–30. Braunschweig.

Pettersson, K. (1988). *Ensiling of forages; Factors affecting silage fermentation and quality.* Diss. Swedish University of Agricultural Sciences, Uppsala.

Piltz, J. W., Kaiser, A. G. (2004). Principles of silage preservation. In: A.G. Kaiser, Piltz, J. W., Burns, H. M., Neil, W. G. (Eds) *Successful silage*. pp 25–56. Dairy Australia and New South Wales Departement of Primary Industries.

Rust, S. R., Kim, H. S., Enders, G. L. (1989). Effects of a Microbial Inoculant on Fermentation Characteristics and Nutritional Value of Corn Silage. *jpa*, 2(3), p 235.

Ruxton, G. D. & Gibson, G. J. (1993). A critical introduction to mathematical modelling of the aerobic deterioration of silage. *Journal of the Science of Food and Agriculture*, 62(3), pp 209–217.

Schenck, J., Müller, C. E. (2014). Microbial Composition before and after Conservation of Grass-Dominated Haylage Harvested Early, Middle, and Late in the Season. *Journal of Equine Veterinary Science*, 34(5), pp 593–601.

Tabacco, E., Piano, S., Revello-Chion, A., Borreani, G. (2011). Effect of Lactobacillus buchneri LN4637 and Lactobacillus buchneri LN40177 on the aerobic stability, fermentation products, and microbial populations of corn silage under farm conditions. *Journal of Dairy Science*, 94(11), pp 5589–5598.

Weinberg, Z. g., Ashbell, G., Hen, Y., Azrieli, A. (1993). The effect of applying lactic acid bacteria at ensiling on the aerobic stability of silages. *Journal of Applied Bacteriology*, 75(6), pp 512–518.

Wilkinson, J. M., Davies, D. R. (2013). The aerobic stability of silage: key findings and recent developments. *Grass and Forage Science*, 68(1), pp 1–19.

Woolford, M. k. (1990). The detrimental effects of air on silage. *Journal of Applied Bacteriology*, 68(2), pp 101–116.

Woolford, M. K. (1976). A Preliminary Investigation into the Role of Yeasts in the Ensiling Process. *Journal of Applied Bacteriology*, 41(1), pp 29–36.

Wu-tai, G., Driehuis, F., Wikselaar, P. van (2002). The influences of addition of sugar with or without *L. buchneri* on fermentation and aerobic stability of whole crop maize silage ensiled under anaerobic silos. *Journal of Animal Science*, 15(8), pp 1128–1133.

Electronic references

Eriksson, T. *Organic matter digestibility - Methods used in the Nordic countries*. Avaible from: http://norfor.info/Files/pdf-dokumenter/pdf_lab/Analyses/OMD_Methods_070925.pdf. [2014-11-21]

Hoffman, P. C. (2005). Ash content of forages. *Focus of forage* 7(1). Available from: http://fyi.uwex.edu/forage/files/2014/01/ASH05-FOF.pdf. [Accessed 2015-02-11].

Appendix 1

Frågeformulär vid provtagning

Kontaktinformation

Gård:	
Kontaktperson:	
Adress:	
Telefon:	
E-post:	

Vallinformation

Skördenummer:	1:a 2:a 3:e
Datum för slåtter: (ungefärlig tidpunkt)	
Datum när provet togs: (ungefärlig tidpunkt)	
Väder vid bärgning/packning:	Regn Sol Molnigt
Vallålder:	
Är vallen gödslad?	Ja Nej Om ja, med vad? Hur mycket? När gödslades den?
Här någon typ av bekämpning gjorts i vallen?	

Botanisk sammansättning i vallen? (olika gräsarter, baljväxtinslag)	
Ogräsförekomst? (omfattning, arter)	
Annan information om vallen	Gammal förna kvar? Ja Nej Risk för jordinblandning? 0 1 2 1=liten, 2= medel, 3=mycket
Hur användes vallen hösten innan skörd? (Bete, slåtter, nysådd etc.)	
Vilken typ av maskin användes vid slåtter? (slåtterbalk, slåtterkross med krimprar, slåtterkross med valsar, slåtterkross som lägger ihop flera strängar m.m.) Arbetsbredd?	
Väder vid slåtter?	Regn Sol Molnigt
Vilken typ av hack?	
Bredspridning eller strängläggning vid slåtter?	Bredspridning Strängläggning
Arbetsbredd på strängen?	
Vilken stubbhöjd?	
Vilken snittlängd?	
Hur länge förtorkades gräset? (från	

slåtter till att det bärgades)	
Väder vid förtorkning:	Regn Sol Molnigt
Behandlades grönmassan under tiden den låg på slag? (t.ex. mekanisk behandling)	Ja Nej Om ja, hur, när, vilken maskintyp?
Hur lagras grödan?	Slang Plansilo
Storlek på slang/silo?	
Täckningstid: (hur lång tid från sista lasset packats klart till dess att täckning sker?) Återbesök	
Maskinvikt på packaren?	
Antal ton/m^3 inkört och packat per timme alt. Storlek på vagnarna som kör hem gräset.	Håll reda på om de räknar ts, ton eller kubik.
Vad täcks silon med?	Plast Annat, nämligen: Om plast, färg, antal lager, lagertjocklek?
Vad täcks plasten med?	Hjulsidor Halmbalar Sand Hela däck Annat, nämligen:
Uttagare:	Blockuttagare Skopa Annat, nämligen:
Hur länge får silon vila mellan täckning och öppning?	

Tidigare problem med varmgång:	Ja Nej Om ja, fråga om några trender märks, såsom efter blöta somrar, skadade åkrar o.s.v.
Ensileringsmedel	Ja Nej

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