Silage quality of grass and red clover-dominated forages as affected by particle size and additive, when ensiled at different dry-matter concentrations

Ensilagekvalitet av gräs och rödklöverdominerat vallfoder med inverkan av strålängd & tillsatsmedel, ensilerat vid olika torrsubstanshalter

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Swedish University of Agricultural Sciences, SLU
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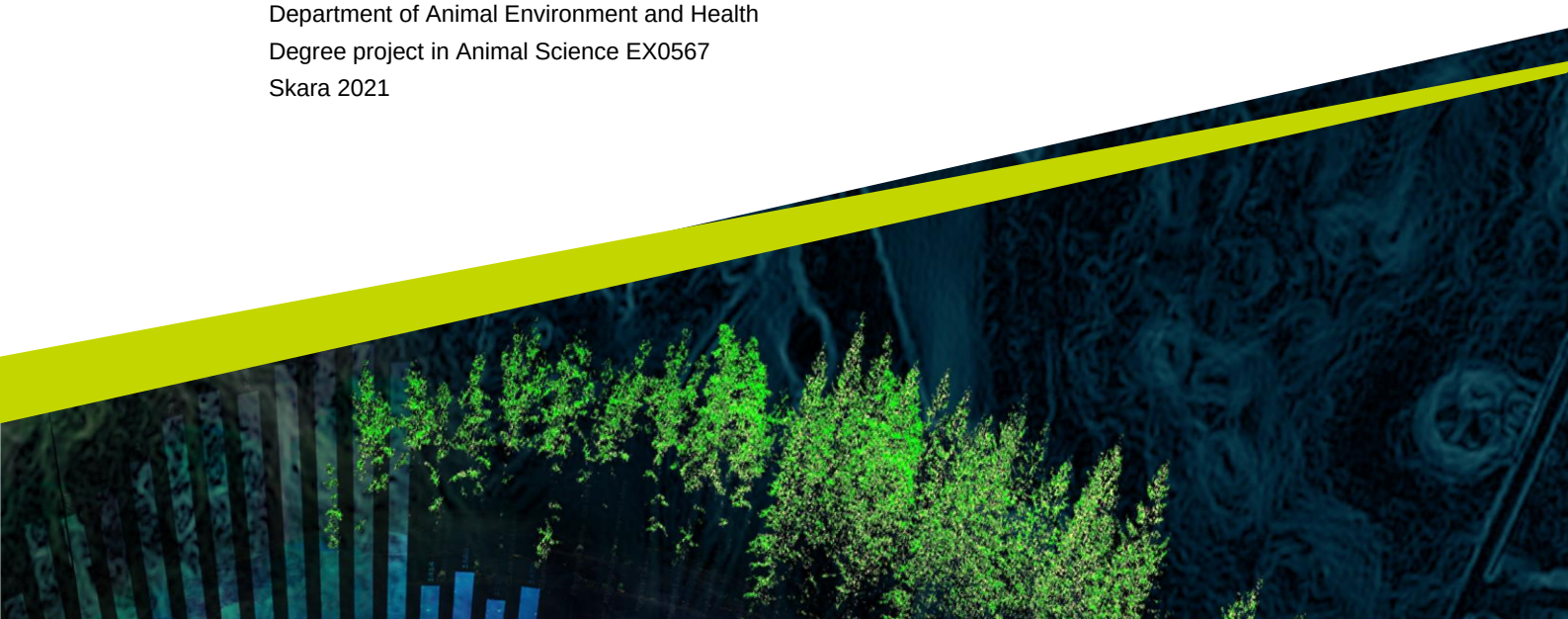

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Sammanfattning

Syftet med denna studie var att undersöka effekterna av partikelstorlek samt typ och dosering av kemiska tillsatser på ensilagekvalitet och lagringsstabilitet hos klöverdominerat ensilage och gräsensilage vid två torrsubstans (TS)-halter. Partikelstorlekarna var ca 24 cm för långt ensilage och ca 5 cm för hackat ensilage. De kemiska tillsatserna som användes var ett saltbaserat tillsatsmedel (natriumnitrit, hexametylentetramin, natriumbensoat) och ett syrabaserat (myrsyra, propionsyra, salter av organiska syror) och doser rekommenderade antingen (R) från tillverkningen eller halva halten rekommenderad dos (H). För klöverdominerat ensilage var doserna av tillsatserna för Salt-R 2,5 l / ton, Salt - H 1,25 l / ton, Syra - R 5,0 l / ton och Syra - H 2,5 l / ton. För gräsensilage var doseringarna av tillsatser Salt-R 2,0 l / ton, Salt-H 1,0 l / ton, Syra-R 3,0 l / ton och Syra-H 1,5 l / ton. Behandlat ensilage jämfördes med ett kontrollensilage utan tillsats. Skördat gräs packades i 1,7-l laboratoriesilor och lagrades i 100 dagar, med tre replikat per behandling. Den statistiska modellen som användes inkluderade fixa effekter av partikelstorlek- och tillsatsbehandlingar samt deras samspel för varje valltyp vid två olika TS-halter. Analysen gjordes i PROC GLM i SAS (vers 9) och parvis jämförelse gjordes med Tukey's t-test.

Resultaten visade att pH sänktes snabbare i hackat än i långt ensilage, vilket visade sig i en högre mjölksyrahalt i hackat än i långt ensilage med en något tydligare effekt i klöver-gräsensilage än i gräsensilage. Resultaten visade vidare på en förbättrad ensilagekvalitet med tillsatsmedel, och en doseringseffekt på protolysen i grödorna. Koncentrationen av mjölksyra var lägre i syra behandlingarna, med en doseringseffekt där Syra – R hade lägre koncentration än Syra – H. Behandling med saltbaserat medel hade liknande nivå på mjölksyrahalten som kontrollensilaget. Syra-R minskade ts-förlusterna i klöver-gräsensilaget vid båda ts-halter jämfört med kontrolledet. Lagringsstabiliteten förbättrades i gräsensilage med hög TS-halt när syra och salt-baserade medel användes vid rekommenderade doseringar.

Slutsatsen av den här studien är att ensilagekvaliteten påverkas av snittlängden och förbättras vid användning av kemiska tillsatsmedel vid rekommenderad dos.

Abstract

The aim of this study was to examine the effects of particle size and type and dosage of chemical additives on silage quality and aerobic stability of clover-grass and grass silages at two dry-matter (DM) concentrations. The particle sizes were ca 24 cm for long silage and ca 5 cm for precision chopped silage. The chemical additives used were a salt-based additive (sodium nitrite, hexamethylene tetramine, sodium benzoate) and an acid (formic acid, propionic acid, salts of organic acids), and dosages were either recommended (R) from the manufacture or half the recommended dose (H). For the clover silages the dosages of the additives were for Salt-R 2.5 l/ton, Salt – H 1.25 l/ton, Acid – R 5.0 l/ton and Acid – H 2.5 l/ton. For the grass silage dosages of additives were Salt – R 2.0 l/ton, Salt-H 1.0 l/ton, Acid-R 3.0 l/ton and Acid – H 1.5 l/ton. Treated silages were compared to a control silage without additive. Forages were ensiled in 1.7 l laboratory silo for 100 days, with three replicates per treatment. The statistical model used included fixed effects of forage particle and additive and their interactions for each forage type and DM concentration. Analysis was done in PROC GLM of SAS (vers. 9) and pairwise comparison was done with Tukey´s t-test.

Result of the study showed that the pH was lowered more rapidly in the chopped silage than in the long silage, which was shown by a higher lactic acid concentration in the chopped than in the long silage, with a stronger effect in clover-grass silage than in grass silage.

Furthermore, the results showed an improved silage quality by using silage additives, and with a dosage effect on proteolysis in the crops. Concentration of lactic acid was decreased by acid treatment and Acid-R had lower concentration than Acid-H. The salt-based additive treatment had the similar lactic acid concentration as the control silage. Recommended dosage of the acid decreased the DM losses in clover-grass silage. Aerobic stability was improved in grass silage of high DM content when acid and salt-based additives were used at recommended dosages.

In conclusion, chopping of the forage and use of chemical additives at recommended dosage improve silage quality.

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Introduction

Grass and legume silage and mixtures thereof are used as feed for ruminants and horses. In Sweden the most common grass species is timothy (*Phleum pratense*) and the most common legume is red clover (*Trifolium pretense*; Swedish Board of Agriculture, 2015ab). The process of ensiling is based on lactic acid bacteria (LAB) fermentation of water soluble carbohydrates (WSC) to organic acids (mostly lactic acid and acetic acid) under anaerobic environment (Weinberg & Muck, 1996).

Factors affecting the ensiling process are wilting (increasing dry matter (DM)), particle length and sward type (grass or legume; Muck *et al*., 2003). Clover has lower content of sugars and higher buffering capacity compared to grasses which results in a slower drop of pH during the ensiling process (Albrecht & Beauchemin, 2003; Harrison *et al.*, 2003). During ensiling undesirable secondary fermentation can occur, which will not be detected before the silo (tower or bunker silo), tube or bales is opened for feed out (Weinberg and Muck, 1996; Pahlow *et al.*, 2003). One way to improve the condition for good quality (nutrient, microbial and aerobic stability) silage is to use some type of additive (Kung, 2010). Additives can either be biological (*i.e.* bacterial inoculant) or chemical (*i.e.* acid or salt based). Chemical additives decrease the pH rapidly (acid based) and contain substances against clostridia and fungi (acid and salt based; Woolford, 1975; Kung, 2010).

The manufacturers state recommended dosages of the additives to prevent the risks of secondary fermentation and aerobic deterioration (Kung, 2010). Correct application rates of chemical additives are crucial for improving silage fermentation and aerobic stability, whereas application rates of the LAB containing inoculants are not as crucial as the bacteria can populate during the normal silage fermentation process. Chemical additives contain substances that can inhibit fermentation and malfermentation (Kung *et al*., 2003; Kung, 2010; Muck *et al.*, 2018). Consequently, there is an interest to investigate silage fermentation characteristics and aerobic stability at lower than recommended dosage of chemical additives.

The effect of various factors, such as sward type (Bodarski *et al*., 2003; Rooke & Hatfield, 2003; Knický, 2005), wilting (Knicky & Lingvall, 2004;), particle length (Nadeau et al., 2012; McEniry *et* al., 2013; Nadeau et al., 2013; Nadeau & Auerbach, 2014) and additive (Knicky & Spörndly 2009; Randby,2000) have been investigated to some extent. However, only limited information is available on how these factors interact and the importance of each one of these factors on silage fermentation characteristics and aerobic stability.

Objective

The aim of this study was to examine the effects of particle size and type and dosage of chemical additives on fermentation characteristics and aerobic stability of clover-grass and grass silages at two DM concentrations.

Hypothesis

Proteolysis will be lower in the additive treated silages compared to the untreated control.

In the unchopped silage the pH will decline slower (higher pH_{3days}) compared to the chopped silage resulting in differences in fermentation characteristics and aerobic stability.

Fermentation characteristics and aerobic stability will differ between half of recommended dosage and recommended dosage of the additives.

Dry matter losses will be greater in the silage treated with half the recommended dosage compared to the recommended dosage of the additive.

Aerobic stability will be improved by use of additive.

Literature review

The ensiling process

The ensiling process is based on the fermentation patterns of LAB (Weinberg & Muck, 1996). The different processes that occur from the sealing of the silos to the opening of the silos can be divided into four phases. These are the aerobic phase, fermentation phase, stable phase and feed out phase (Muck & Pitt, 1988; Weinberg & Muck, 1996; Pahlow *et* al., 2003; Rooke & Hatfield, 2003; Oude Elferink *et al*., 2008). Microorganisms, both desirable and undesirable, are present during the ensiling process and they have different desirable environments for growth and fermentation pathways (see Table 1). The ensiling process starts after the silo is closed.

Lactic acid bacteria

Lactic acid bacteria are naturally present on the crop (McDonald *et al.*, 2002; Pahlow *et al*., 2003). Water soluble carbohydrates (WSC) are simple sugars and fructans (Longland & Byrd, 2009) that are metabolized by LAB to produces organic acids (i.e. lactic acid, acetic acid). The production of organic acids decreases the pH to 3.8 – 5.0 depending on the dry-matter (DM) concentration and sward type (Weinberg & Muck, 1996; Oude Elferink *et al*., 2008). The metabolic patterns of LAB can be divided into three groups; obligate homofermentative, facultative heterofermentative and obligate heterofermentative. Obligate fermenters can only use one metabolic pathway; in comparison to facultative fermenters can use more than one. Homofermentative LAB produces only lactic acid and heterofermentative LAB produce lactic acid, acetic acid and ethanol (see table 1). Homofermentative LAB converts WSC to lactic acid more efficiently than heterofermentative LAB (McDonald *et al.*, 1991; Weinberg & Muck, 1996; Pahlow *et al*., 2003).

Ensiling phases

Aerobic phase

During filling of the silo and for some time after the silo is closed, oxygen is still present in the silo and the plant cells still respire and oxygen is consumed (Muck 1988; Muck *et al*., 2003). During respiration the first thing that happens is a loss of DM, due to the loss of fermentable carbohydrates (McDonald *et al.,* 1991). During the aerobic phase the enzymes of the plant (proteases and carbohydrases) degrade the plant nutrients when pH is 6.0 – 6.5 (Weinberg & Muck, 1996). Starch and hemicellulose are degraded by hydrolysis to monosaccharides (Muck, 1988; Rooke & Hatfield, 2003). True protein is degraded by the plant proteases to soluble non-protein nitrogen (NPN), CO_2, amines and volatile N (i.e. NH_3; Rotz & Muck, 1994; Rooke & Hatfield 2003). The rate of proteolysis during ensiling is determined by the DM concentration of the crop, pH, and temperature of the silage and presence of proteolysis inhibitors (Slottner and Bertilsson, 2006).

Extensive respiration (due to slow silo filling or imperfect sealing, air leakage) causes loss of DM, sugars and lack of decrease of pH, which leads to prolonged microbial activity (Shao *et al.*, 2005; Kung, 2010). The silage can increase temperature during prolonged respiration, leading to deterioration of the silage (McDonald et al., 1991; Pahlow *et al*., 2003). Thus, DM losses correlate negatively to silage density and feed out rate (Köhler *et al*., 2013).

Fermentation phase

The next phase starts when all oxygen is consumed in the silo and the environment is anaerobic. The monosaccharides are used as easily metabolizable sugar for LAB to produce organic acids (i.e. lactic acid, acetic acid; Muck, 1988; Pahlow *et al*., 2003). Production of organic acids inhibits microbial life. The undissociated acids pass by passive diffusion through the cell wall. When the acids dissociate inside the cell (i.e. bacteria, yeast) hydrogen ions are released, which reduce pH (McDonald *et al.,* 1991; Pahlow *et al*., 2003). The end-point of the phase is reached when the supply of available substrate has been exhausted; microbial growth is inhibited by the low pH, or there is a lack of available water (a_w). Bacterial growth is affected by a decreased a_w to a greater degree than yeasts and moulds (Auerbach, 2003; Pahlow *et al.,* 2003; Rooke & Hatfield, 2003). During the fermentation phase the population of LAB is growing if the fermentation is successful (Weinberg & Muck, 1996; Pahlow *et al*., 2003).

Storage phase & Feed-out phase

The storage phase starts when the microorganisms are inhibited by the stable low pH (Weinberg & Muck, 1996; Pahlow *et* al., 2003). When the pH has decreased to a sufficient level (3,8-4,5; Duniere *et al*., 2013) the microbiological activity is inhibited, and the silage is stable for storage if anaerobic environment is kept (Weinberg & Muck, 1996). During this phase the number of microorganisms decreases, but acid-tolerant species can survive (Weinberg & Muck, 1996; Pahlow *et al.,* 2003). Micro-organisms that are still active in storage phase can become a problem when the silo is opened. Yeast and other acid-tolerant micro-organisms can start respiring when oxygen is present. Increasing pH proliferate other bacteria and moulds, which contributes to an increase in temperature and the silage is spoiled (Muck & Pitt, 1988; Weinberg & Muck, 1996; Pahlow *et al.,* 2003). Once the temperature rises above 45 °C the amount of yeasts present declines and other microbial organisms (i.e. moulds bacilli, clostridia and enterobacteria) begin to accumulate (Vissers *et al*., 2007; Borreani & Tabacco, 2008).

Table 1: The desirable environment and fermentation pathways of microorganisms in silage (Muck, 1988; McDonald *et al.*, 1991; Driehus and Oude Elferink, 2000; McDonald *et al.*, 2002; Pahlow *et al.*, 2003; Seglar, 2003; Pedroso *et al.*, 2005; Vissers *et al*., 2006; Oude Elferink *et al.*, 2008)

	Desirable environment	Fermentation pathways
Bacteria		
Lactic acid bacteria - desirable	Facultative anaerobe Optimal pH 5-6 Acid tolerant (pH 4-3.8)	Obligate homofermentative: Glucose → 2 Lactic acid + 2 H_2O Fructose → 2 Lactic acid + 2 H_2O Facultative heterofermentative: Glucose → Lactic acid + ethanol + CO_2 Pentose → Lactic acid + Acetic acid Obligate heterofermentative: Glucose → 2 Lactic acid
Enterobacteria - undesirable	Facultative anaerobic Optimal pH 7.0	Saccharolytic: (strict anaerobe) Glucose →acetic acid + ethanol + 2 CO_2 + 2 H_2 + 2 H_2O Butanediol → acetoin + 2,3-butanediol Proteolytic: Amino acids → biogenic amines + CO_2 Nitrate → Ammonia + N_2O
Clostridia spp. - undesirable	Most species strict anaerobe Optimal pH 7.0-7.4 Low DM High a_w	Saccharolytic: Glucose → butyric acid + 2 CO_2 + 2 H_2 2 Lactic acid → butyric acid + 2 CO_2 + 2 H_2 + H_2O Proteolytic: *Deamination* Glutamic acid → acetic acid + pyruvic acid + NH_3 Lysine → acetic acid + butyric acid + 2 NH_3 *Decarboxylation* Amino acids → biogenic amines + CO_2
Bacilli - undesirable	Facultative anaerobic Acid sensitive	Carbohydrates → organic acids (i.e. butyrate, acetate, lactate) Carbohydrates → Ethanol + 2,3-butanediol + glycerol
Fungi		
Yeasts - undesirable	Anaerobe Aerobe Acid tolerant	Anaerobic: Glucose, → Ethanol + 2 CO_2 + 2 H_2O Aerobic: Lactic acid → CO_2 + H_2O
Moulds - undesirable	Aerobe Optimal pH>4.5	Various carbohydrate degradation

Undesirable microorganisms

Microorganisms categorised as undesirable for the ensiling process can impair silage preservation (Duniere *et al*., 2013). Undesirable bacteria in grass and legume silage are, for example; enterobacteria, bacilli and clostridia and undesirable fungi are yeasts and moulds (see table 1).

Clostridia

Clostridia are anaerobic bacteria and can form endospores (Pahlow *et al*., 2003). Legumes with low sugar contents and high protein contents are prone to clostridia activity during ensiling (Nadeau and Auerbach, 2013b). Fermentation pathway of clostridia species are divided into two categories; saccharolytic (ferments sugars) or proteolytic (ferments proteins; Pahlow *et al*., 2003). Silage with extended clostridia fermentation is characterised by high pH (>7) and high content of products from lactic acid fermentation. Most growth occurs at pH 7.0-7.4 and growth is inhibited by pH 4.2 (Flythe & Russel, 2003; Vissers *et al*., 2006b). Clostridia have a higher optimal a_w than LAB and tolerate higher a_w (Driehus, 2013). Increasing DM concentration (decrease a_w) thereby inhibits clostridia growth (Muck & Pitt, 1993; Kung, 2001; Kung, 2010). Dry matter concentration above 300 g/kg restricts clostridia (McDonald *et al*., 2002). Tabacco *et al*. (2009) showed that clostridia spores can return to a vegetative cell and start proliferating in both the fermentation stage and the feed-out phase during aerobic deterioration. The pH value inhibiting clostridia activity also depends on a_w -value of the silage (McDonald *et al*., 1991; Pahlow *et al*., 2003).

Nitrate (NO_3) occurs naturally in green forage (McDonald *et al.* 1991), and the content is affected by maturity, fertilization and plant species. During the ensiling phase, NO_3 is reduced with the product of either NH_3 or nitrous oxide gas (N_2O; Rooke & Hatfield, 2003). This reduction inhibits clostridia and other spore forming bacteria (Spoelstra, 1983). During the pH drop in the silage the NO_3 is partially reduced and later in lactate degradation (second fermentation to butyric acid) reduced further. With NO_3 present in the silage, to inhibit clostridia, the pH drop is not needed to be as low compared to nitrate-free silages (Kaiser *et al*. 2002). The effect of nitrite (NO_2) as clostridia inhibitor is used in silage additives (Woolford, 1975).

Silage with clostridia fermentation has an increased content of butyric acid and amines, which due to the reduced palpability decrease the feed intake (McDonald *et al.*, 1991; van Os *et al.*, 1996; Driehus *et al*., 2013). Contamination of *Clostridia tyrobutyricum* spores from silage to milk are a problem for cheese production with late blowing cheeses. The spores during cheese making proliferate and metabolise lactic acid to H_2 and CO_2 (Pahlow *et al*., 2003; Vissers *et al*., 2006). Some clostridia species produce toxins that are pathogenic to animals and humans, i.e. *C. botulinum* produce the neurotoxic botulinum toxin (McDonald *et al*., 1991; Pahlow *et al.*, 2003; Bok *et al*., 2012).

Enterobacteria

Enterobacteria (i.e. *Escherichia coli*) are facultative anaerobes and compete with LAB for WSC as nutrient prior to and during ensiling. Fermentation pathway of enterobacteria can both be saccharolytic and proteolytic. The protein degradation of enterobacteria affects the feed value and decrease palatability due to the production of biogenic amines and fatty acids (van Os & Dulphy, 1996). Some enterobacteria metabolize glucose to ethanol, acetic acid and hydrogen (McDonald et al., 1991). Some enterobacteria can degrade NO_3 to NO_2 and NO_3 to NH_3 and

N_2O. This ability has been shown to inhibit clostridia (Pahlow *et al*., 2003; Knický, 2005). Rapid drop of pH to a level below 4.5 is efficient to decrease the amount of enterobacteria (McDonald *et al.,* 1991; Pahlow *et al*., 2003).

Bacilli

Bacilli are endospore forming bacteria and facultative anaerobic (Pahlow *et al.,* 2003). They are found widespread on harvested grass, but inhibited by the fermentation of LAB (Pahlow *et al.,* 2003; McDonald *et al*., 2002; Oude Elferink *et al*., 2008; Muck, 2010). Bacilli are unable to start aerobic deterioration but continue the deterioration that yeasts have initiated (Driehus & Oude Elfrinke, 2000; Pahlow *et al.,* 2003). Bacilli spores are a problem for dairy production, such as spoilage of milk and outbreak of foodborne illness. Silage is an important contamination source to milk (Te Giffel *et al*., 2002).

Yeast and Moulds

Yeast is facultative anaerobic eukaryotes and the most important group involved in aerobic degradation, in the aerobic phase or feeding phase (Driehus and Oude Elferink, 2000; Pedroso *et al.,* 2005). Aerobic degradation is often initiated by yeast, because many yeast species are acid tolerant, they are able to grow at pH 4-5 (Moon, 1983). Yeast uses lactic acid and thus pH increases enabling undesirable bacteria (i.e. bacilli) and mould to proliferate (McDonald *et al*., 1991; Kung, 2001, Pahlow *et al*., 2003). Yeasts are also saccharolytic, fermenting carbohydrates. These yeasts compete with LAB for carbohydrates to ferment in the beginning of the fermentation phase (Muck *et al*. 2003).

If obligate aerobe moulds are present when oxygen is present, such as before anaerobic environment occurs in the silo, oxygen leakage in the silo during storage or when the silo is opened creates a suitable environment for moulds. (Pahlow *et al*., 2003). Growth of mould occurs in silage when the pH has increased above 4.2 (Seglar, 2003) and thus proliferate after yeast fungi have initiated the aerobic deterioration (Driehus and Oude Elferink, 2000). Moulds reduce feed value and palatability of the silage, but also health risks with respiratory diseases from spores both for animals and humans can occur (Adesogan, 2006) A couple of mould species can produce secondary metabolites, mycotoxins (i.e. *Aspergillus flavus* produce aflatoxins; Auerbach, 2003; Driehuis, 2013). Lethal doses of mycotoxins do not often occur in grass and legume silages (Muck & Pitt, 1993), but chronic low doses during a long time may lead to reduced immune response and imbalance of hormones in the animals (Morgavi & Riley, 2007). Mycotoxins can transfer to meat and milk and thereby being a potential risk for the consumer (Fink – Gremmels, 2008).

Quality parameters for silage

The quality of grass/legume silage can be distinguished by analyzing the pH value, ash content and end-products of microbial fermentation (see Table 2).

Table 2: Quality parameters of silage.

Hygienic parameters	Indicator of
Ethanol	Yeast activity
Ammonia nitrogen	Poor/extensive fermentation
	Clostridia fermentation
pH	Inhibits bacterial activity
	Silage quality
Ash	Soil contamination
	DM loss
	Clostridia fermentation
Lactic acid	Fermentation quality
Acetic acid	Fermentation quality, Clostridia fermentation
Propionic acid	Fermentation quality
Butyric acid	Clostridia fermentation

pH

Bacterial activity in the silage can be inhibited by the pH. For storage stability of the silage both the pH value and the DM content is to consider. A measurement of stability during storage is critical pH (see equation 1; Weissebach, 1996). The critical pH shows the maximum pH value for a anaerobically stable silage (Pahlow *et al*., 2003).

$$pH < (DM\ (\%) \times 0{,}0257) + 3.71$$

Equation 1: Critical pH

Organic acids

Lactic acid is the most important acid for silage production and is the acid that lowers the pH most (Seglar, 2003). The extent of acid dissociation in silage depends on pH and dissociation constant (pKa) of the acid. Strong acids have a value of under 1 to 5, where the strongest dissociate completely (Bruice, 2006). Lactic acid has a higher acidification rate than acetic acid because of a lower pKa-value (3.86 compared to 4.75; Rooke & Hatfield, 2003; Bruice, 2006). Propionic acid and butyric acid have also higher pKa than lactic acid (4.87 and 4.82 respectively; Bruice, 2006). In silage with high DM concentration, lactic acid cannot be used to determine fermentation quality, due to the lower extent of fermentation (Cherney & Cherney, 2003). Acetic acid in high concentration suppresses the growth of yeast and moulds and improves the aerobically stability (Weinberg & Muck, 1996). The concentration of acetic acid in grass and legume silage is usually 1-3 % of DM (Kung, 2001) but varies depending on type of fermentation. Propionic acid concentration in silage is generally less than 0.1% of DM (Seglar, 2003), but often used in chemical additives (Kung *et al*., 2003). Undissociated propionic acid inhibits yeast and moulds and thus increases aerobic stability for the silage (Kung *et al*., 2003; Rooke & Hatfield, 2003).

Butyric acid

Good quality silage should have a butyric acid concentration of less than 0.1 % on a fresh weight basis (Seglar, 2003). High concentration indicates secondary fermentation by clostridia and deterioration by proteolytic activity with end-products such as amines and amides. Silage containing excessive amounts of butyric acid is undesired but is aerobically stable (McDonald *et al*., 1991; Pahlow *et al*., 2003; Seglar, 2003).

Ethanol

The primary indicator of yeast fermentation is ethanol (Pahlow *et al*., 2003; Seglar, 2003). For good quality silage the ethanol concentration should be below 0.5% of DM (Kung, 2001; Seglar, 2003). Enterobacteria can also produce ethanol from glucose (McDonald et al., 1991).

Ammonia Nitrogen

A measurement on extensive fermentation and proteolytic activity is ammonia nitrogen (Seglar, 2003). Secondary fermentation by the proteolytic clostridia activity can also increases the ammonia concentration (Kung, 2000). Recommended levels are less than 10% of total nitrogen (Seglar, 2003).

Ash

The total mineral content of the feed is in the ash content. A high ash content of the silage may indicate soil contamination or dry matter losses from aerobic instability or clostridia fermentation during storage (Seglar, 2003; Knický, 2005).

Sward type & ensilability

Ensilability of the crop is determined by WSC concentration, DM content and buffering capacity (Weissbach, 1996). Buffering capacity is defined as the amount of acids needed to change the pH from 6 to 4 (Muck, 1988; Knický, 2005). The ability to buffer is done by the content of organic acid salts, nitrate and sulfate. Legumes have higher buffering capacity than grasses, but buffering capacity is also affected by nitrogen fertilization (Tommila *et al.*, 1996). For temperate forages the WSC content is determined by the species and stage of maturity (Buxton & O'Kiely, 2003; Knický, 2005).

Fermentability coefficient (FC) is a measurement of the crops ensilability and is calculated by the formula shown in equation 2. The formula contains DM content, quantity of fermentable substrate and buffering capacity of the crop (Pahlow & Weissbach, 1999). As a measurement for good fermentation quality the value should be above 45 (Pahlow *et al*., 2001). One large part of the ensilability of the crop is the flora on the crop (Mogodiniyai Kasmaei *et al.* 2014), also the nitrate content of the crop has been reported to increase the fermentation quality (Knicky *et al*., 2017).

$$FC = DM\ (\%) + 8\ WSC\ BC^{-1}$$

Equation 2: Fermentability coefficient

Grass

The content of WSC in temperate grasses (i.e. timothy, perennial ryegrass;) includes monosaccharides (i.e. glucose and fructose), disaccharide (i.e. sucrose) and polysaccharides (i.e. fructans) (Rooke & Hatfield, 2003; Knický, 2005). The most important WSC for ensilability are glucose and fructose, because these are free sugars that provide substrate for LAB at the start at the ensiling process. The sucrose and fructans are available later in the ensiling process, when they are hydrolyzed to monosaccharides by acid hydrolysis (Rooke & Hatfield, 2003). In comparison to legumes, grasses typically contain higher cell-wall concentration (Buxton & Martens, 1995), but are more digestible (Nadeau *et al*., 1996).

Legumes

Legumes are more difficult to ensile than grasses due to the lower sugar content, higher protein and higher buffering capacity (Albrecht & Beauchemin, 2003; Harrison *et al.*, 2003). In contrast to grasses, legumes store polysaccharides as starch which is unavailable for LAB metabolism

(Albrecht & Beauchemin, 2003; Knický, 2005). Protein in red clover degrades to a less extent than lucerne during ensiling (Owens *et al*., 1999; Owens *et al*., 2002), which is due to the content of polyphenol oxidase in red clover (Lee *et al.*, 2008). Polyphenol oxidase inhibits enzymatic degradation by proteases by cross-linking protein complex (Kroll & Rawel, 2001).

The harvest time of lucerne and red clover affects the protein degradation (Owens *et al*.,2002). Red clover had significantly lower NPN levels than lucerne regardless of cutting time. The study also showed increased preservation and silage quality with afternoon harvest due to a lower silage pH and lower sugar concentration from the LAB metabolism (Owens *et al*., 2002). Lucerne silage has higher BC than red clover (McDonald *et al*., 1991; Pahlow *et al*, 2001) which results in higher final pH (Owens *et al*., 2002).

Aerobic stability & deterioration

Aerobic stability is defined as the time which elapses before the silage shows clear evidence of aerobic deterioration (Ranjit & Kung, 2000), which is standardized as the time elapsed when the temperature of the silage is 2°C above the ambient temperature (O'Kiely 1993). The most common symptom of aerobic deterioration is heating, due to the metabolism by microorganisms (Muck & Pitt, 1993). Aerobic deterioration occurs when the silo is opened, exposing the silage to oxygen, and the end-products of fermentation is used as substrate for microbial growth (Pahlow *et al*., 2003).

Yeast often initiates the deterioration and use lactic acid as substrate and degrading it to carbon dioxide, water and heat (Pahlow *et al*.,2003; Kung, 2010). Spörndly & Persson (2015) found that aerobic stability was negatively correlated to the yeast count in the silage. The subsequent decrease of organic acids increases the pH. Thus, the inhibition of bacteria and fungi by the organic acids ends (Pahlow *et al*., 2003; Borreani & Tabacco, 2010). The increased pH enables both increases in the number of yeast and enable more microorganisms to grow (i.e. clostridia; Borreani and Tabacco, 2010). Silage temperature increases during yeast fermentation, which may lead to maillard reaction ("browning reaction"), which is a chemical reaction between carbohydrates and amino acids. The products of the reaction are larger compounds that are slowly digestible i.e. acid detergent fibre (ADF) and acid detergent insoluble nitrogen (ADIN; Muck & Pitt, 1993). The ADF is the crude lignin and cellulose fraction, after refluxing the fibre with 0.5M sulphuric acid and acetyltrimethyl-ammonium bromide, and ADIN is the nitrogen remaining in the acid detergent fibre residue (McDonald *et al.*, 2002). Aerobic activities of yeast increase the DM losses and temperature which enables other aerobic microorganisms to be active (Weinberg & Muck, 1996). Moulds are the last microorganisms to grow after the pH value is increased (Muck, 1988).

Factors affecting aerobic stability

The stability of the silage in an aerobic environment is affected by biochemical and microbial factors, physical factors, management, silo sealing and if used; type of additive (Wilkinson & Davies, 2012). Microbial and biochemical factors influencing the stability are the number of yeasts, concentrations of organic acids and WSC. Crops with high starch or sugars often have a greater amount of yeast, not desirable for aerobic stability (Kung, 2001). Well fermented silage with yeast contamination and residual sugars, high lactic acid and low acetic acid can be aerobically unstable during aeration (Nadeau & Auerbach, 2013a).

Density, permeability, porosity and temperature are physical and management factors affecting the aerobic stability (Savole & Jofriet, 2003). Elevated environmental temperature during silage making decreases the aerobic stability of the silage. The increased temperature promotes

undesirable microorganisms (enterobacteria, clostridia; Adesogan, 2009). Proper sealing of the silo prevents penetration of oxygen into the silage during storage, promoting an anaerobic environment (Kung, 2010). Porosity is a measure of the voids between the solid particles (Williams, 1994) and is influenced by fresh weight density, DM content and rate of harvest (Holmes & Muck, 2007). At an increased DM concentration, by wilting of the crop, the fermentation becomes restricted with higher WSC residuals (Wilkinson & Davies, 2012). There is no direct relationship with aerobic stability and high WSC residual (Wrobel et al., 2008). At high DM content the acidification is lower due to a lower amount of LAB. High DM content in the raw material, over 50%, makes the silage more susceptible to self-heating and growth of fungi (Purwin *et al*., 2006). If the porosity is high (low density) the ingress of air is higher, and stability of the silage is lowered (Holmes & Muck, 2007). Silage density is lower at the edges of the bunker silo (Craig *et al*., 2009) hence permeability and porosity make it possible for oxygen to penetrate the silage (Holmes & Muck, 2007). Additives, especially chemical salts (benzoate and sorbate) and organic acids (i.e. propionic acid), have antifungal properties which improves aerobic stability (Kung *et al.*, 2003; Kung, 2010; Muck et al., 2018).

Factors affecting the ensiling process

The ensiling process is influenced both by plant components (Buxton & O'Kiely, 2003) and by harvest conditions and technique (Muck *et al*., 2003). Applying additives to the crop affect the ensiling process, either by inhibiting or stimulating fermentation (Kung *et al*., 2003; Knický, 2005; Kung, 2010).

Particle size

Chopping, decreasing particle length, affects the fermentation quality (McEniry *et al*., 2008), density and effluent production (Muck *et al*., 2003). Similar chop lengths improve the possibility for more uniform silage in the silo. Precision chopping decreases the clostridia activity and increases lactic acid concentration. High density of the silage mass reduces respiration and aerobic losses (Pauly & Lingvall, 1999).

Chopping the crop releases the nutrients form the cell sap (Pauly & Lingvall, 1999), which can be metabolised by LAB. Chopping legumes can lead to a greater loss compared to grasses, with a loss of leaves, and thereby valuable protein (Muck *et al*., 2003). McEniry *et al*. (2008) compared the fermentation characteristic of precision-chopped (19 mm) to unchopped grass silage. The precision-chopped was ensiled in laboratory silos and the unchopped was ensiled in either laboratory silos or round bales. The decline in pH was slower in unchopped baled silage than in precision chopped, indicating a slower rate of fermentation. Concentrations of lactic and acetic acids were greater in the chopped silage, but the content of WSC was smaller (McEniry *et al*., 2008).

Nadeau *et al*. (2012a) studied the effect of particle size of the crop and additive use on fermentation quality of the silage and aerobic stability. Wilted grass was, after running through a baler, either precision chopped (20 mm) or cut (250 mm). The acidification of the long cut grass was significantly slower than that of chopped silage Nadeau *et al*., 2012a).

Effect of wilting

Wilting increases the DM concentration and affects the microbial population and activity on the crop (McDonald *et al*., 1991). Wilting of the crop improves fermentation by increasing the number of LAB (Muck, *et al*., 2003). Clostridia activity is decreases by wilting (Knicky & Lingwall, 2004) but yeast numbers increased which may decrease the aerobic stability of silages (Pahlow *et al*., 2003).

Rainfall during the wilting process of the forage can leak sugar and if the wilting time is prolonged can increased respiration result in increased nutrient leaks. The loss of nutrients can affect the ensiling process, were fermentation can be limited and the risks for unwanted microbiology can increase (McDonald *et al., 2002;* Muck *et al*., 2003).

Spörndly *et al*. (2008) studied the economic aspects and effects on silage quality by different wilting techniques; either swaths or wide spreading. By wilting the cut forage widespread the drying was faster and more controlled. This also led to an increased silage quality, with lower loss of DM and a decrease of clostridia spores. By wide spreading the forage the production costs were lowered by 10 % compared to swaths spreading. This was due to a higher bale density and faster mowing work, even though wide spreading had a higher machinery cost (Spörndly *et al*., 2008).

During the wilting process the plant enzymes contribute to respiration and proteolysis. Wilting influences the protein quality, limit protein degradation before ensiling depending on the wilting condition (Muck *et al*., 2003). Edmunds *et al* (2013) studied the effect on nitrogen components by wilting grass forage to 4 different dry matter concentrations (20, 35, 50 and 65% DM) at either fast or slow rate. The metabolizable protein increased by fast wilting to the highest DM concentration. The content of NPN decreased by increased DM concentration, which also was reported by Muck *et al*. (2003). Wilting at a fast rate was observed to improve the protein quality of the grass silage of all DM-concentrations (Edmunds *et al*., 2013).

Nadeau *et al*. (2012b) evaluated the effect of wilting and ensiling on protein quality. The grass-legume silage (77% grass, 18% clover, 5% lucerne) was wide spread and wilted during ~23 h to 35 % of DM. Nadeau *et al.* (2012b) concluded that wilting grass-legume silage during favorable conditions increased the concentration of rumen undegraded protein.

Chemical Additives

Decreasing pH is a crucial part of the fermentation in silage (Pahlow *et al*., 2003) and by using chemical additives the fermentation can be improved. Chemical additives, such as acids and salts, restrict partly the microbial growth, whereas bacterial inoculants stimulate growth of LAB (Kung *et al*., 2003; Kung, 2010; Muck *et al.*, 2018). Low sugar (< 2% of the green mass fresh weight) content in grass can result in a low acid production and impair silage quality. Propionic acid partially dissociates and lowers the pH (Kung, 2010). Formic acid is a stronger acid (pKa 3.8) compared to the organic acids produced by LAB, i.e. lactic acid (3.8), acetic acid (4.7) and propionic acid (4.9; Rooke & Hatfield, 2003; Brucie 2006). With a higher dissociation rate the pH drops more and faster (Rooke & Hatfield, 2003). The effect of chemical additives can be divided into two groups; improving the fermentation quality and improving the aerobic stability. Chemical additives to improve the aerobic stability often contain substances that inhibit yeast and mould growth (Kung, 2010). At a pH range of 3 to 6 potassium sorbate and sodium benzoate inhibit spore-forming bacteria, yeasts and moulds. At low pH levels sodium nitrite inhibits growth of spore forming bacteria (Woolford, 1975).

Studying the effect of acid-based additives on grass silage (24 % DM) were done by Rinne *et al*. (2016). The seven additive mixtures used is described in table 3. The application rate was 5 l/ton for all additive treatments. Buffering substances, that stabilize the pH, used in the silage additive are sodium and ammonium salts. Using the additive mixtures decreased the ammonium nitrogen, concentration of lactic acid and acetic acid compared to control. The concentration of WSC increased by using additive. Restricting the fermentation was most prominent in the silage

were found in AIV 2 Plus Na and AIV Ässä Na. Aerobic stability was mostly improved by using AIV Ässä Na, with a low content of ethanol and yeast count, but all additives improved the aerobic stability (Rinne et al., 2016).

Table 3: The content in the additive mixture used in Rinne *et al.* (2016)

AIV 2 Plus	Formic acid, ammonium formate
Blend 1	Formic acid, sodium formate, propionic acid, sodium benzoate
Blend 2	Formic acid, sodium formate, propionic acid
AIV 2 Plus Na	Formic acid, sodium formate
AIV Ässä Na	Formic acid, sodium formate, propionic acid, potassium sorbate
Blend 3	Formic acid, sodium formate, lactic acid
Blend 4	Formic acid, sodium formate

Prolonging the respiration increase the risk of deterioration of the silage (McDonald et al., 1991; Pahlow *et al*., 2003). Randby (2000) examined the effect of acid additives on grass silage that either was sealed direct after harvest or sealed 24 hours after. Additives used were both formic based commercial additives and acid mixtures included in the manufactured additives, both recommended dose (R) and half of the recommended dose (H) were tested (see table 4). The acids and acid mixture used were to test the manufactural additives active ingredients to compare the effect with the commercial additive. As control formic acid (85%) was used as a negative control and as positive control no additives were added. Using the recommended application dose of the additives the sugar content was higher compared to using half the recommended dose.

Sealing the silos 24 h after harvesting increased concentration of butyric acid, ethanol and pH value. This indicates that waiting with the sealing of the silo effects the fermentation negatively. The use of additives could not fully stop the fermentation of propionic acid, and the application dose of the additive did not affect the ethanol concentration. Under good fermentation conditions lactic acid concentration was negatively correlated with application rate, but under bad fermentation conditions the correlation was positive (Randby, 2000).

Table 4: The composition in the acid additives and application used by Randby (2000).

	Additives	Composition	Application dose R-dose (l/t)	Application dose H - dose(l/t)
1	Foraform (F)	formic acid (645 g/kg), ammonia (60 g/kg)	4.0	2.0
2	Formic acid (85%)	Formic acid (850 g/kg)	3.0	1.5
3	Ensimax (E)	213 g/kg formic acid, 200 g/kg acetic acid, 190 g/kg of DM from WPL	4.0	2.0
4	Acetic acid	Acetic acid (1000 g/kg)	0.8	0.4
5	Formic acid	Formic acid (850 g/kg)	1.0	0.5
6	Acetic acid + formic acid	Acetic acid (1000 g/kg) & Formic acid (850 g/kg)	0.8 Acetic acid 1.0 Formic acid	0.4 Acetic acid 0.5 Formic acid
7	Wood pulp liquor	Wood pulp liquor (340 g/kg)	2.0	1.0
8	Acetic acid + wood pulp liquor	Acetic acid (1000 g/kg) & Wood pulp liquor (340 g/kg)	0.8 Acetic acid 2.0 WPL	0.4 Acetic acid 1.0 WPL
9	Formic acid + wood pulp liquor	Formic acid (850 g/kg) & Wood pulp liquor (340 g/kg)	0.8 Acetic acid 2.0 WPL	0.4 Acetic acid 1.0 WPL
10	Ensimax modifed (EM)	360 g/kg formic acid, 50 g kg, acetic acid, 190 g kg of DM from WPL	4.0	2.0
11	Acetic acid + formic acid	Acetic acid (1000 g/kg) & Formic acid (850 g/kg)	0.2 Acetic acid 1.7 Formic acid	0.1 Acetic acid 1.7 Formic acid

Combination of the factors

Studies testing the interaction between the different factors (i.e. DM, particle size, additive) for ensiling grass and clover silage have been done.

The effect of DM and particle size was studied by (McEniry *et al*., 2007), were grass silage with different wilting times (0, 24 h and 48 h), particle size (chopped or unchopped), compaction and air infiltration (complete or incomplete silo sealing). Particle size for the chopped was 19 mm. Air filtration were achieved by only sealing the laboratory silos bottom and top by hand, enabling air to seep in. The chopped silage had a lower concentration of ethanol compared to the unchopped, but particle size did not affect microbial composition (i.e. LAB, yeast, clostridia). Wilting and particle size affected clostridia activity. Air filtration treatment for unwilted silage increased the clostridia activity. Chopping the grass silage

decreased the ammonia nitrogen content. Pore space decreased in compacted silage and decreased penetration of oxygen in the silage. In comparison, uncompact silage increased the penetration of oxygen, due to high prevalence of air pockets. The DM concentration and air infiltration were concluded to affect the silage fermentation to a larger degree than chopping and compaction (McEniry *et al*., 2007). Aerobic stability was not affected by chopping, but wilting, compaction and air filtration affected aerobic stability.

The additive effect on fermentation with different DM concentration was studied by Knicky and Spörndly (2009) and Knicky & Spörndly (2011).

Knicky and Spörndly (2009) tested the effect of five salt additive mixtures and two commercially produced additives (see table 5), on the fermentation and hygienic quality of clover-grass silages (ratio 8:92) at high and low DM-concentrations. The mixtures tested contained sodium benzoate, potassium sorbate and sodium nitrite in different ratios. For the high DM (46 %) yeast was analysed and clostridia was analysed in the low DM (23%) clover-grass silages. Dosage of the additives was 5l/ton FM. The additives inhibitory effect on clostridia fermentation was tested by adding a strain of *Clostridium tyrobutyricum.* In the low DM silage, all tested additives reduced significantly the concentration of butyric acid and the formation of ammonia-N compared to the control. The treatments that reduced the number of clostridia spores in the low DM silage were the commercially additive KU and the study's mixture A1 and A5. The mixtures A1 and A5 did contain no hexamine and lower concentration of nitrate than the commercial additive. For the high DM silage treated with the mixture A1, A2 or A5 contained less yeast compared with the control silage and the commercial additive PNF. Nitrite –N concentration in all silages decreased during the fermentation. The use of additive resulted with aerobically stable silages (Knicky & Spörndly, 2009).

Table 5: Concentrations of the content in the additives and additive mixture used in Knicky & Spröndly (2009)

Additive name	**Content (per ton fresh forage)**
Kofasil Ultra (KU)	750 g/kg Sodium benzoate, 600 g/kg sodium nitrate, 400 g/kg hexamine, 250 g/kg sodium propionate
Promyr NF (PNF)	Formic acid, Propionic acid, Sodium formate (authors did not have permission to print the proportions)
A1	600 g/kg sodium nitrite, 250 g/kg sodium propionate & 750 g/kg sodium benzoate
A2	250 g/kg sodium nitrite, 1000 g/kg sodium benzoate
A3	500 g/kg potassium sorbate, 250 g/kg sodium nitrite
A4	1000 g/kg sodium benzoate, 500 g/kg potassium sorbate
A5	250 g/kg sodium nitrite, 1000 g/kg sodium benzoate, 500 g/kg potassium sorbate

The fermentation quality, with focus on yeast and clostridia activity, in various forages treated with salt-based additive was studied by Knicky & Spörndly (2011). The study tested 13 crops divided into three groups depending in ensilabilty and DM (see Table 6). Additive mixture used consisted of sodium benzoate (200 g /kg), potassium sorbate (100 g /kg) and of sodium nitrite (50 g /kg). Application rate of the additive was 3 ml/kg FM for the low DM silage (<35 %) and for the high DM (>35 %) silage 5ml/kg FM.

Table 6: Crops and DM-content of the three groups in Knicky & Spörndly (2011)

Group	Crops	DM (%)
Difficult to ensile	Legume dominated	<20
Easy to ensile with high DM	Grass dominated	>35
	Whole – crop barley	
Easy to ensile with low DM	Grass dominated (85 - 90%)	<35
	Maize (100%)	

Using additive to ensiles the difficult to ensile crop improved the silage quality, decreasing the secondary fermentation (measured in ammonia nitrogen content) and increased the concentration of lactic acid compared to the untreated control silage of the same ensilability. Clostridia spores and butyric acid concentration decreased with additives use. The dry matter loss decreased with additive use. Using additive in the easy to ensile corps at low DM concentration had similar result as the difficult to ensile group. Yeast count was decreased in the high DM-concentration silages with additive use. All tested silages were aerobically stable, and the use of additives improved the stability compared to the untreated control. The study concluded that the additive mixture of sodium benzoate, potassium sorbate and sodium nitrate effectively decreased the activity of undesirable microorganisms (Knicky & Spörndly, 2011).

The effect on fermentation and particle size have been studied by Nadeau *et al*. (2012a) & Nadeau & Auerbach (2014). The effect of fermentation quality in grass silage by additive use and particle size was studied by Nadeau *et al.* (2012a). Two particle sizes were tested, chopped (20 mm) and cut (250 mm). Additive tested were two acid-based additives, three salt-based additives and two biological inoculants. See table 7 for the content and application rate of the chemical additives used in the study.

Table 7: Content and application rate of the additives used in Nadeau et al. (2012a)

Additive name	Type of additive	Content	Application rate
GrasAAT SP	Acid-based	Formic acid (350 g/kg) propionic acids (120 g/kg) sodium formate (255 g/kg), sodium benzoate (15 g/kg)	3 l/ton
ProMyr NT 570	Acid-based	Formic acid (500 g/kg), propionate (171 g/kg), sodium (56 g/kg)	3 l/ton
Kofasil Ultra K	Salt- based	Sodium nitrite (65 g/kg), hexamethylene tetramine (110 g/kg), potassium sorbate (81 g/kg), sodium benzoate (22 g/kg), sodium propionate (8 g/kg)	2 l/ton
Kofasil LP	Salt- based	Sodium nitrite (202 g/kg), hexamethylene tetramine (135 g/kg), sodium benzoate (50 g/kg)	2 l/ton
Safesil	Salt- based	Sodium benzoate (180 g/kg), potassium sorbate (74 g/kg), sodium nitrite (50 g /kg)	3 l/ton

The acidification during ensiling was faster in the chopped compared to the cut, but after 90 days of ensiling the final pH was low for all treatments. Using salt-based additives decreased the concentration of ethanol in both particle size compared to the untreated control (only water

added), and average over particle size proteolysis decreased with salt-based additive and decreased the DM losses. The silages treated with acid-based additive resulted in lower acetic acid concentration compared to the untreated control. Using the acid-based additives the ethanol concentration only decreased in the chopped silages. The content of residual WSC was also higher in the chopped than the cut silage. As conclusion of the study the additive use may improve the fermentation quality, decreasing the undesirable microorganisms and their fermentation. By chopping the forage acidification is faster, which improves the fermentation quality (Nadeau *et al*., 2012a).

Soil contamination during harvest increases the risk of clostridia in the silage. Nadeau & Auerbach (2014) examined the effect of additive use and particle size on the fermentation of clostridia contaminated grass-clover silage (50 % grass and 50% red clover of DM). To achieve the contamination clostridia contaminated soil (50 g/kg forage) was added before ensiling. The forage was wilted (30 % DM) and either cut (180 mm) or chopped (17 mm). Additive treatments in the study were five salt-based and two acid-based additives (see table 8). As untreated control the study had one with and one without soil contamination.

Table 8: Dosages and content of the additives in the study by Nadeau & Auerbach (2014)

Additive name	Type of additive	Content	Application rate
Kofasil Liquid	Salt -based	Sodium nitrite (245 g/kg), hexamine (164 g/kg)	2.5 mL/kg
Kofasil Liquid plus	Salt - based	Sodium nitrite (245 g/kg), sodium benzoate (50 g/kg), potassium sorbate (50 g/kg)	2.5 mL/kg
Kofasil LP	Salt - based	Sodium nitrate (202 g/kg), hexamine (135 g/kg), sodium benzoate (50 g/kg)	2.5 mL/kg
Kofasil Ultra KS	Salt-based	Sodium nitrite (165 g/kg), hexamine (110 g/kg), potassium sorbate (81 g/kg), sodium benzoate (22 g/kg), sodium propionate (8 g/kg)	2.5 mL/kg
Safesil	Salt-based	Sodium benzoate (180g/kg), potassium sorbate (74 g/kg), sodium nitrite (50 g/kg)	4.0 mL/kg
GrasAAT SX	Acid-based	Formic acid (400 g/kg), sodium formate (200 g/kg), propionic acid (200 g/kg), benzonic acid (10 g/kg), sorbic acid (10 g/kg)	4.0 mL/kg
Promyr XR680	Acid-based	Formic acid (488 g/kg) , propionic acid (184 g/kg), sodium (61 g/kg)	4.0 mL/kg

Treating the silage with the acid-based treatment resulted in a significant decrease in pH and the concentration of acetic acid and ethanol compared to the clostridia contaminated control. Butyric acid was not produced in additive treated silages, indicating inhibition of clostridia growth. Proteolysis in the additive treated silages was significantly lower compared to the untreated control, resulting in lower concentration of ammonia-nitrogen. DM losses decreased significantly in additive treated silage, the acid treatment had a lower loss compared to salt and control treatments. Average over additive treatment, the aerobic stability was better in the cut silage compared to the chopped silage. For fermentation quality the particle size had no effect

on lactic acid concentration, but acetic acid concentration was higher in the chopped silage. Clostridia contamination impact on silage fermentation can be eased by using chemical additives. Aerobic stability and fermentation of the silages were more influenced by usage of additives than by particle size. The authors concluded that particle size affect the fermentation to a lesser degree than chemical additives. Inhibiting clostridium fermentation can be achieved by using chemical additives (Nadeau & Auerbach, 2014).

The effect of DM concentration, particle size and use of additive on fermentation and proteolysis of the silage was studied by Slottner & Bertilsson (2004). Grass-clover silage was either precision chopped or cut and stored either in bales or steel silos (25 l). The DM concentrations used in the study were one low DM (~300 g/kg) and high DM (400 g/kg).
Four different additives were used, two acid-based additives, one salt-based additives and one inoculant (LAB; see table 9).

Table 9: Additive and application rate in Slottner & Bertilsson (2004)

Additive name	**Type of additive**	**Content**	**Application rate**
Formic acid	Acid-based	Formic acid (850 g/kg)	6 l /ton
Proens	Acid-based	Propionic acid (1/3), formic acid (2/3)	4 l/ton
Kofasil Ultra	Salt-based	Hexamethylenetetraamine (80 g/kg), sodium propionate (50 g/kg), sodium nitrite (120 g/kg), sodium benzoate (150 g/kg)	4 l/ton

The use of chemical additives resulted in a restricted fermentation; the salt-based additive had higher pH than the other additives. Additive use decreased the degradation of protein significantly. The study concluded that increased DM concentration and additive use reduced the protein degradation, which is similar to the results by Nadeau *et al.*, (2012b) were the content of NPN in salt-based additives was lower than in untreated silage. The untreated chopped silage had greater fermentation than cut material in bales at the same DM concentration (Slottner & Bertilsson, 2004) which also was shown by Nadeau and Auerbach (2014).

Table 10: Outline of the experiment. Dry-matter concentration of the forage, average particle size of the forage, type and dosage of additives used.

Sward type	DM of herbage (%)	Forage particle size (cm)	Additive treatments	Additive dosage (l/ton forage)
Clover dominated	41.4	5.7±3.4	Control treatment	0
			Salt -Half dose[1]	1.25
			Salt - Recommended dose[2]	2.5
			Acid - Half dose	2.5
			Acid - Recommended dose	5.0
		23.6±9.0	Control treatment	0
			Salt -Half dose	1.25
			Salt - Recommended dose	2.5
			Acid - Half dose	2.5
			Acid - Recommended dose	5.0
	24.8	4.8±2.9	Control treatment	0
			Salt -Half dose	1.25
			Salt - Recommended dose	2.5
			Acid - Half dose	2.5
			Acid - Recommended dose	5.0
		24.8±10.7	Control treatment	0
			Salt -Half dose	1.25
			Salt - Recommended dose	2.5
			Acid - Half dose	2.5
			Acid - Recommended dose	5.0
Grass	38.2	4.8 ±2.6	Control treatment	0
			Salt -Half dose	1.0
			Salt - Recommended dose	2.0
			Acid - Half dose	1.5
			Acid - Recommended dose	3.0
		24.2±11.7	Control treatment	0
			Salt -Half dose	1.0
			Salt - Recommended dose	2.0
			Acid - Half dose	1.5
			Acid - Recommended dose	3.0
	26.2	5.3± 2.5	Control treatment	0
			Salt -Half dose	1.0
			Salt - Recommended dose	2.0
			Acid - Half dose	1.5
			Acid - Recommended dose	3.0
		24.4± 10.4	Control treatment	0
			Salt -Half dose	1.0
			Salt - Recommended dose	2.0
			Acid - Half dose	1.5
			Acid - Recommended dose	3.0

[1] sodium nitrite, hexamine and sodium benzoate
[2]formic acid, propionic acid and salts of organic acids

Design of the trial

During 2014 two fields at Överstegården, Norra Lundby, Skara, Västra Götaland, Sweden were harvested during the second cut. The fields had different sward types, one clover dominated sward and one grass sward. The experiment had the following treatments for each sward type; DM concentration, forage particle size and additive type and dosage (See Table 10).

Forage particle size was either long or chopped. Additives used were the acid product Promyr NT570 (formic acid, propionic acid and salts of organic acids; Perstorp AB, Perstorp, Sweden and the salt-based additive Kofasil LP (sodium nitrite, hexamine and sodium benzoate; Addcon Europe GmbH) using two dosages of each additive for each sward type. The application rates of the additives were recommended dosage and half of the recommended dosage for each of the additives. The experimental unit was the 1.7-litre silos used, which were replicated three times. The experiment had a completely randomized design.

Harvest, wilting and particle size

The botanical compositions of the leys are shown in Table 11. Harvest occurred on 9 – 10 July. The swards were harvested with a mower (John Deere 730) and made into swaths by a rotary rake (Pöttinger Eurotop 771A). Half of each forage from the two sward types was passed through a baler (John Deere 678) and the other half of the forages were chopped by a precision chopper (Sahlströms 40 cubic Torps 480).

Table 11: Proportion (%) of grass and clover in the two sward types

Sward type	Grass	Red clover *Trifolium pretense*	White clover *Trifolium repens*
Clover dominated	27.5	65.9	6.3
Grass	96.0	-	3.5

The weather conditions during wilting were sunny and warm (25-27°C) and to extend the wilting time the forages were wilted in swaths.

The target DM concentrations were 25 % (low DM) 40% (high DM). High DM grass was mowed at 16.30 July 9 and wilted for 18 hours. The next day at 10.00 the harvested grass was transported to SLU Skara and packed into mini silos at 11.00. Clover of high DM concentration was mowed at 17.15 on the 8th of July, wilted for 17 hours and transported at 10.00 on the 9th of July to SLU Skara, wilted for 3 more hours before being packed into 1.7 litre laboratory silos at 14.00. The high DM concentrations of the sward types were 41.4 % for the clover and 38.2 % for the grass. Grass and clover of low DM concentrations were mowed 8.00-9.00 on July the 10th, wilted in swaths for 2 to 3 hours before being transported to SLU Skara. The low DM concentrations were 24.8% for the clover-grass and 26.3% for the grass.

For the study two forage particle sizes were used; long and chopped. The long particle size was produced by baling using 14 knives in the baler, and the chopped forage was produced by a precision chopper. Transportation of the forage from the fields to SLU Skara was done by car and trailer, which was covered by a tarpaulin. To prevent the risks for heating of the forage, ice packs were used during transport and during storage until packing into the silos.

Ensiling

The silage experiment was conducted at the Department of Animal Environment and Health, Swedish University of Agricultural Sciences, Skara, Sweden.120 laboratory silos (volume of 1.7 liter glass jar) were used for ensiling the grass and clover/grass forages, which were stored for 100 days. The silos were weighed before packing (including lid) and when the silo was filled and closed. The water locks on the lid of the laboratory silos enable the air to leave but not to enter. To be able to measure pH at day 3 of after harvest, every silo had a corresponding smaller silo (500 ml). The amount of additive applied to the forage was mixed with tap water to a total volume of 10 ml/kg of forage and sprinkled on the forage with a hand spray to ensure an even distribution of the additive to the forage, which was mixed well before filling of the silos. In the control treatment 10 ml of tap water/kg forage were added. The 500-ml silos were opened after 3 days and pH was measured at SLU Skara. 50 g of silage was mixed with 50 ml distilled water and stored in refrigerator (4 °C) overnight. The pH was measured on the water extract by a calibrated pH-meter (TES 1380). DM losses in the silage during 100 days of storage were determined by the weight difference at ensiling and at opening of the 1.7-litre silos divided by the dry weight of the wilted forage with addition of the factor 2.5 according to Weissbach (2005).

Determination of aerobic stability of the silages

Aerobic stability of the silage after 100 days of storage was analyzed according to Honig (1990). 350 to 400 g of silage, depending on the DM content, was loosely put in PVC-pipes, with small holes in the bottom for air flow. Wireless temperature loggers (Tinytag Talk 4014, Gemini, Chichester, UK) were inserted in the middle of the silage in the PVC pipes, the pipes were put into individual Styrofoam boxes that allowed airflow for 14 days in room temperature (20 °C). The number of days until the temperature of the silage increased 2 °C above ambient temperature of 20 °C was registered.

Determination of dry matter concentrations in wilted forage and silage

To determine the time of ensiling, to get the wanted DM concentrations, the forage was measured by microwave-oven. The method comprised of weighing in 100 g of cut forage from the field and drying it in a microwave-oven (700 W) in time-sessions (5 min, 3 min, 2 min, 1 min etc.) with stirring and weighing of the forage between the time sessions, until the weight of the forage did not decrease any more. At packing the DM concentration was determined by drying 200 g of forage at 105 °C for 24 h in a forced-air cabinet. The long cut forage was cut by scissors in the lab before drying. The DM concentration of the forage was determined by drying 100 g of forage at 60 °C for 21h followed by 105°C for 3 h. This DM concentration was corrected for volatile losses during fermentation (Weissbach and Strubelt, 2008).

Laboratory analysis

For microbiological analysis (Eurofins Food & Agro Sweden AB, Jönköping, Sweden) 150 g of the unensiled forage was analyzed for pH, and counts of live lactic acid bacteria (NMKL, 140, 1991), bacillus spores, enterobacteria, Escherichia coli, clostridia spores, total yeast (NMKL 98, 2005), and mould (NMKL 98). Enterobacteria analysis had an over limit of 6.2. Table 12 shows the microbiological content of the unensiled forage.

Table 12: Microbiological content of the wilted forage for the two sward types with the two different particle sizes and dry matter contents. The unit is log cfu/g if nothing else is specified.

	Clover dominated				Grass			
	Chopped		Long		Chopped		Long	
	High DM	Low DM	High DM	Low DM	High DM	Low DM	High DM	Low DM
pH	5.7	7.1	5.9	5.8	6.1	6.0	6.0	6.0
Lactic acid bacteria	4.4	6.3	4.8	5.8	5.0	6.3	3.4	4.5
Mould	5.3	5.5	5.4	5.5	5.3	5.2	4.7	5.6
Yeast	4.6	4.9	4.4	5.2	4.7	4.7	3.0	4.7
Escherichia coli	2.6	3.0	2.6	2.5	3.6	4.8	3.6	2.7
Enterobacteria	>6.2	6.2	5.7	5.5	>6.2	6.2	5.8	4.5
Bacillus spores	3.6	3.3	4.4	4.9	4.3	4.0	4.3	4.7
Clostridia spores	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0

Water-soluble carbohydrates (WSC) and crude protein of wilted forage and WSC, pH, ammonia-N, organic acids and alcohols of the silage were analyzed at the Central laboratory, Humboldt Universität zu Berlin, Germany. The WSC concentration was determined by the colormetric method with anthrone (Lengerken & Zimmermann, 1991) and the crude protein concentration was determined by multiplying the analyzed total nitrogen (Kjeldahl N method) by 6.25. Ammonia concentration was determined colorimetrically based on the Berthelot reaction by use of a continuous flow analyzer (SKALAR analytical B.V., Breda, Netherlands) and pH was determined potentiometrically using a calibrated pH electrode. Volatile fatty acids and ethanol were analyzed by gas chromatography according to Weiss (2001) and lactic acid was analyzed by HPLC according to Weiss & Kaiser (1995).

Analyses of ash, in vitro organic matter digestibility (IVOMD) and NDF of wilted forages were conducted at the research laboratory of the Department of Animal Nutrition and Management, SLU Uppsala. Ash was determined at 525ºC for 16 hours. The IVOMD was analyzed by the VOS method by incubation of 0.5 g sample at 38 ºC for 96 hours in 49 ml buffer and 1 ml rumen fluid (IVOMD; Lindgren, 1979) and the metabolizable energy was calculated from IVOMD (Lindgren, 1983, 1988). To determine the ash content the wilted forage was incinerated at 550 °C for 16 h. The NDF content was analyzed according to Chai and Udén (1991) with addition of alpha-amylase and sodium sulphite to the ND solution. The content of nutrients in the wilted forage is shown in Table 13.

Table 13: Nutrient content of the wilted forage for the two sward types with the two different particle sizes and dry matter contents. The unit is % of DM if nothing else is specified.

	Clover dominated				Grass			
	Chopped		Long		Chopped		Long	
	High DM	Low DM	High DM	Low DM	High DM	Low DM	High DM	Low DM
DM, %	41.2	24.1	39.5	24.3	40.7	27.7	42.1	18.1
CP[1]	18.0	19.2	18.6	20.0	18.9	16.2	20.4	17.3
WSC[2]	9.1	6.5	8.5	6.8	9.6	9.0	9.9	8.6
NDF[3]	39.0	42.8	42.1	41.8	50.7	53.6	49.6	54.0
IVOMD[4], %	79.5	82.0	81.3	83.4	87.3	85.9	86.6	85.2
Ash	9.0	8.9	9.1	9.1	9.1	7.4	7.4	7.5
ME[5], MJ/kg DM	10.3	10.6	10.5	10.7	11.0	10.9	11.1	10.8

[1] Crude protein

[2] Water soluble carbohydrate

[3] Neutral detergent fibre

[4] In vitro organic matter digestibility

[5] Metabolizable energy

Statistical model

Data on silage fermentation characteristics and aerobic stability were analyzed by sward type and DM content. The statistical model used for analysis of the data included fixed effects of forage particle size and additive treatment in PROC GLM of SAS (vers. 9). Three replications (=silo) per treatment were used. Main effects of treatment and particle size and their interactions were analyzed for the variables studied for each sward type at each DM concentration.

When the *F*-test was significant ($P \leq 0.05$) pairwise comparisons were done between the least square means with Tukey´s t-test adjustment.

Results

Tables 12-15 shows the effect of particle length and additive and their interactions on fermentation characteristics and aerobic stability in clover-grass and grass silages of high and low dry matter contents.

Clover-grass silage of high dry-matter content

The clover-grass silage classified as high DM silage had DM contents of 40 to 42% (Table 14). The content of WSC in the clover-grass silage was lower in the chopped than in the long silage, when averaged over additive treatments (5.13 vs. 7.65% of DM, $P < 0.001$). Silage treated with the acid at 5.0 l/t had higher WSC content than the control and salt-treated silages, when averaged over particle sizes ($P < 0.001$). The acid treatment had the lowest lactic acid concentrations in both long and chopped clover-grass silage and the effect was dose dependent ($P < 0.01$). The salt-based additive had similar lactic acid concentrations as the control in the chopped silage whereas the additive caused a decrease in the lactic acid content in the long clover-grass silage, but the effect was not dose dependent. Concentrations of lactic acid and acetic acid were higher in the chopped than in the long silage (5.41 vs. 4.30% of DM and 1.26 vs. 0.92% of DM, respectively, $P < 0.001$). Silage treated with the acid at 5.0 l/t had the lowest acetic acid content followed by the acid treatment at 2.5 l/t, which had lower acetic acid content than the untreated and salt-treated silages, which did not differ ($P < 0.001$, Table 14)

Clover-grass silage treated with the acid product, contained small amounts of propionic acid, which originated from the additive, which contained propionic acid in addition to formic acid and salts of acids (Table 12). The ethanol content was similar between treatments. Long silage had higher NH_3-N content than chopped silage, when averaged over treatments (8.7 vs. 6.8% of total N; $P < 0.001$). Silage treated with the salt-based additive at 2.5 l/t had the lowest NH_3-N concentration whereas the untreated control silage had the highest NH_3-N content ($P < 0.001$). Silage treated with the salt-based additive at 1.25 l/t had similar NH_3-N content as the silage treated with the acid at 5.0 l/t. The effect of the additives on silage NH_3-N content was dose dependent ($P < 0.001$). The pH after 3 days of ensiling ($pH_{\text{three days}}$) and the pH at the opening of the silos (pH_{final}) after 100 days of ensiling was lower in the chopped silage compared to the long silage (5.34 vs. 5.42 and 4.55 vs. 4.93, respectively, $P < 0.001$). Clover-grass silage treated with the salt-based additive had lower pH at 3 days of ensiling than the control, whereas the acid-treated clover-grass silage had similar pH as the control silage. After 100 days of ensiling, the pH was lower in the control than in both the salt-treated silages and in the silage treated with 5.0 l/t of the acid ($P < 0.001$). Clover-grass silage treated with the acid at 5.0 l/t had lower DM losses during storage than the untreated control silage, whereas the other additive treatments did not significantly decrease DM losses compared to the control silage ($P < 0.05$). Long silage had lower DM losses than chopped silage (4.7 vs. 5.4%, $P < 0.01$). All silages were aerobically stable (Table 14).

Table 14: Effects of particle size (S) and additive treatment (T) on fermentation characteristics and aerobic stability of clover-grass silage of high DM content. Least-square means are expressed in % of DM unless stated otherwise (n=6).

	Chopped					Long					SEM [1]	*P*- value S*T	*P*-value S	*P*-value T
	C[2]	Salt-H[3]	Salt-R[4]	Acid-H[5]	Acid-R[6]	C	Salt-H	Salt-R	Acid-H	Acid-R				
DM, %	41.5^{ab}	41.3^{abc}	41.1^{abc}	41.2^{abc}	41.1^{abc}	40.8^{bc}	42.6^{a}	41.6^{ab}	39.6^{c}	41.4^{bc}	0.36	0.007	NS	<0.001
pH $_{\text{three days}}$	5.48	4.97	5.24	5.59	5.41	5.72	5.11	5.40	5.76	5.70	0.028	0.081	<0.001	<0.001
pH $_{\text{final}}$	4.48	4.53	4.61	4.54	4.60	4.80	4.98	4.95	4.91	5.01	0.027	NS	<0.001	<0.001
LA[7]	6.48^{a}	6.52^{a}	6.07^{ab}	4.48^{cd}	3.48^{e}	5.65^{b}	4.85^{c}	4.60^{c}	3.87^{de}	2.54^{f}	0.122	0.001	<0.001	<0.001
AA[8]	1.32	1.44	1.38	1.16	0.98	1.13	1.05	1.03	0.85	0.56	0.055	NS	<0.001	<0.001
PA[9]	0.00^{c}	0.00^{c}	0.01^{c}	0.08^{b}	0.15^{a}	0.00^{c}	0.02^{c}	0.01^{c}	0.08^{b}	0.12^{a}	0.006	0.020	NS	<0.001
BA[10]	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.001	-	-	-
Ethanol	0.48	0.67	0.43	0.67	0.55	0.51	0.55	0.42	0.32	0.27	0.20	NS	NS	NS
WSC[11]	4.45	3.67	5.29	4.66	7.58	4.90	6.65	5.85	8.87	11.99	1.05	NS	0.001	<0.001
ASTA h[12]	336	307	336	307	336	324	336	336	336	336	13.4	NS	NS	NS
ASTA d[13]	14.0	12.8	14.0	12.8	14.0	13.5	14.0	14.0	14.0	14.0	0.56	NS	NS	NS
pH $_{\text{ASTA}}$[14]	4.45	5.61	4.55	5.26	4.94	5.22	4.87	4.78	4.76	4.81	0.418	NS	NS	NS
Clostridia[15]	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	0.00	-	-	-
NH_3-N[16]	8.0	6.5	5.6	7.3	6.5	9.9	8.5	7.8	9.0	8.0	0.17	NS	<0.001	<0.001
DM loss, %	5.6	6.0	5.1	5.7	4.7	5.8	4.5	4.5	4.6	3.9	0.37	NS	0.008	0.023

[1]Standard error of the mean
[2]Control
[3] Salt-based (sodium nitrite, hexamine and sodium benzoate) additive half dose 1.25 l/t
[4]Salt-based (sodium nitrite, hexamine and sodium benzoate) additive recommended dose, 2.5 l/t
[5] Acid (formic acid, propionic acid and salts of organic acids) half dose, 2.5 l/t
[6] Acid (formic acid, propionic acid and salts of organic acids) recommended dose, 5.0 l/t
[7]Lactic acid
[8]Acetic acid
[9]Propionic acid
[10] Total Butyric acid
[11]Water soluble carbohydrates
[12] ASTA: Aerobic stability test, hours before temperature increase of 2°C
[13] ASTA: Aerobic stability test, days before temperature increase of 2°C
[14]pH after aeration
[15] cfu of Clostridium spores
[16]Ammonia nitrogen % of total N
a,b,c,d,e mean values with different superscripts differ significantly ($P < 0.05$)
NS = none significance

Table 15: Effects of particle size (S) and additive treatment (T) on fermentation characteristics and aerobic stability of clover-grass silage of low DM content. Least-square means are expressed in % of DM unless stated otherwise (n=6).

	Chopped					Long					SEM [1]	P-Value S*T	P-Value S	P-value T
	C[2]	Salt-H[3]	Salt-R[4]	Acid-H[5]	Acid-R[6]	C	Salt-H	Salt-R	Acid-H	Acid-R				
DM, %	23.9	23.9	23.0	24.4	24.7	25.6	26.7	26.7	26.0	25.1	0.529	0.058	<0.001	NS
pH $_{\text{three days}}$	4.57^{ef}	4.76^{d}	4.45^{f}	4.63^{e}	4.55^{ef}	4.97^{bc}	5.03^{ab}	4.95^{bc}	5.12^{a}	4.89^{c}	0.025	0.001	<0.001	<0.001
pH $_{\text{final}}$	4.26	4.20	4.24	4.25	4.19	4.48	4.44	4.42	4.32	4.28	0.037	0.098	<0.001	NS
LA[7]	9.17^{b}	11.71^{a}	9.27^{b}	7.89^{cd}	5.89^{f}	10.69^{a}	8.26^{bcd}	7.47^{de}	7.89^{cd}	6.77^{ef}	0.217	<0.001	<0.0005	<0.001
AA[8]	2.48^{ab}	2.09^{abc}	2.26^{abc}	1.54^{cde}	0.96^{de}	2.89^{a}	2.22^{abc}	1.74^{bcde}	0.92^{e}	0.97^{de}	0.170	0.029	NS	<0.001
PA[9]	0.04	0.00	0.024	0.18	0.27	0.04	0.01	0.02	0.13	0.22	0.013	0.062	0.033	<0.001
BA[10]	0.01	0.014	0.10	0.01	0.00	0.03	0.00	0.00	0.08	0.00	0.029	0.099	NS	NS
Ethanol	0.51^{a}	0.19^{b}	0.15^{bc}	0.15^{bc}	0.09^{c}	0.43^{a}	0.21^{b}	0.14^{bc}	0.20^{b}	0.16^{bc}	0.018	0.007	NS	<0.001
WSC[11]	0.84^{e}	0.85^{e}	0.80^{e}	2.25^{cd}	5.19^{a}	0.61^{e}	0.84^{e}	1.38^{de}	3.21^{bc}	3.66^{b}	0.231	<0.001	NS	<0.001
ASTA h[12]	168	211	213	191	231	336	336	303	246	336	36.9	NS	<0.001	NS
ASTA d[13]	7.0	8.8	8.9	8.0	9.6	14.0	14.0	12.6	10.2	14.0	1.54	NS	<0.001	NS
pH $_{\text{ASTA}}$[14]	8.55	8.30	7.61	6.72	7.26	4.44	4.49	5.46	5.84	4.21	0.69	NS	<0.001	NS
Clostridia[15]	<1.0	<1.0	1.2	<1.0	<1.0	<1.0	<1.0	1.1	2.4	<1.0	0.51	NS	NS	NS
NH_3-N[16]	9.9	7.7	6.4	8.4	6.9	10.8	8.7	6.5	8.5	7.6	0.35	NS	0.019	<0.001
DM loss, %	6.4	5.8	6.2	5.6	5.0	6.5	6.6	6.0	5.4	5.0	0.23	NS	NS	0.001

[1]Standard error of the mean
[2]Control
[3] Salt-based (sodium nitrite, hexamine and sodium benzoate) additive half dose 1.25 l/t
[4]Salt-based (sodium nitrite, hexamine and sodium benzoate) additive recommended dose, 2.5 l/t
[5] Acid (formic acid, propionic acid and salts of organic acids) half dose, 2.5 l/t
[6] Acid (formic acid, propionic acid and salts of organic acids) recommended dose, 5.0 l/t
[7]Lactic acid
[8]Acetic acid
[9]Propionic acid
[10] Total Butyric acid
[11]Water soluble carbohydrates
[12] ASTA: Aerobic stability test, hours before temperature increase of 2°C
[13] ASTA: Aerobic stability test, days before temperature increase of 2°C
[14]pH after aeration
[15] cfu of Clostridium spores
[16]Ammonia nitrogen % of total N
a,b,c,d,e, f mean values with different superscripts differ significantly ($P < 0.05$)
NS = none significance

Clover-grass silage of low dry-matter content

The clover-grass silage classified as low DM silage had DM contents from 23% to 26% (Table 15). Silage treated with the acid at 5.0 l/t had higher WSC content than the acid treatment at 2.5 l/ton ($P < 0.001$), which both had higher WSC concentrations than the salt-treated and the untreated clover-grass silages, which did not differ. The acid treatment at 5.0 l/t had the lowest lactic acid concentration in chopped clover-grass silage and the effect was dose dependent. There also was a lower lactic acid content in the acid-treated long silage at 5.0 l/ton than at 2.5 l/ton ($P < 0.05$). The salt-based additive at 2.5 l/t had similar lactic acid concentration as the control, whereas the salt treatment at 1.25 l/t had higher lactic acid concentration than the control in the chopped silage. The salt-based additive at both dosages resulted in silage with lower lactic acid concentrations than in the control, when the silage was long, which also was true for the acid-treated silage. When averaged over additive treatments, the lactic acid concentration was higher in the chopped than in the long silage (8.79 vs. 8.21% of DM, $P < 0.001$), whereas the opposite was true for the DM concentration (24.0 and 26.0%, $P < 0.001$). The acid treated silages had lower acetic acid concentrations than the control silage at both particle sizes. The salt-treated silage at 2.5 l/t of long silage had lower acetic acid concentration than the long control silage ($P < 0.05$; Table 15).

Clover-grass silage treated with the acid product, contained small amounts of propionic acid, which partly originated from the additive (Table 15). Butyric acid concentration was low in the silage. No significant differences in Clostridia spores were found between the treatments although there was a numerically higher spore counts in the acid-treated long silage at 2.5 l/t compared to the other treatments. All the additive treatments decreased the ethanol content of the silage ($P < 0.01$).

Long silage had higher NH_3-N content than chopped silage, when averaged over treatments (8.4 vs. 7.8% of total N; $P < 0.05$). Treated silages had lower NH_3-N concentrations than the untreated silages, when averaged over particle sizes, and the decrease was dose dependent for both the salt and the acid ($P < 0.001$). The pH after 3 days of ensiling ($pH_{\text{three days}}$) was higher in silages treated with half dosages of the salt and the acid compared to the control silages.

The final pH after 100 days of ensiling was lower in the chopped silage compared to the long silage (4.23 vs. 4.39, $P < 0.001$). Clover-grass silage treated with the acid at 5.0 l/t had lower pH at 100 days of ensiling than the untreated control silage, when averaged over particle sizes ($P < 0.01$). Furthermore, the acid treated silage at 5.0 l/t had lower DM losses than the control silage, when averaged over particle sizes ($P < 0.001$). Long silage was more aerobically stable than chopped silage, when averaged over additive treatments (13.0 vs. 8.5 days, $P < 0.001$). This difference in aerobic stability resulted in differences in silage pH after the aeration between long and chopped silage (4.89 vs. 7.69, $P < 0.001$; Table 15).

Grass silage of high dry-matter content

The high-DM grass silage had DM contents from 36% to 39% (Table 16). Silage treated with the acid at 3.0 l/t had higher WSC content than the control silage ($P < 0.001$) , whereas the WSC content of the other treatments did not differ from the control but had a moderate content of 4 to 6% of DM. The acid treatment at 3.0 l/t had the lowest lactic acid concentration in chopped grass silage and the effect was dose dependent. There also was a lower lactic acid content in the acid-treated long silage at 3.0 l/ton compared to 1.5 l/ton ($P < 0.001$). The salt-based additive at both dosages had similar lactic acid concentrations as the control silages of both particle sizes. The acid treated silage had lower acetic acid concentration than the control silage of the chopped particle size and the salt treatment at 2.5 l/t of long silage had a lower

acetic acid content than the long control silage ($P < 0.05$; Table 16). Furthermore, the acetic acid content was lower in the chopped than in the long silage, when averaged over additive treatments (0.75 vs. 0.94% of DM, $P < 0.01$; Table 16).

Grass silage treated with the acid product, contained small amounts of propionic acid, which partly originated from the additive (Table 16). Chopped grass silage treated with the salt-based additive at 2.0 l/t had lower NH_3-N content than the chopped control silage ($P < 0.001$). All additive treatments decreased the NH_3-N content of long grass silage with the greatest effects by the salt and the acid at the highest application rates ($P < 0.001$). Long silage had higher NH_3-N content than chopped silage, when averaged over treatments (11.6 vs. 9.3% of total N; $P < 0.001$). All the additive treatments, except the acid at 1.5 l/t, decreased pH after 3 days of ensiling ($pH_{\text{three days}}$) , when it was chopped ($P = 0.001$), whereas no effect of the treatments on the pH after 3 days of ensiling was found in the long silage. The pH at opening of the silos after 100 days of storage was not affected by additive treatments. There were small but significant differences in pH after 3 days of ensiling and of final pH between particle sizes of the silages. Chopped silage had lower pH than long silage, when averaged over additive treatments ($pH_{\text{three days}}$: 5.67 vs. 5.78, $P < 0.01$; pH_{final}: 4.35 vs. 4.45, $P < 0.01$; Table 16).

Chopped control silage contained yeast that was decreased significantly by the acid treatment at 3.0 l/t ($P < 0.05$; Table 16). No differences in ethanol contents and DM losses could be found between treatments. Although the chopped grass silage contained more yeast than the long silage (2.4 vs. 1.3 cfu/g, $P < 0.05$), the chopped silage was more aerobically stable than the long silage, when averaged over additive treatments (11.3 vs. 7.2 days, $P < 0.001$). This difference in aerobic stability resulted in differences in silage pH after the aeration between chopped and long silage (5.67 vs. 8.32, $P < 0.001$; Table 16). Both the salt at 2.0 l/t and the acid at 3.0 l/t improved the aerobic stability of the silages as shown by the significant *P*-value for treatments averaged across particle sizes (10.8 and 10.9 days vs. 7.2 days, $P < 0.05$; Table 16).

Table 16: Effects of particle size (S) and additive treatment (T) on fermentation characteristics and aerobic stability of grass silage of high DM content. Least-square means are expressed in % of DM unless stated otherwise (n=6).

	Chopped					Long					SEM [1]	P-Value S*T	P-Value S	P-value T
	C[2]	Salt-H[3]	Salt-R[4]	Acid-H[5]	Acid-R[6]	C	Salt-H	Salt-R	Acid-H	Acid-R				
DM, %	39.4	39.3	39.0	39.0	39.7	36.4	37.9	38.3	36.4	36.7	0.623	NS	<0.001	NS
pH $_{\text{three days}}$	5.94^{a}	5.49^{d}	5.64^{bcd}	5.77^{abc}	5.53^{cd}	5.74^{abcd}	5.71^{abcd}	5.74^{abcd}	5.82^{ab}	5.88^{ab}	0.052	0.001	0.005	0.0013
pH $_{\text{final}}$	4.37^{ab}	4.19^{b}	4.42^{a}	4.35^{ab}	4.44^{a}	4.39^{ab}	4.52^{a}	4.47^{a}	4.40^{ab}	4.45^{a}	0.044	0.008	0.003	NS
LA[7]	6.00^{abc}	6.75^{a}	5.66^{bcd}	6.09^{abc}	5.04^{d}	6.36^{ab}	6.16^{abc}	6.42^{ab}	6.31^{ab}	5.52^{cd}	0.154	0.004	0.021	<0.001
AA[8]	0.84^{abc}	0.83^{abc}	1.05^{ab}	0.61^{bc}	0.42^{c}	1.23^{a}	0.85^{abc}	0.89^{ab}	0.93^{ab}	0.80^{abc}	0.088	0.018	0.003	<0.001
PA[9]	0.00^{e}	0.01^{e}	0.02^{cde}	0.06^{bc}	0.09^{a}	0.04^{cd}	0.00^{e}	0.01^{de}	0.05^{bc}	0.07^{ab}	0.006	0.001	NS	<0.001
Ethanol	0.29	0.32	0.50	0.39	0.45	0.43	0.23	0.22	0.37	0.29	0.140	NS	NS	NS
WSC[11]	4.68	4.82	4.10	5.84	8.82	3.64	5.97	6.05	6.02	8.37	0.696	NS	NS	<0.0001
ASTA h[12]	188	203	336	289	336	156	170	180	178	185	30.8	NS	<0.001	0.025
ASTA d[13]	7.8	8.5	14.0	12.1	14.0	6.5	7.1	7.5	7.4	7.7	1.28	NS	<0.001	0.025
pH $_{\text{ASTA}}$[14]	7.30	6.62	4.52	5.44	4.47	8.40	8.34	8.25	8.31	8.33	0.656	0.183	<0.001	NS
Yeast[15]	4.4^{a}	3.5^{ab}	1.6^{ab}	1.5^{ab}	1.0^{b}	1.0^{b}	1.1^{b}	2.0^{ab}	1.2^{b}	1.4^{ab}	0.61	0.034	0.011	0.033
NH_3-N[16]	10.3^{bc}	9.3^{cd}	7.6^{d}	10.0^{c}	9.1^{cd}	15.1^{a}	11.7^{b}	9.5^{c}	12.0^{b}	9.8^{c}	0.35	<0.001	<0.001	<0.001
DM loss	5.2	5.0	6.5	5.2	4.8	5.7	5.5	5.0	5.3	5.0	0.58	NS	NS	NS

[1]Standard error of the mean
[2]Control
[3]Salt-based additive (sodium nitrite, hexamine and sodium benzoate) half dose, 1.0 l/t
[4]Salt-based additive (sodium nitrite, hexamine and sodium benzoate) recommended dose, 2.0 l/t
[5] Acid (formic acid, propionic acid and salts of organic acids)half dose, 1.5 l/t
[6] Acid (formic acid, propionic acid and salts of organic acids) recommended dose, 3.0 l/t
[7]Lactic acid
[8]Acetic acid
[9]Propionic acid
[10] Total Butyric acid
[11]Water soluble carbohydrate
[12] ASTA: Aerobic stability test, hours before temperature increase of 2°C
[13] ASTA: Aerobic stability test, days before temperature increase of 2°C
[14]pH after aeration
[15] cfu of yeast
[16]Ammonia nitrogen % of total N
a,b,c,d,e mean values with different superscripts differ significantly ($P < 0.05$)
NS = none significance

Table 17: Effects of particle size (S) and additive treatment (T) on fermentation characteristics and aerobic stability of grass silage of low DM content. Least-square means are expressed in % of DM unless stated otherwise (n=6).

	Chopped					Long					SEM [1]	P-Value S*T	P-Value S	P-value T
	C[2]	Salt-H[3]	Salt-R[4]	Acid-H[5]	Acid-R[6]	C	Salt-H	Salt-R	Acid-H	Acid-R				
DM %	26.5^{b}	26.5^{b}	27.1^{ab}	26.2^{b}	26.3^{b}	26.7^{b}	25.9^{b}	26.5^{b}	27.9^{a}	26.9^{ab}	0.247	0.0005	NS	0.0386
pH $_{\text{three days}}$	4.45^{de}	4.35^{ef}	4.32^{f}	4.54^{d}	4.45^{def}	5.03^{ab}	5.10^{a}	5.02^{ab}	4.95^{b}	4.79^{c}	0.025	<0.001	<0.001	0.0002
pH $_{\text{final}}$	3.92^{e}	3.99^{d}	3.98^{d}	3.88^{ef}	3.83^{f}	4.22^{a}	4.16^{b}	4.18^{ab}	4.15^{bc}	4.09^{c}	0.012	0.001	<0.001	<0.001
LA[7]	7.33^{ef}	7.96^{abcde}	7.36^{cdef}	8.46^{a}	7.57^{bcde}	8.35^{a}	7.83^{abcde}	7.57^{bcde}	8.09^{ab}	6.80^{f}	0.140	<0.001	NS	<0.001
AA[8]	0.85	1.08	1.04	0.83	0.59	1.38	1.43	1.33	0.84	0.74	0.1233	NS	0.0029	0.0004
PA[9]	0.00^{d}	0.00^{d}	0.00^{d}	0.09^{b}	0.14^{a}	0.03^{c}	0.00^{d}	0.01^{cd}	0.07^{b}	0.12^{a}	0.0038	<0.001	NS	<0.001
Ethanol	0.45	0.38	0.12	0.44	0.27	0.46	0.47	0.22	0.55	0.60	0.0539	0.0903	0.0011	<0.001
WSC[11]	2.01	1.69	2.35	2.72	5.04	1.02	1.26	1.58	2.68	4.20	0.172	0.0835	<0.001	<0.001
ASTA h[12]	104^{ab}	87^{ab}	183^{a}	132^{ab}	159^{ab}	158^{ab}	203^{a}	136^{ab}	150^{ab}	33^{b}	27.6	0.003	NS	NS
ASTA d[13]	4.3^{ab}	3.6^{ab}	7.6^{a}	5.5^{ab}	6.6^{ab}	6.6^{ab}	8.5^{a}	5.7^{ab}	6.2^{ab}	1.4^{b}	1.15	0.003	NS	NS
pH $_{\text{ASTA}}$[14]	8.26	7.96	8.05	8.14	8.06	8.44	8.36	8.45	8.17	8.40	0.090	0.210	<0.001	NS
Yeast[15]	4.6^{ab}	5.0^{ab}	0.7^{c}	4.5^{ab}	3.8^{abc}	2.3^{bc}	4.0^{ab}	2.2^{bc}	4.7^{ab}	5.8^{a}	0.63	0.017	NS	0.0002
NH_3-N[16]	11.2	9.93	10.0	10.4	10.1	9.7	10.1	9.1	10.4	10.1	0.39	NS	0.093	NS
DM loss	5.5	5.8	4.7	5.5	4.8	6.4	6.4	5.4	6.3	6.4	0.28	NS	0.0001	0.023

[1]Standard error of the mean
[2]Control
[3] Salt-based additive half dose, 1.0 l/t
[4]Salt-based additive recommended dose, 2.0 l/t
[5] Acid half dose, 1.5 l/t
[6] Acid recommended dose, 3.0 l/t
[7]Lactic acid
[8]Acetic acid
[9]Propionic acid
[10] Total Butyric acid
[11]Water soluble carbohydrates
[12] ASTA: Aerobic stability test, hours before temperature increase of 2°C
[13] ASTA: Aerobic stability test, days before temperature increase of 2°C
[14]pH after aeration
[15] cfu of yeast
[16]Ammonia nitrogen % of total N
a,b,c,d,e,f mean values with different superscripts differ significantly ($P < 0.05$)
NS = none significance

Grass silage of low dry-matter content

The low-DM grass silage had DM contents from 26% to 28% (Table 17). Silage treated with the acid had greater WSC content than the control silage, whereas the WSC content of the salt treatment did not differ from the control, when averaged across silage particle sizes. Also, the WSC content of the acid treated grass silage was higher for the application at 3.0 l/t compared to 1.5 l/t ($P < 0.001$). The acid-treated chopped grass silage had greater lactic acid content than the other treatments for chopped silage. For the long silage, all additive treatments, except the acid treatment at 1.5 l/t, had lower lactic acid content than the control silage. For both particle sizes, the lactic acid content decreased with increased application rate of the acid ($P < 0.001$). The acetic acid content was lowest for the acid-treated silage at the dosage of 3.0 l/t, when averaged across silage particle sizes ($P < 0.001$). When averaged across additive treatments, the chopped silage had a greater content of WSC (2.76 vs. 2.15% of DM, $P < 0.001$) and a lower content of acetic acid than the long grass silage (0.88 vs. 1.14% of DM, $P < 0.01$).

Grass silage treated with the acid product, contained lesser amounts of propionic acid, which partly originated from the additive (Table 17). Butyric acid was not present in the silages. No differences in NH_3-N content were found between additive treatments and silage particle sizes.

Silage pH after 3 days of fermentation was decreased by the salt-based additive at 2.0 l/t when the silage was chopped, whereas the acid at 3.0 l/t decreased the pH after 3 days of ensiling of the long silage ($P < 0.001$; Table 17). Silage pH after 100 days of storage was lower for the acid treatment at 3.0 l/t than for the control treatment, when the silage was chopped, whereas almost all additive treatments decreased the pH of the control silage, when the silage was unchopped ($P < 0.001$). Chopped silage had lower pH than long silage, when averaged over additive treatments ($pH_{\text{three days}}$: 4.42 vs. 4.98, $P < 0.001$; pH_{final}: 3.92 vs. 4.17, $P < 0.001$)

Chopped control silage contained yeast that was decreased significantly by the salt-based additive treatment at 2.0 l/t, whereas the acid treatment at 3.0 l/t increased the yeast content compared to the control silage, when the silage was ensiled long ($P < 0.05$; Table 17). When averaged across silage particle sizes, the salt-based additive at 2.0 l/t decreased the ethanol content of the control silage ($P < 0.001$). Furthermore, the ethanol content of the silage was lower in chopped than in long silage, when averaged across additive treatments (0.33 vs. 0.46% of DM, $P < 0.01$). The DM losses during storage were less for the grass silage treated with the salt-based additive at 2.0 l/t compared to the control silage, when averaged across silage particle sizes (5.1 vs. 6.0%, $P < 0.05$). Also, the DM losses were smaller in the chopped than in the long grass silage, when averaged across additive treatments (5.3 vs. 6.2% of DM, $P < 0.001$). The salt-based additive at 1.0 l/t produced a more aerobically stable silage than the acid at 3.0 l/t, when the grass was ensiled long (8.5 days vs. 1.4 days, $P < 0.01$; Table 17).

Discussion

This study aimed to examine the effects of particle size and chemical additives at two dosages on fermentation characteristics and aerobic stability of clover-grass and grass silages at low and high DM concentrations.

Clover-dominated silage

High DM content

By chopping the clover-grass silage, the acidification can be faster due to that WSC is easily available for LAB with the leakage of cell sap (Pauly & Lingvall, 1999; McEniry *et al*., 2008). The chopped clover-grass silage had, compared to the long silage, a lower content of WSC, higher concentration of lactic and acetic acid and a lower $pH_{three\ days}$ and pH_{final}. Chopping the silage seems to decrease the proteolysis (Pauly & Lingvall, 1999; Pahlow *et al*., 2003), as reflected by the decreased concentration of ammonia nitrogen in the chopped silage in this experiment.

The chopped clover-grass silage had a greater loss of DM than the long silage, which indicate that microbial activity was prolonged (Shao *et al.,* 2005; Kung, 2010) during the ensiling phase (McDonald et al., 1991; Pahlow *et al*., 2003). Packing the silage is essential for a low DM loss, and the result indicate low DM with air-pockets, which delays the ensiling phase. The easily available nutrients for LAB and increased packing density (McEniry et al., 2008) should decrease the loss of DM in chopped silage but the high DM content of 40-42% probably made the herbage more difficult to pack, leaving air pockets in the silo, resulting in lower density (McEniry et al., 2008). This is supported by no differences in DM losses between long and chopped silages in clover-grass silage of low DM content.

The acid contains formic acid, which has a direct acidification effect (Kung *et al.,*2003; Kung 2010; Knicky & Sprödly, 2009). The acid – R silage had restricted fermentation of WSC to lactic acid and acetic acid, resulting in a lower DM loss compared to the control silage. The acid decreased the lactic acid concentration regardless of particle size compared to the control. The lowest concentration of lactic acid was observed in the unchopped acid treated silage at recommended dose,

The dose of the acid treatment had effect on the concentration of lactic acid. Treatment with Acid – R resulted in a lower concentration compared Acid - H. The loss of DM of the silage treated with half the dose was on the same level as the control treated silage, suggesting that inhibition of LAB was dose dependent. This corresponds with the conclusion by Randby (2000), who showed that lactic acid concentration correlated negatively with application rate of the acid. Formic acid dissociates to a higher rate compared to the lactic, acetic and propionic acid (Brucie, 2006), resulting in a lower pH. As shown in this experiment, the acidification is dose dependent with a lower silage pH at recommended compared to half recommended dosage, which corresponds with the results by Rinne *et al*. (2016).

All additive treatments decreased proteolysis, as measured by content of ammonia-N, compared to the control. The silage treated with Salt – R had the lowest concentration of ammonia nitrogen, and the effect was dose dependent. These results agree with results by Nadeau & Auerbach (2014) where the salt treatments had lower ammonia nitrogen than the acid treatments. Similarly, Slottner & Bertilsson (2004) concluded that chemical additives (salt and acid additives) and high DM concentrations decrease proteolysis in the silage. The effect of acid on ammonium nitrogen was also dose dependent. A good fast acidification is important for inhibition of microbial growth, thereby decreasing proteolysis (Weinberg & Muck, 1996).

The salt treatment had a lower $pH_{three\ days}$ compared to the other treatments, but after ensiling the pH was higher than the untreated control. Slottner & Bertilsson (2004) showed similar results, where the pH was high in the salt-treated silage. The salt treatment contains antimicrobial and antifungal substances (Kung et al., 2003) which results in stable conditions of the silage despite a somewhat higher pH due to a lower acid production (Woolford, 1975; Slottner & Bertilsson, 2004; Kung, 2010).

Low DM-concentration

The chopped clover-grass silage was of good quality with low pH and high concentration of lactic acid compared to the long silage. Nadeau & Auerbach (2014) showed that chopping clover-grass silage did not result in increased concentration of lactic acid but increased the concentration of acetic acid. The aerobic stability on the other hand was significantly better in the long silage compared to the chopped clover-grass silage (8.5 days vs 13.0 days), which agree with the results in this experiment. High concentration of acetic acid inhibits yeast (Weinberg & Muck, 1996). The pH after aeration in this study was also higher in the chopped silage, indicating deterioration of the silage during aeration. Often yeast initiate the deterioration (Borreani & Tabacco, 2010), but in this study yeast was not analyzed in the clover-grass silage, only in the wilted forage before ensiling. The clover-grass herbage before ensiling contained yeast, spores of bacillus and enterobacteria. Ethanol concentration, an indicator of yeast activity (Pahlow et al., 2003), did not differ between particle sizes, when averaged over additive treatments. Residual WSC and lactic acid have been linked to lowered aerobic stability, as they are easily available nutrients to yeast (Wrobel et al.,2008; Nadeau and Auerbach, 2013). The concentration of WSC in the clover-grass silage did not differ between the particle sizes but the concentration of lactic acid was greater in chopped than in long silage. Lactic acid is used as substrate for yeast and is degraded to carbon dioxide, water and heat (Pahlow et al.,2003; Kung, 2010). The degradation of lactic acid increases the pH enabling other bacteria and fungi to grow and proliferate (Borreani & Tabacco, 2010). Silage with high concentration of lactic acid and low concentration of acetic acid decrease the aerobic stability, shown in Nadeau and Auerbach (2013). Another factor that influences the aerobic stability is density and porosity (Savole & Jofriet, 2003), with low density/high porosity, the aerobic stability is lowered. The silos in this study were densely packed at filling and air ingress was eliminated by use of water locks on the lid.

Additives, both acid and salt, used in the clover-grass silage with low DM had a favorable effect on decreasing protein degradation (Slottner & Bertilsson, 2004; Nadeau & Auerbach, 2014) and decreased the concentration of ethanol. The additives used in this study contained antimicrobial and antifungal substances (Kung, 2001). The effect on protein degradation was dose dependent, with less degradation (lower content of NH_3-N) in the silages when the additives were applied at recommended dosages compared to using half the dose. This supports the importance of a fast acidification rate (quick drop in pH) and/or inhibition of the microbial growth (Pahlow *et* al., 2003).

Acid-R decreased the pH more after 100 days of storage compared to the untreated silage. Formic acid has a higher dissociation rate than the organic acids naturally occurring in untreated silages (Rooke & Hartfield, 2003). The loss of DM during storage decreased with acid treatment at recommended dose compared to the other treatments. Nadaeu & Auerbach (2014) presented similar results, but the salt treatments had in their study also a lower DM loss compared to the control. This effect did not occur in this study.

The content of WSC was in the acid treatment dose dependent in the chopped silage, with the highest content in the silage, which was treated with the Acid- R. Likewise, Nadeau *et al*. (2012b) reported a higher WSC content when using the same acid product at 3 l/t forage compared to untreated silage. The use of Acid - R dose led to the lowest concentration of lactic acid. Dosage of the acid influenced the acidification rate at the beginning of the ensiling process, treating the clover-grass silage with Salt- H and Acid-H, the $pH_{\text{three days}}$ were higher compared to the untreated control and the Salt- R and Acid-R. The salt treatment as well as the acid was dose dependent for the pH after three days of ensiling and for the extent of fermentation. The pH at the silo opening was on the same level for all the additive treatments, indicating that the slower acidification in the beginning of the ensiling process but did not affect the pH at opening. Randby (2000) reported the similar effects for acids at recommended dose and half the recommended dose.

Ethanol concentration decreased in both particle sizes by using additives, which shows that the additives (in both dosages) inhibit yeast (Pahlow *et al*., 2003). This complies with Nadeau & Auerbach (2014), were the additive treatment decreased the ethanol concentration.

Grass silage

High DM-concentration

Even though the yeast count was higher in the chopped silage, it had a better aerobic stability and a lower pH after aeration. The ethanol concentration did not differ between the particle sizes, indicating that yeast activity did not differ during the ensiling (Pahlow *et al*., 2003). Acetic acid suppresses the growth of yeast and moulds (Weinberg & Muck, 1996), whereas lactic acid stimulates growth of yeast. However, both contents of lactic and acetic acid only differed marginally between the silages of different particle sizes. Therefore, it is a combination of factors causing the improved aerobic stability of chopped compared to long silage.

By chopping the grass silage, the acidification was improved, lowering the pH, complying with the result of McEniry *et al*. (2007). Proteolysis (reflected by ammonia nitrogen content) was lower in the chopped compared to the long silages which probably was due to the more rapid drop in pH of chopped silage (Pahlow *et al*., 2003; Rinne *et al*., 2016).

The aerobic stability of the high DM grass silage was improved by using Acid- R and Salt -R, complying with results by Nadeau *et al*. (2012a) and Knicky & Spörndly (2009). Treating the grass silage with Acid-R decreased the lactic concentration compared to the other treatments, and the effect was less when using half the recommended dose. The direct acidification from the additive inhibited LAB from fermenting WSC to lactic acid, reflected by the higher residual concentration of WSC (Pahlow *et al*., 2003).

The acidification of the chopped grass silage in the beginning of ensiling was decreased by salt treatment (both dosages) and Acid- R compared to the control treatment. By treating the chopped silage with Salt –R the pH after ensiling was higher compared to half of the recommended dose. These results on pH show that additives containing salts and/or acids restricts the fermentation of WSC by LAB(Woolford, 1975; Kung, 2010) .

Lactic acid concentration decreased by a higher dose of acid treatment, complying with results by Rinne *et al*. (2016). The formic and propionic acids have an antimicrobial effect due to the fast acidification (Kung, *et al*., 2003; Kung, 2010). In the chopped silage yeast content was decreased by using the Acid – R. Nadeau *et al*. (2012a) reported that both salt-based additive

(2 l/t) and acid (3 l/t) lowered the ethanol concentration, but only the acid had effect in the chopped silage. In this study the protein degradation was decreased by using additives in silages of both particle sizes. In the long grass silage, the protein degradation was dosage dependent for both additives; ammonia nitrogen concentration decreased by increasing the dose of additive. This result corresponds with Slottner & Bertilsson (2004).

Low DM-concentration

The long grass silage contained a higher concentration of ethanol, compared to the chopped silage but the content of yeast did not differ between the particle lengths, indicating that the fermentation by yeast was more active in the long silage. McEniry *et al*. (2008) saw similar result with higher concentration of ethanol in the long silage compared to the chopped. The loss of DM decreased by chopping the grass silage, suggesting that respiration phase was shorter than for the long grass silage (McDonald *et al*., 1991).

Both particle sizes were similar in aerobic stability but were only stable for 5 days. After aeration the pH was high (pH>8) and deterioration occurred in silages of both particle sizes, possibly by the yeast initiation (Driehus and Oude Elferink, 2000; Pedroso *et al.*, 2005**).** Nadeau *et al*. (2013) and Spörndly & Persson (2015) concluded in their study that high yeast count is negatively correlated with aerobic stability.

In contrast to McEniry *et al*. (2008), where chopping decreased the content of WSC, the result in this study showed a higher WSC content in the chopped silage compared to the long. The lactic acid concentration was not affected by the particle size and the acetic acid concentration decreased by chopping, which did not comply with McEniry *et al*. (2008). High concentration of acetic acid has an inhibitory effect on yeast and moulds (Weinberg & Muck, 1996), but in this grass silage, the acetic acid concentration was low to have a strong effect on yeast growth.

For the grass silage with low DM, pH at the silo opening was lowest in the acid treated silage. Furthermore, acid treated long silage with Acid-R had the lowest lactic acid concentration. Treating the grass with Acid –H resulted in a higher concentration of lactic acid compared to recommended dose, which agree with results by Randby *et al*. (2001). Knicky & Spörndly (2009) showed similar results with salt-based additive and Randby (2000) showed that the lactic acid concentration was correlated with dosage of acid treatment. Chemical additives are fermentation inhibitors, half of recommended dose inhibit to a lesser degree than using recommended dose. Residual WSC was low in the salt treatments and the untreated control. Loss of DM was dose dependent for the salt treatment, where Salt-R decreased the loss compared to Salt-H but did not differ from the control silage. Nadeau *et al*. (2012) had similar results with the same salt-based additive. The salt-based additive decreased contents of ethanol and yeast, which agrees with results by Knicky & Spörndly (2011).

Salt- R inhibited yeast growth more than the Acid-R, resulting in a silage that was more aerobically stable. Knicky and Spörndly (2009) found that all their tested additives (both acid and salt) improved the aerobic stability, but yeast content was only affected in the high DM (46,4%) and the clostridia spore count in the low DM (22,9%).

The long grass silage treated with the Acid-R increased the count of yeast compared to the control. Using additive containing formic acid and propionic acid has an antifungal effect and decrease the yeast content (Kung *et al*., 2003; Rooke & Hatfield, 2003). Long silage has a higher porosity than chopped, and the compression is less than chopped silage (Pauly & Lingwall, 1999). A high porosity (Holmes & Muck, 2007), with air pockets, leads to a longer respiration

time at the start of the ensiling process (Muck *et al*., 2003). The aerobic stability did not significantly differ between the control and the acid additive in the long grass silage, but the acid at recommended dose had the shortest time before temperature increased, following the result by Rinne *et al*. (2016).

Conclusions

To conclude this study, the hypothesis did to some extent correspond with the results.The additive-treated silages, except the low-DM grass silage, had lower concentrations of NH_3-N than the untreated control, indicating reduced proteolysis in the silage. This effect was dependent on the additive dose for clover-dominated silage at both DM-concentrations and for the grass silage at high DM content, with the recommended dose resulting in the lowest proteolysis in the silage.

For the long silage the acidification rate (higher pH_{3days}) was slower compared to the chopped silage, which was shown in lower lactic acid content in the long clover-grass silage compared to its chopped counterpart but the differences in lactic acid between the particle sizes were less evident in the grass silage. No difference in aerobic stability was found, except for clover-grass silage at low DM content, where long silage was more aerobically stable than chopped silage.

Acid treatment decreased the lactic acid concentration in clover-grass silages, whereas no or only small effect was found in the grass silages. The decrease of the lactic acid concentration was dependent on the additive dose with the recommended dose being most effective in reducing the lactic acid concentration. The salt-based additive had often similar lactic acid content to the untreated control silage. Furthermore, acid at recommended dose decreased the DM losses in clover-grass silage at both DM concentrations compared to the control. The aerobic stability of high-DM grass silage was improved by use of salt or acid at recommended dosage.

Further studies

Further studies in this area are needed:

- The effect of particle size on aerobic stability in clover-dominated silage at lower DM.
- Studying the effect of using half the recommended dose of chemical additives for grain silage and maize silage.

Recommended guidelines to improve silage quality:

- Use the manufacturers recommended dose of the additive.
- Chopping the silage increases its ensilability.

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