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# Controlling aerobic stability of silage based total mixed rations

Doctoral Dissertation

Arja Seppälä

Luke

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Academic dissertation

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# Abstract

Arja Seppälä

Total mixed ration (TMR) is a feeding method for ruminants. Prior to feeding different types of feed ingredients are mixed together to create a uniform moist mass. TMR provides favourable conditions for microbial growth, which can cause rapid spoilage and spontaneous heating of the feed especially during the warm season. Potential negative consequences of the TMR heating are dry matter (DM) losses, reduced feed intake and subsequent reduction in animal production.

The objective of this thesis was to explore the factors affecting TMR stability. Silage is normally the most important component of a TMR. The effect of silage stability on TMR stability was evaluated by varying both silage additive and silage DM concentration. The ensiled crops were grass (a mixture of timothy and meadow fescue) and faba bean-wheat and field pea-wheat whole crops. The grass was ensiled at two dry matter levels: 218 (LDM) or 539 (HDM) g $kg^{-1}$. The whole crops were dominated by legumes as their proportions were 0.84 (faba bean) and 0.89 (pea) and DM was low (173 and 181 g DM $kg^{-1}$, respectively). Ensiling trials were conducted in 12 L silos. Each silage from the ensiling trials was used to prepare a TMR by adding various concentrate components. Aerobic stability of both the silage and the TMR was measured by following the temperature rise in the feed materials during aerobic exposure.

Silage stability was directly linked to TMR stability of the additive treated silages within crop. On the contrary, non-additive treated LDM silage had stability of 13.8 days while the TMR prepared from that silage had a stability of only 10 hours.

TMR DM was 590 g $kg^{-1}$ and 330 g $kg^{-1}$ for HDM and LDM, respectively. Numerically TMR prepared from HDM silages had 69 hours longer stability than TMR prepared from LDM silages. Conversely, TMR prepared from whole crop legume silages had only 280 g DM $kg^{-1}$, and yet their stability was relatively good (> 45 hours). It is notable, that silages were introduced into TMR immediately after silo opening without any semi-aerobic feedout phase.

The stability of the LDM silages was on average 12 times longer than the stability of the respective TMR. Legume whole crop silages had a stability 1.4 times higher than the respective TMR. The range of the silage additive effect on the TMR stability was 13 hours for faba bean-wheat silages, 30 hours for LDM silages, 57 hours for pea-wheat silages and 82 hours for HDM silages.

Preservatives can be added into TMR at the time of mixing to delay the spoilage. Two trials were conducted to explore the effects TMR hygieny and preservative additions. Effects of both liquid and solid preservatives were investigated as a method to delay TMR heating. The hygiene level of TMR was varied by an inclusion (10 % on DM basis) of one-week-old TMR into the mixture, by inclusion of brewers grains (13 % on DM basis) or by the quality of the grass silage (fresh or after aerobic exposure).

The preservative added into TMR at the time of mixing improved TMR stability on average by 1.5 to 41 hours depending on the microbial status of the TMR and the application level of the preservative. The effects of chemical stabilizers were larger, when the Control TMR had good stability.

Low yeast count (< 3 $\log_{10}$ cfu $g^{-1}$) of the TMR was related to good stability. A high yeast count (> 5 $\log_{10}$ cfu $g^{-1}$) and consequent rapid TMR heating may be caused by inoculation in the form of high yeast containing components such as by brewers grains or sometimes by silages. Together with yeasts, also aerobic bacteria may play a role in TMR spoilage, as suggested by the high count of aerobic bacteria (9.8 $\log_{10}$ cfu $g^{-1}$) in aerobically spoiled silage.

Poor hygiene reduced TMR stability by 50 hours. The amount of yeasts, moulds and aerobic bacteria, respectively, were 55, 257 and 126 times higher in TMR containing 26 % spoiled silage (on DM-basis) compared to the TMR prepared from high hygienic quality ingredients.

It was concluded that silage additives can manipulate silage and respective TMR aerobic stability. Within additive treated silages there was a strong correlation between silage stability and TMR stability. High dry matter grass silages and TMR prepared from them had good stability in the trial conditions, which may not reflect practical situation on farms where presense of oxygen is more variable during silo filling, during ensiling as well as during feed out potentially causing substantially higher count of aerobic spoiling microbes than in trial silos. Chemical TMR preservatives can improve TMR stability especially effective when the initial number of spoiling organisms is low.

Keywords: aerobic stability, hygiene, preservative, spoilage, total mixed ration, yeast

# Tiivistelmä

Arja Seppälä

Seosrehuruokinta on märehtijöiden ruokintamenetelmä, jossa erilaiset rehukomponentit sekoitetaan tasaiseksi kosteaksi massaksi ennen rehun jakamista elämille. Seosrehussa olosuhteet mikrobien kasvulle ovat suotuisat. Seosrehun pilaantuminen ja siitä johtuva rehun lämpeneminen voivat tapahtua nopeasti erityisesti lämpimänä vuodenaikana. Seurauksena seosrehun lämpenemisestä ovat ravintoainetappiot, rehun syönnin heikkeneminen ja eläintuotoksen lasku.

Tämän väitöstyön tavoitteena oli tutkia seosrehun lämpenemiseen vaikuttavia tekijöitä. Säilörehu on tyypillisesti seosrehun tärkein komponentti. Väitöstyöhön sisältyvissä osakokeissa tutkittiin säilörehun säilöntäaineen ja säilörehun kuiva-ainepitoisuuden vaikutusta seosrehun stabiilisuuteen. Säilörehuja tehtiin timotei-nurminataseoksesta sekä härkäpapuvehnä- ja hernevehnäkokoviljoista. Nurmikasvuston kuiva-ainepitoisuus säilöttäessä oli joko 218 tai 539 g kg-1. Kokoviljoissa palkokasvin osuus oli 0.84 (härkäpapu) ja 0.89 (herne) ja kuiva-ainepitoisuus oli 173 (härkäpapu-vehnä) ja 181 (herne-vehnä) g kg-1. Rehut säilöttiin 12 l siiloihin. Säilöntäjakson jälkeen valmiit säilörehut yhdistettiin väkirehukomponenettien kanssa seosrehuiksi. Sekä säilörehun että siitä valmistetun seosrehun stabiilisuus mitattiin seuraamalla rehunäytteen lämpötilaa aerobisissa olosuhteissa.

Yhteys säilörehun ja vastaavan seosrehun aerobisen stabiilisuuden välillä oli suoraviivainen säilöntäaineella tehtyjen rehujen osalta kasvimateriaalikohtaisesti. Sen sijaan, ilman säilöntäainetta tehty märkä nurmisäilörehu oli aerobisesti hyvin stabiilia (stabiilisuus 13.8 vrk), kun taas siitä tehty seosrehu lämpeni jo 10 tunnissa.

Nurmisäilörehuista tehtyjen seosrehujen kuiva-ainepitoisuus oli 590 g kg-1 and 330 g kg-1. Numeerisesti stabiilisuus oli kuivemmilla rehulla 69 tuntia pidempi kuin märemmällä seosrehulla. Toisaalta, kokoviljasäilörehuista tehtyjen seosrehujen kuiva-ainepitoisuus oli vain 280 g DM kg-1 ja kuitenkin niiden stabiilisuus oli varsin hyvä (> 45 tuntia). Käytännön tiloilla siilon syöttövaiheen puoliaerobisen vaiheen vaikutusta ei huomioitu näissä laboratoriomittakaan kokeissa, mikä on huomioitava tulosten tulkinnassa.

Märän nurmisäilörehun aerobinen stabiilisuus oli 12 kertaa pidempi kuin samasta säilörehusta tehdyn seosrehun stabiilisuus. Kokoviljojen osalta säilörehun aerobinen stabiilisuus oli 1,4-kertainen verrattuna samasta rehusta tehdyn seosrehun stabiilisuuteen. Säilörehun säilöntäainevalinta heijastui seosrehun stabiilisuuteen siten, että vaikutuksen suuruus oli härkäpapuvehnällä 13 tuntia, märällä nurmirehulla 30 tuntia, hernevehnällä 57 tuntia ja pitkälle esikuivatulla nurmirehulla 82 tuntia.

Seosrehuun voidaan lisätä säilöntäaineita sekoituksen yhteydessä hidastamaan rehun pilaantumista. Kahdessa kokeessa tutkittiin seosrehun hygieniatason ja säilöntäaineen lisäyksen vaikutusta. Seosrehuun lisättiin sekoitusvaiheessa nestemäistä tai kiinteää säilöntäainetta. Hygienian vaikutusta tutkittiin joko sisällyttämällä seokseen 10 % viikon vanhaa seosrehua, mäskiä (13 % kuiva-aineesta) tai säilörehua, joka oli altistunut

aerobiselle pilaantumiselle. Seosrehuun sekoitusvaiheessa lisätty säilöntäaine lisäsi stabiilisuutta 1,5 - 41 tuntia riippuen lähtötilanteen mikrobiologisesta tasosta ja säilöntäaineen käyttömäärästä. Seosrehun stabiilisuus parani säilöntäainelisäyksellä enemmän lähtökohtaisesti stabiililla seoksella verrattuna pilaantumisherkkään seokseen.

Seosrehun vähäinen hiivojen määrä (< 3 log10 pmy g-1) oli yhteydessä hyvään stabiilisuuteen. Seosrehun suuri hiivamäärä (> 5 log10 pmy g-1) ja siitä seuraava lämpenemisherkkyys voivat johtua yksittäisen komponentin (esim. mäskin) korkeasta hiivamäärästä tai säilörehun hiivoista. Hiivojen ohella myös aerobiset bakteerit voivat olla osallisia seosrehun lämpenemisessä, esim. aerobisesti pilaantuneessa säilörehussa aerobisten bakteerien määrä oli 9.8 log10 pmy g-1.

Huono hygienia lyhensi seosrehun stabiilisuutta 50 tunnilla. Hiivojen, homeiden ja aerobisten bakteerien määrät olivat 55-, 257- ja 126 -kertaiset seosrehussa, jossa oli 26 % pilaantunutta säilörehua kuiva-aineesta verrattuna seosrehuun, jossa kaikki raaka-aineet olivat hyvälaatuisia.

Johtopäätöksenä voidaan todeta, että säilörehun säilöntäaineet voivat vaikuttaa sekä säilörehun että siitä tehdyn seosrehun aerobiseen stabiilisuuteen. Säilöntäaineen kera tehdyissä rehuissa yhteys säilörehun ja seosrehun stabiilisuuden välillä oli voimakas. Pitkälle esikuivattun säilörehu ja siitä tehty seosrehu olivat koeolosuhteissa hyvin stabiileja. Tulos ei sellaisenaan heijasta käytännön tilannetta, sillä käytännön maatiloilla rehu altistuu hapelle vaihtelevasti siilon täyttövaiheessa, säilönnän aikana ja varsinkin syöttövaiheessa, jolloin aerobisten pilaajamikrobien määrä on voinut kasvaa huomattaavsti suuremmaksi kuin koesiiloissa. Kemialliset seosrehun stabilointiin tarkoitetut säilöntäaineet voivat parantaa seosrehun stabiilisuutta erityisesti jos ne lisätään rehuun, kun pilaajamikrobien määrä on rehussa alhainen.

Avainsanat: aerobinen stabiilisuus, hiivat, hygienia, pilaantuminen, seosrehu, säilöntäaine

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# List of original publications

This thesis is based on the following publications:

I Seppälä, A., Heikkilä, T., Mäki, M. & Rinne, M. 2016. Effects of additives on the fermentation and aerobic stability of grass silages and total mixed rations. Grass and Forage Science 71: 458 – 471.

II Seppälä, A., Rinne, M. & Huuskonen, A. 2019. Efficacy of different additives in ensiling faba bean and field pea based whole crop silages. Agricultural and Food Science 28: 165 – 175.

III Seppälä, A., Heikkilä, T., Mäki, M., Miettinen, H. & Rinne, M. 2013. Controlling aerobic stability of grass silage-based total mixed rations. Animal Feed Science and Technology 179: 54 – 60.



The publications are referred to in the text by their Roman numerals.

All experiments were conducted at the Natural Resources Institute Finland (Luke; until end of 2014 MTT Agrifood Research Finland).

# Contribution

The contributions of all authors to the original articles of this thesis are presented in the following table:

| | I | II | III |
|---|---|---|---|
| Planning the experiment | AS, MR | AS, MR, AH | AS, HM, MR |
| Data analysis | AS | AS | AS |
| Calculating and interpreting the results | AS, MR | AS, MR, AH | AS, MR |
| Manuscript preparation | AS, MR, TH, MM | AS, MR, AH | AS, MR, HM, TH, MM |

AS = Arja Seppälä, MR = Marketta Rinne, MM = Maarit Mäki, HM = Harri Miettinen, TH = Terttu Heikkilä, AH = Arto Huuskonen

# Abbreviations

General abbreviations:

| | |
|---|---|
| DM | dry matter |
| D-value | digestible organic matter in dry matter |
| LAB | lactic acid bacteria |
| NDF | neutral detergent fibre |
| SEM | standard error of the mean |
| TMR | total mixed ration |
| VFA | volatile fatty acids |
| WSC | water soluble carbohydrates |

| | |
|---|---|
| LDM | low dry matter silage |
| HDM | high dry matter silage |
| $_{ho}LAB_1$ | homofermentative lactic acid bacteria |
| $_{ho+he}LAB$ | mixture of homofermentative and heterofermentative lactic acid bacteria |
| $_{ho}LABcel_1$ | homofermentative lactic acid bacteria and cellulase, product 1 |
| $_{ho}LABcel_2$ | homofermentative lactic acid bacteria and cellulase, product 2 |
| $_{ho}LABcelSB$ | homofermentative lactic acid bacteria, cellulase and sodiumbenzoate |
| SALT | mixture of sodium benzoate, potassium sorbate and sodium nitrite |
| FAPA | formic acid, sodium formate, propionic acid, benzoic acid, glycerol |
| FA | formic acid, ammonium formate |

Names of treatments in the publications:

| | |
|---|---|
| FBW | faba bean-wheat whole crop |
| PW | field pea-wheat whole crop |
| LAB1 | silage additive including homofermentative and heterofermentative lactic acid bacteria strains |
| LAB2 | silage additive including homofermentative and heterofermentative lactic acid bacteria strains and enzymes |
| ACID | silage additive including formic acid, propionic acid, ammonium formate, potassium sorbate |
| $TMR_{bob}$ | TMR including whole crop silage, barley, oats and brewers grains |
| $TMR_{bobs}$ | TMR including whole crop silage, barley, oats, brewers grains and straw |
| | |
| TMR 1A | TMR including farm grass silage and pelleted concentrate |
| TMR 1B | 90 % TMR 1A + 10 % one week old TMR 1a |
| 2A | TMR including grass silage, rape seed expeller, barley, oats and brewers grains mixed in a mixer wagon |
| 2B | same as 2A but mixed in laboratory |
| 2C | same as 2B but grass silage was partly aerobically deteriorated |
| 2D | same as 2B but without brewers grains |

# Contents

# 1. Introduction

## 1.1. Background

### 1.1.1. Total mixed ration (TMR as a feeding method

Ruminants are fed with diverse feeds varying in moisture concentration, physical structure and chemical and microbial composition. Despite the versatile feeding options, the diet should not vary too much between days and the diet composition should be balanced to fulfil nutritional needs and to optimize production economy. Total mixed ration (TMR) feeding is a well-established method to feed cattle (Schingoethe 2017). In the method, all feed ingredients are mixed into one ration that is delivered to animals. There are many types of mixers available for that purpose. Some of the TMR feeding systems are fully automated.

A well managed TMR feeding system helps to avoid digestive upsets. Success is dependent on sound ration planning and reliable feed analytics as well as careful follow-up of dry matter (DM) content of the feed ingredients, function of electronic load cells in the mixer and the mixing operation itself (Schingoethe 2017). Optimal DM concentration of TMR has been suggested to be between 450 and 600 g $kg^{-1}$ (Schingoethe 2017). Practical recommendations related to DM concentration vary to some extent. In Finland the normal range is 350–450 g $kg^{-1}$ (Kyntäjä et al. 2010) since the main TMR component is normally relatively low DM grass silage. In so called “compact TMR” the target DM concentration is 37 %, which is achieved by adding water or high moisture ingredients into the mixture (Kristensen 2015). The aim is to ensure even distribution of all the components in the consumed feed.

One of the major challenges of TMR feeding is, that TMR presents conditions favorable for aerobic micro-organisms. Availability of oxygen, moisture and easily degradable substrates especially in warm ambient temperature enable rapid growth of aerobic microbes that are normally present in the feed ingredients. Microbes may also be derived from any remains of previous feed batches left in the mixer. All these factors contribute to a more rapid rate of heating of the TMR than occurs in silage alone (Kung 2005). Vigorous growth of aerobic microbes in feed and the consequent heating of TMR seems to be a common problem in practical conditions, especially at high ambient temperatures (Kung 2005, Hilgefort 2014, Kristensen 2019). Potential consequences of TMR heating are DM losses, reduced feed intake (Whitlock et al. 2000, Windle & Kung 2013), subsequent production losses (Salvo et al. 2015), issues related to animal health, additional labour caused by removal of left overs or a need for more frequent feed mixing (Kung 2005, 2014, Hilgefort 2014).

### 1.1.2. Aerobic stability of TMR

Aerobic stability describes the length of time that silage or TMR remains stable, i.e. no heating due to microbial activity is detected in the feed (Ranjit & Kung 2002, Kung 2005). Aerobic stability of silages has been widely examined (Lindgren et al. 1985, Muck & O'Kiely 1992, Kung et al. 2003a, Jaakkola et al. 2009, Pahlow & Muck 2009; Tabacco et al. 2011; Wilkinson & Davies 2013) and the aerobic stability of ensiled TMR (i.e., rations based on forage and concentrate components ensiled together) has also been a subject of previous research (Nishino & Hattori 2007, Wang & Nishino 2008, 2009, Uddin et al. 2009, Weinberg et al. 2011, Hao et al. 2015, Wang et al. 2016). While silage is normally fed as TMR, the silage stability is directly correlated to TMR stability (Kung 2005). However, the complexity is increasing when other ingredients bring their micro-organisms and nutrients into the TMR. Thus, based on practical observations, silage itself can be stable, but still TMR heating may be a problem.

Reports on the aerobic stability of TMR have mostly been based on maize silage (Kung et al. 1998, Kung 2005, Kristensen 2019, Hao et al. 2015). However, other types of forages have also been studied to some extent. Taylor et al. (2002) evaluated the stability of TMR prepared from barley silage, lucerne silage and concentrate, and da Silva (2018) studied the stability of TMR based on maize silage, lucerne silage and grain mixture. Kung et al. (2003b) assessed the aerobic stability of TMR prepared from two different lucerne silages, corn silage, lucerne hay and concentrates. Seppälä et al. (2012) evaluated the aerobic stability of TMR prepared from grass silage and crimped grains, while Rinne et al. (2019) studied the effect of additive treatment of high moisture faba bean on TMR stability when other TMR ingredients were grass silage and dry ground barley. Nuβbaum (2005), Jaakkola et al. (2017) and Rinne et al. (2018) have also explored the effects of additives on grass silage based TMR.

Kung (2005) detected TMR heating within 12 hours from mixing and suggested that the silages used had potentially already gone through some degree of aerobic spoilage before being mixed into the TMR. The heating problem may be boosted by the additional water into the TMR (Hao et al. 2015, Pries et al. 2018, Rinne et al. 2018). Water may be a necessity to ensure TMR homogeneity and to prevent feed selection (Kristensen 2015) thus increasing intake and milk production (Pries et al. 2018, Denißen et al. 2019). However, negative responses to added water have also been reported, including enhanced sorting and reduced DM intake (Miller-Cushon & DeVries 2009).

### 1.1.3. Preservatives for improving TMR stability

To avoid the negative consequences of aerobic deterioration, new feed batches may be prepared at shorter intervals and leftovers may need to be removed carefully causing economic losses caused by increased labour costs and feed wastage. Normally a new batch of TMR is prepared once or twice per day, but e.g. for dry cows and heifers farmers prefer to prepare a new batch less often, e.g. once per three days. Mixing a new batch once per day can be enough during cold ambient temperature (Kurdna 2003, Mäntysaari

et al. 2006), while during the summer season increasing mixing frequency from two to three improved milk production by 1.24 kg per cow (Kurdna et al. 2001).

Increasing TMR mixing frequency may be impossible or too expensive due to labour costs. As another option there are several preservatives available on the market for TMR stabilizing. Preservatives inhibit microbial growth and thus delay the spoilage and temperature increase in the TMR. Preservatives often consist of mixtures of different organic acids and their salts to balance the price, technical properties and effectiveness of the products. Both liquid and solid products are on the market. However scientific literature related to TMR stabilizers is scarce.

Among preservatives, undissociated propionic acid is known to have strong antifungal properties (Kung et al. 2003a), and it has been examined as a preservative for both maize silage-based TMR (Kung et al. 1998, Kung 2005, Nuβbaum 2005) and grass silage-based TMR (Nuβbaum 2005). Effectiveness of propionic acid-based preservatives increases with high application rates (Kung et al. 1998). da Silva (2018) proved that mixture of sodium benzoate, potassium sorbate and sodium nitrate can improve TMR stability either when used as silage additive or when added into silage just prior to mixing into TMR. Rinne et al. (2018) tested calcium propionate, mixtures of calcium propionate and sodium benzoate and a blend of propionic acid and formic acid in TMR stabilizing. All the additives were able to improve stability, especially if the hygienic quality of the feeds were high at the time of TMR preparation.

Nuβbaum (2005) tested eight products in TMR stabilizing including propionic acid - based products, potassium sorbate and a mixture of benzoate, fumaric acid and common salt. In that trial, a strong propionic acid-based product gave the best response in easily heating grass silage based TMR while the maize silage based TMR had inherently longer stability than grass silage based TMR. In contrast, according to Kristensen (2019) heating problems are more commonly linked to poor stability of maize silage. The statement is supported by findings of Kung (2005) in US and Arnessons et al. (2009) in Swedish farms.

Despite numerous commercial products being marketed to control TMR heating, the number of scientific publications is limited, especially when TMR is based on grass silage or leguminous whole crop silage. Most of the information related to control TMR heating is practical advisory material for farmers or commercial material supporting marketing of preservatives to control TMR heating, and full composition of the products may be missing from the reports (e.g. Nuβbaum 2005, Kristensen 2019). Lack of detailed information may be due to the commercial interests of the companies manufacturing those products.

### 1.1.4. Methods to monitor aerobic spoilage of feeds

Aerobic spoilage is caused by the growth of aerobic spoiling organisms, which consume nutrients and oxygen and simultaneously produce carbon dioxide and water. Part of the chemical energy is turned into heat. Heat may accumulate so that temperature rise can be detected. Temperature rise is not the primary change but a result of quite significant microbial growth and chemical changes.

Methods to monitor progress of feed spoiling in aerobic conditions include $CO_2$ measurements either by fixing $CO_2$ into potassium hydroxide (Daniel et al. 1970, Crawshaw et al. 1980, Lindgren et al. 1985, Ashbell et al. 1991) or by measuring $CO_2$ directly from the air space (Östling and Lindgren 1995, Maier et al. 2010, Lehman & Rosentrater 2012, Franco et al. 2019), temperature measurements (e.g. Hara et al. 1979, Lindgren et al. 1985, Jungbluth et al. 2016, Pauly & Wyss 2018), visual inspection (Franco et al. 2019, Rinne et al. 2019a) and microbial and chemical analytics (especially pH) at different time points (Pitt et al. 1991). Devices suitable to follow oxygen levels during aerobic phases of ensiling were compared by Shan et al. (2016).

In respect to monitoring feed spoiling, TMR is a very heterogenous feed as nutrients and micro-organisms are not evenly distributed throughout it. Thus, any attempt to monitor changes in chemical composition or in microbial numbers are seriously hindered by difficulties to sample the TMR representatively. Further, several time points are needed, which multiplies the costs. Thus, preferably, monitoring spoilage should be automated and low-cost, which is the case with temperature recording. Additionally, Gerlach et al. (2013) found that temperature change in corn silage was the most accurate predictor for silage intake when compared to chemical parameters.

When temperature is monitored at laboratory scale, the feed sample is placed into an insulated box, which is not closed to ensure aerobic conditions. Eliminating variation in ambient temperature is a perquisite for successful use of the method.

## 1.2. Objectives and hypotheses of the study

The objective of this thesis was to explore at laboratory scale the factors affecting TMR stability. Explored factors included silage additive (no additive, LAB strains with or without enzymes, formic acid with or without propionic acid, mixture of salts: benzoate-sorbate-nitrate), silage DM concentration, inclusion of brewers grains or straw, hygienic quality (inclusion of spoiled silage or TMR) or use of chemical preservatives at the time of mixing. Main component in the TMR was either grass silage or whole crop pea-wheat or faba bean-wheat silage.

It was hypothesized that:

1. Silage aerobic stability is directly reflected to TMR stability.
2. Silage additives can manipulate silage and TMR aerobic stability.
3. Silage DM content affects TMR stability.
4. Chemical TMR preservatives improve TMR stability irrespective of the TMR hygienic quality.

# 2. Materials and methods

## 2.1. Description of the data

The study comprised four experiments that were documented in three publications (I–III, Table 1). The experimental procedures used are described in detail in publications (I-III) and only brief summaries are presented here. All the trials were performed at Natural Resources Institute Finland (Luke; until end of 2014 MTT Agrifood Research Finland) either in Jokioinen (I, II) or in Siikajoki (II).

**Table 1.** A general overview of the experimental material included in this thesis. In Experiments 1 and 2, the ensiling trial was followed by testing the stability of each silage as such or as a part of TMR. Experiments 3 and 4 started from the TMR mixing and additive treatments were applied at the time of TMR mixing.

| Pub. | Exp. | Ensiling experiment | TMR stability experiment |
|---|---|---|---|
| I | 1 | Two dry matter levels of grass silage and nine additive treatments (Control + four additives) | All silages as a TMR component |
| II | 2 | Pea-wheat whole crop and faba bean-wheat whole crop and four additive treatments (Control + three additives) | All silages as a TMR component with or without straw |
| III | 3 | None | Two different TMR, treated at the time of mixing (Control + liquid additives at two levels + solid additive at two levels) |
| III | 4 | None | Four different TMR, treated at the time of mixing (Control + liquid additives at three levels + solid additive at one level) |

Pub. = publication, Exp. = experiment

## 2.2. Ensiling trials

Two pilot scale ensiling trials were conducted in order to explore the effects of silage crop, silage fermentation quality and microbial quality and dry matter concentration on aerobic stability of the silages and TMR prepared from the silages. Silage raw materials are described in Table 2 and silage additive treatments in Table 3. The grass (I) was cut using mower-conditioner and prewilted for either one hour (low dry matter, LDM) or overnight

(high dry matter, HDM) before harvesting with a precision chopper. The whole crops (II) were harvested using a direct cut precision chopper. The weather was dry and sunny in all cases.

Additive treatments were evenly applied and manually well mixed into the forage batches. Three replicate silos (12 L) were filled for each additive treatment. Silos were closed immediately after filling and a 10 kg weight was added on top of each silo. Effluent was not separately removed from the silos during ensiling, but some effluent was spontaneously lost in the latter trial (II). During ensiling, the silos were stored either in room temperature (I) or in an uninsulated storage building and transported to a cool storage room (2–10 °C) before outside temperature dropped below zero (II).

After an approximately 100-day ensiling period the silos were opened and the top layer was removed before sampling for chemical and microbiological analysis, for TMR preparation and for aerobic stability measurements.

## 2.3. Preparing total mixed rations

The trials (Table 4) included 52 TMR differing from each other. The number of replicates was three in all cases. Grass silage was the main component in the TMR in publications I and III and the faba bean-wheat (FBW) and pea-wheat (PW) whole crop silages were the main ingredient in II. Most of the TMR were mixed at laboratory scale either by hand (I, II) or using a pilot-scale (50 kg) horizontal mixer, but TMR 2A was mixed using a farm-scale Storti Labrador 160 mixer wagon (Storti S.p.A., Belfiore, Italy). All the grass silages were prepared from mixed sward of timothy-meadow fescue (*Phleum pratense-Festuca pratensis*). The farm grass silage (III) was prewilted, precision chopped, and ensiled in a bunker silo using a formic acid-based additive.

After mixing the TMR ingredients (III) the preservative additions (Table 5) were made on separate batches, which were after that sampled (3 samples from each combination) for measuring aerobic stability.

**Table 2.** Silage raw materials in the ensiling trials.

| Pub. | Abbreviation | Crops | Dry matter, g $kg^{-1}$ | Filling density, g $l^{-1}$ | ensiling time, days |
|---|---|---|---|---|---|
| I | LDM | Mixed sward of timothy (*Phleum pratense*) and meadow fescue (*Festuca pratensis*), primary growth | 218 | 542 | 102 |
| | HDM | | 539 | 375 | 116 |
| II | FBW | Faba bean (*Vicia faba* cv. Fuego) dominated (0.84) whole crop with wheat (*Triticum aestivum* cv. Anniina) | 173 | 925 | 105 |
| | PW | Field pea (*Pisum sativum* cv. Florida) dominated (0.89) whole crop with wheat (*Triticum aestivum* cv. Anniina) | 181 | 833 | 105 |

**Table 3.** Additive treatments in the ensiling trials.

| Pub. | Abbrevation | Composition | Application level |
|---|---|---|---|
| I | Control | Nothing added | -- |
| | $_{ho}$LAB$_1$ | *Lactobacillus plantarum* | $10^6$ cfu g$^{-1}$ |
| | $_{ho+he}$LAB | *Enterococcus faecium, Lactobacillus brevis*, *Lactobacillus plantarum* | $2 \cdot 10^5$ cfu g$^{-1}$ |
| | $_{ho}$LABcel$_1$ | *Lactobacillus plantarum*, *Pediococcus acidilactici*, cellulase | $10^6$cfu g$^{-1}$ |
| | $_{ho}$LABcel$_2$ | *Lactobacillus plantarum*, *Pediococcus acidilactici*, cellulase | $10^6$cfu g$^{-1}$ |
| | $_{ho}$LABcelSB | *Lactobacillus plantarum*, *Pediococcus acidilactici, Lactococcus lactis*, *Enterococcus faecium*, cellulase, sodium benzoate | $2 \cdot 10^5$ cfu g$^{-1}$ + 300 g t$^{-1}$ sodium benzoate |
| | SALT | Sodium benzoate, potassium sorbate, sodium nitrite | LDM: 2.62 l t$^{-1}$ HDM: 3 l t$^{-1}$ |
| | FAPA | Formic acid, sodium formate, propionic acid, benzoic acid, glycerol | 5 L t$^{-1}$ |
| | FA | Formic acid, ammonium formate | 5 L t$^{-1}$ |
| II | Control | Nothing added | -- |
| | LAB1 | *Lactobacillus plantarum, Lactobacillus paracasei, Lactobacillus buchneri, Lactococcus lactis* | $2.5 \cdot 10^5$ CFU g$^{-1}$ |
| | LAB2 | *Lactobacillus plantarum, Pediococcus acidilactici, Pediococcus pentosaceus, Propionibacterium acidipropionici*, α-amylaze, cellulase, β-glucanase, xylanase | $1.0 \cdot 10^6$ CFU g$^{-1}$ |
| | ACID | Formic acid, propionic acid, ammonium formate, potassium sorbate | 5 L t$^{-1}$ |

**Table 4.** Total mixed rations (TMR) prepared in the trials.

| Pub. | TMR | TMR ingredients | Number of factor levels within each row | | | |
|---|---|---|---|---|---|---|
| | | | Silage DM | Silage additive | Silage crop species | Preservative added into TMR |
| I | | Experimental grass silages, pelleted concentrate, brewers grains | 2 | 9 | | |
| II | $TMR_{bob}$ | Experimental whole crop silages, barley, oats and brewers grains | | 3 | | |
| II | $TMR_{bobs}$ | TMRbob + straw | | 4 | 2 | |
| III | 1a | Farm grass silage, pelleted concentrate | | | | 5 |
| III | 1b | 1a + 10 % one week old TMR | | | | 5 |
| III | 2A | Farm grass silage, rapeseed expeller, barley, oats, brewers grains, mixed in farm scale | | | | 5 |
| III | 2B | Same as 2A but mixed in laboratory | | | | 4 |
| III | 2C | Same as 2B but used grass silage was partly aerobically deteriorated | | | | 5 |
| III | 2D | Same as 2B but excluding brewers grains | | | | 5 |

**Table 5.** Preservative treatments for total mixed ration (TMR) stabilizing in III.

| Preservative treatment | TMR | g $kg^{-1}$ |
|---|---|---|
| Control without preservative | 1A, 1B<br>2A, 2B, 2C, 2D | |
| Propionic acid, ammonium propionate and ammonium formate (liquid) | 1A, 1B | 2 and 3 |
| Propionic acid, formic acid, ammonium formate (liquid) | 2A, 2B, 2C, 2D | 2, 4 and 6 |
| Sodium-calcium propionate (solid) | 1A, 1B<br>2A, 2C, 2D | 2 and 3<br>4 |

## 2.4. Aerobic stability measurements

The aerobic stability of the silages and the TMR was determined by monitoring temperature changes during air exposure. A polystyrene container (2.5 $dm^3$) was filled with the sample and temperature was followed either twice a day using manually readable electronic thermometers (III: 1A and 1B) or at 10-minute intervals using a thermocouple wire connected to a data logger (I, II, III: 2A, 2B, 2C, 2D). Aerobic stability was defined as the time taken to increase the temperature of the feed by 2.0 °C above the ambient temperature.

## 2.5. Chemical and microbiological analyses

Table 6 includes the analyses conducted for feed samples. Crude protein content was calculated as 6.25 × N content.

**Table 6.** Chemical and microbiological analyses conducted for feed samples.

| Publication | I | | | II | | | III | |
|---|---|---|---|---|---|---|---|---|
| | Silage raw materials | Silages | Other TMR ingredients | Silage raw materials | Silages | Other TMR ingredients | TMR ingredients, 1a, 1b | TMR ingredients, 2A, 2B, 2C, 2D |
| Dry matter | × | × | × | × | × | × | × | × |
| Ash | × | | × | × | | | × | × |
| Nitrogen | × | | × | × | | | × | × |
| Crude fat | | | × | | | | | |
| Water soluble carbohydrates | × | × | | × | × | | | |
| Starch | | | × | × | | | × | × |
| Neutral detergent fibre | × | | × | × | | | × | × |
| D-value (*in vitro*) | × | | | × | | | | |
| Buffering capacity | × | | | × | | | | |
| pH | | × | | | × | | | |
| Formic acid | | | | | x | | | |
| Acetic acid | | × | | | × | | | |
| Propionic acid | | × | | | × | | | |
| Butyric acid | | × | | | × | | | |
| Lactic acid | | × | | | × | | | |
| Ethanol | | × | | | × | | | |
| Ammonium-N | | × | | | × | | | |
| Soluble N | × | | | | | | | |
| Yeasts and moulds | | × | | × | × | × | | × |
| Enterobacteria | | | | × | | | | |
| Coliform bacteria | | | | × | × | × | | |
| Lactic acid bacteria | | | | × | | | | |
| Aerobic bacteria | | | | | | | | × |

## 2.6. Statistical methods

Statistical analyses were performed using SAS GLM procedure. The interaction between the DM levels and additive treatments (I) was significant ($p < 0.001$) for the majority of fermentation quality parameters. Thus, both DM levels were tested separately using a model where additive treatment was a fixed effect (9 levels and 3 replicates). Differences between treatments (LS means) were compared using the Tukey test. Contrasts were performed to test differences between different types of additives (I). Differences in aerobic stability were analyzed separately for silages and TMR on both DM levels because HDM silages had zero variance in aerobic stability.

The statistical model in II included the fixed effects of plant (FBW or PW) and additive treatment (four levels, three replicates). Differences between treatments (LS means) were compared using the Tukey test.

In III, the aerobic stability measurements from TMR were not regarded as true replicates so the results of analytical replicates were averaged before statistical analysis. The statistical model in III included the type of TMR and the preservative application as independent classification variables. Contrasts were performed to examine the effect of application (preservative vs. control, liquid vs. solid preservative, application level 2 vs. 3 g $kg^{-1}$ or linear effect of dosage).

# 3. Results and Discussions

## 3.1. Methodological considerations related to aerobic stability measurements

### 3.1.1. Temperature monitoring

Typical microbial growth curves include successive lag, growth (exponential), and asymptotic (stationary) phases (Zwietering et al. 1990, Meletiadis et al. 2001). Before TMR mixing the microbes in the individual ingredients may be dormant due to low water activity, low pH, high concentration of organic acids, low temperature or lack of oxygen. Conditions change during TMR preparation enabling flourishing of aerobic microbes followed by a temperature increase.

The TMR temperature curves resemble microbial growth curves with a lag phase followed by a rapid temperature increase. The observed temperature curve is the outcome of the metabolism of several microbial species in stages of exponential growth and stationary phases. Probably, the microbial consortia present at any point of time are replaced by others in short intervals in a similar manner as in composting (Insam & de Bertoldi 2007). The TMR spoiling resembles the early stage of composting (mesophilic phase (25-40 °C), where energy-rich, easily degradable compounds are degraded by fungi, actinobacteria and bacteria, which cause the temperature rise (Insam & de Bertoldi 2007).

Methods to follow TMR heating are normally the same as used when measuring silage aerobic stability after silo opening. Recording silage temperature once per day has been used in past (e.g. Saarisalo et al. 2006, Pursiainen & Tuori 2008, Heikkilä et al. 2010, Jaakkola et al. 2010). Interpretation of temperature data was based on the accumulated heat, e.g. total temperature rise during 7-d exposure to air (Pursiainen & Tuori 2008). In III, the TMR temperature was measured either manually twice per day or by a data logger at 10 minutes intervals. Automated temperature monitoring enables detecting more detailed temperature curves and defining aerobic stability as hours needed for a certain temperature increase. The upper limit for stability has been either one (Adesogan and Salawu 2004), two (Pitt et al. 1991) or three (Pauly and Wyss 2018) degrees above the ambient temperature.

In this context, the most important aspect is to detect the switch from the lag phase into the exponential phase (Figure 1). Any fluctuation in ambient temperature disturbs defining the end of aerobic stability, because fluctuation in ambient temperature is reflected into sample temperature with some delay (Figure 2). Setting the upper temperature limit for stability is thus crucial. It should be high enough to omit any variation in ambient temperature. E.g. if ambient temperature drops rapidly, that might cause erroneously small stability results in case the upper limit is too low. On the other hand, the lower the limit, the more sensitive the method is. In the optimal case, temperature rises sharply several degrees above the upper limit (Figure 3).

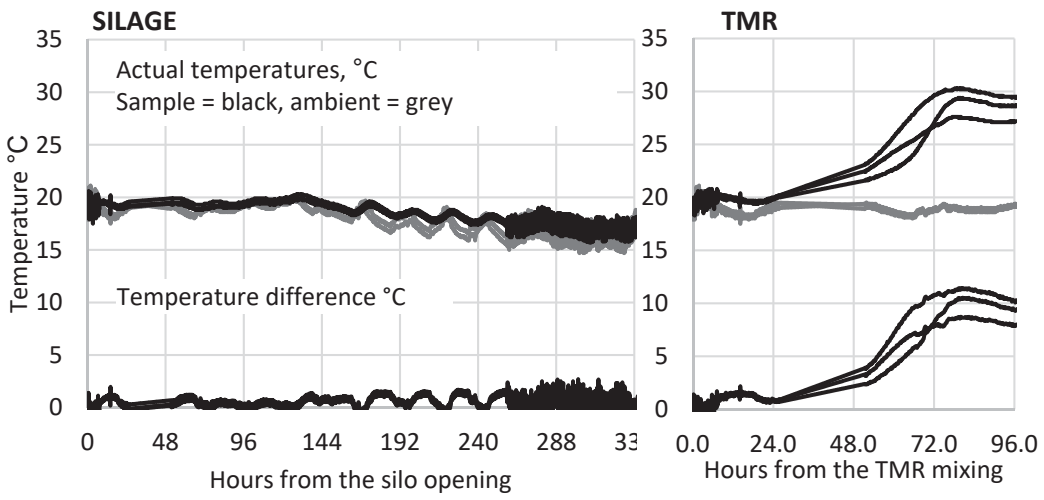



**Figure** 1. Examples of stability measurements based on temperature recording (I). SALT (sodium benzoate, potassium sorbate, sodium nitrate) treatment on (low dry matter) LDM grass clearly made stable silage (left). When the same silage was used as a TMR component, the temperature curves show separate lag phases before the start of the heating (right). There were three replicate samples in I. Hysteresis of the heating device caused variation after 260 hours.

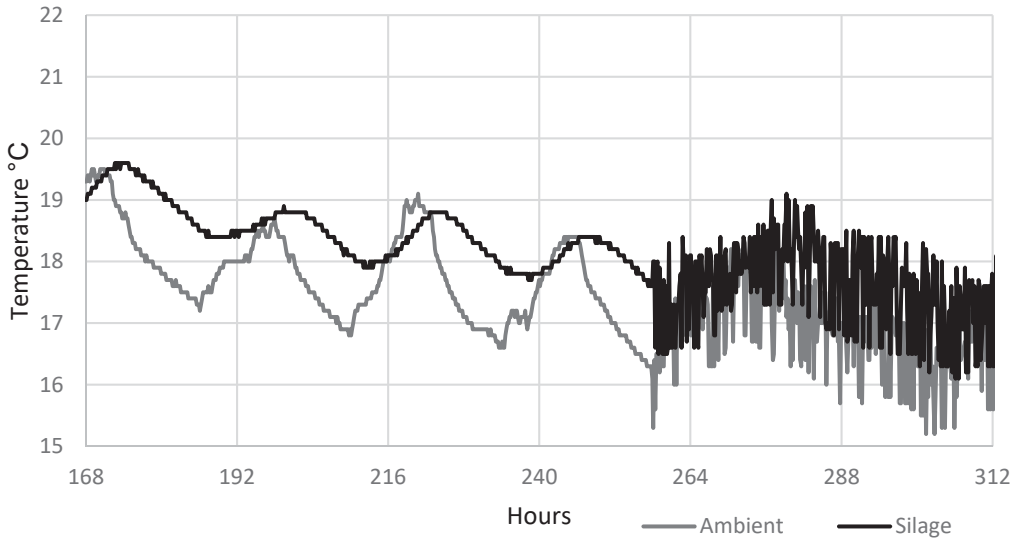



**Figure** 2. Variation in the ambient temperature may seriously hinder defining aerobic stability of a feed sample accurately and precisely (I). Sample temperature follows ambient temperature by some delay. Any fluctuation in ambient temperature may thus either increase or decrease the real temperature difference between a sample and the ambient. For clarity, the temperature of only one replicate shown and only part of the time series included. By cooling down the ambient temperature, the heater started to operate at approximately 260 hours. The hysteresis of the heater clearly created additional variation into the temperature curve.

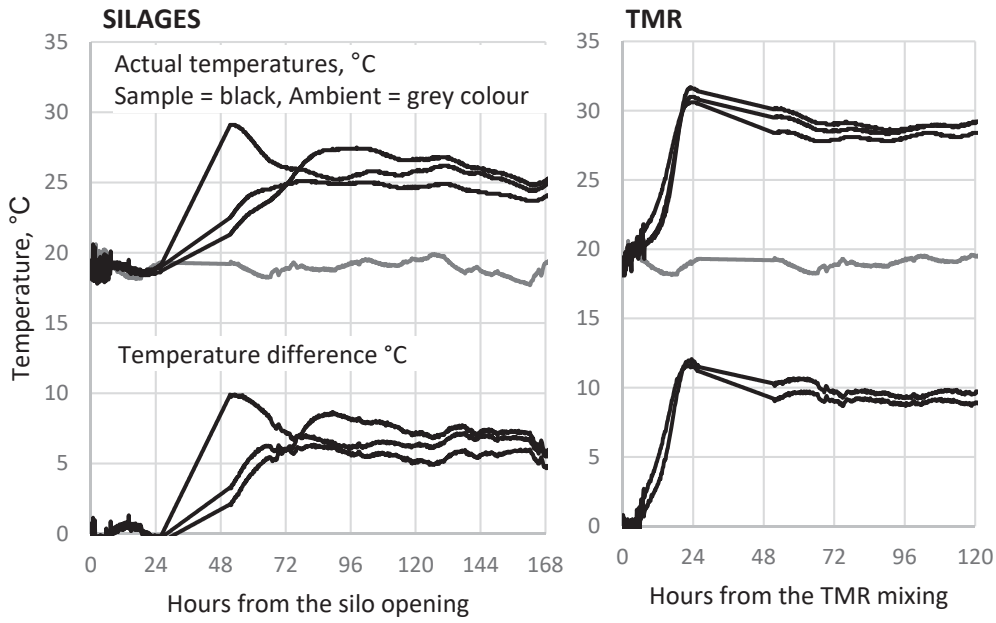



**Figure 3.** Examples of stability measurements based on temperature recording (I). Homofermentative lactic acid bacteria ($_{ho}LAB_{1,}$) treatment on low dry matter (LDM) grass clearly made unstable silage (left). When the same silage was used as a TMR component, the temperature curves show a rapid heating without a lag phase (right). There were three replicate samples in I.

### 3.1.2. Limitations of temperature monitoring

Specific heat capacity and thermal conductivity of a certain feed are mainly dependent on feed moisture concentration (Muck et al. 2003, Berk 2018). High moisture feed needs more energy for the same temperature change than the drier feeds. Furthermore, the sample size and insulation around the sample have effect on the measured temperature rise. Thus, temperature readings are very much related to the circumstances where the measurement is conducted.

*Wet silages and TMR.*

Available energy in PW or FBW silages or TMR was not able to cause a large temperature increase, just slightly over two degrees above ambient in some cases (Figure 4). Anyway, the differences between treatments were quite clear. The numeric stability would have been 10-15 hours less, if the upper limit for stability would have been 0.5 degrees instead of 2 degrees. However, the ranking of treatments would still be the same.

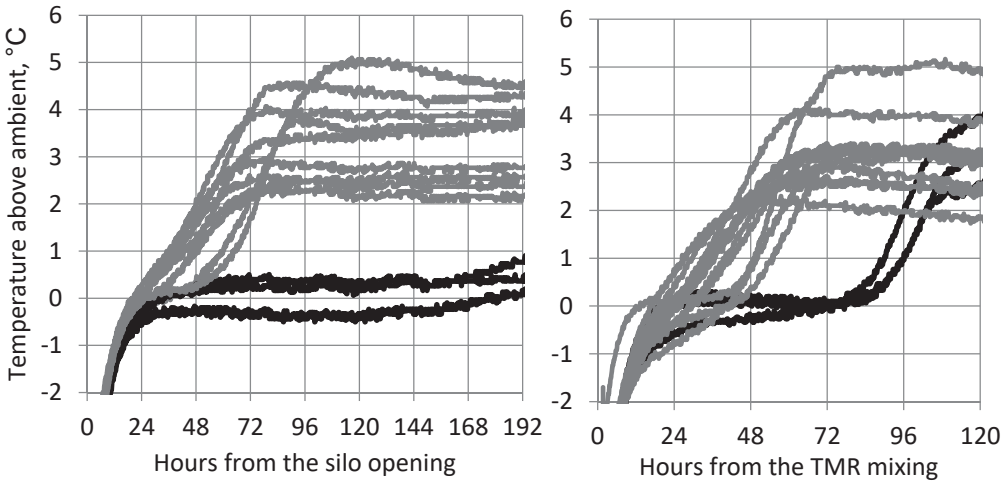



**Figure** 4. The stability of the wet pea-wheat silages (left) and respective TMR (right, II). Heating of samples generated only limited amounts of heat, most of the cases the highest recorded temperature was just slightly above the predefinied two-degrees limit for stability. Nonetheless, the curves showed either clear stability or clear heating, so the outcome of that trial was clear. Black colour was silage ensiled with ACID (formic and propionic acid blend) treatment, while grey colour lines represent non-additive treated or lactic acid bacteria treated silages.

*High dry matter silages and TMR.*

Low water activity is limiting the growth of spoiling micro-organisms. Heat generated from slow spoilage of a small sample may be lost to ambient without causing detectable temperature rise. In those cases, feed will be spoiled, but no spoilage will be detected by the temperature recording. E.g. when aerobic spoilage of crimped grains was followed by Seppälä et al. (2015a), in some cases temperature increase was less than two degrees but the grains were moulding. In that trial grain DM concentration was 816 g $kg^{-1}$.

Examples from the trial of Särkijärvi et al. (2012, Figure 5) show, that haylages having DM of 675 or 692 g $kg^{-1}$ show clear heating and end of stability after 197 and 212 hours. Those heating curves were made using the same methodology as in I. The haylage in Särkijärvi et al. (2012) had numerically higher yeast count (6.26 or 4.67 $log_{10}$ cfu $g^{-1}$) than HDM Control silages in I (4.37 $log_{10}$ cfu $g^{-1}$). The clear heating detected by Särkijärvi et al. (2012) suggests, that the used sample volume is enough for detecting heating of haylages having less than 700 g DM $kg^{-1}$. Based on that backround the high stability of HDM silages (I) seems to be a reliable result. That interpretation ignores the effect of porosity, which in practise has crucial role in aerobic spoilage of silages in clamp silos (Holmes & Bolsen 2009). Due to porosity the oxygen will penetrate into silage causing semi-aerobic conditions several days before silage is mixed into TMR. In trials I and II silages were mixed into TMR immediately after silo opening, which represents a practical system when feeding baled silage.

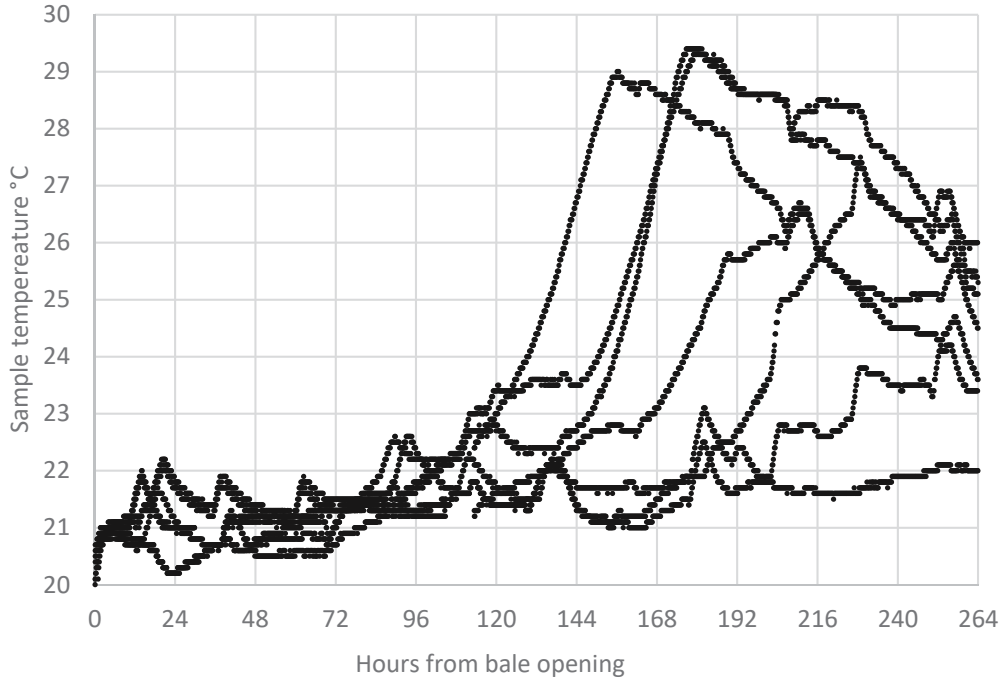



**Figure 5**. Aerobic stability of the wrapped non-additive treated haylages from the trial of Särkijärvi et al. (2012).

The HDM silages (I) were measured to have a stability of over 9 days. Only a small amount of heating could be detected in temperature curves of Control silages and one replicate of $_{ho}LAB_1$ HDM silage towards the end of the recording period (Figure 6). Addition of spoiling micro-organisms via the other TMR ingredients made the spoiling quicker, also in HDM feeds. However, a rapid exponential heat increment was not detected, and it was not that easy to identify when heating started.

In farm silos high DM silages (DM above 400 g $kg^{-1}$) are often those showing mouldy surfaces or heating problems. The problem may appear a few weeks after silo opening, and due to potentially high porosity of such material, the air may penetrate deep into the silo (McEniry et al. 2007, Holmes & Bolsen 2009). In a large volume, accumulated heat is not lost and the spoiling process itself is increasing the feed moisture concentration. Due to these reasons, spoiling may hasten in an exponential manner after the initial start. The method used in the trials included in the current thesis (I, II, III) is not capable of describing the prolonged spoiling in large silos weeks after silo opening. In those conditions silages which appear stable in laboratory (HDM) may not be stable.

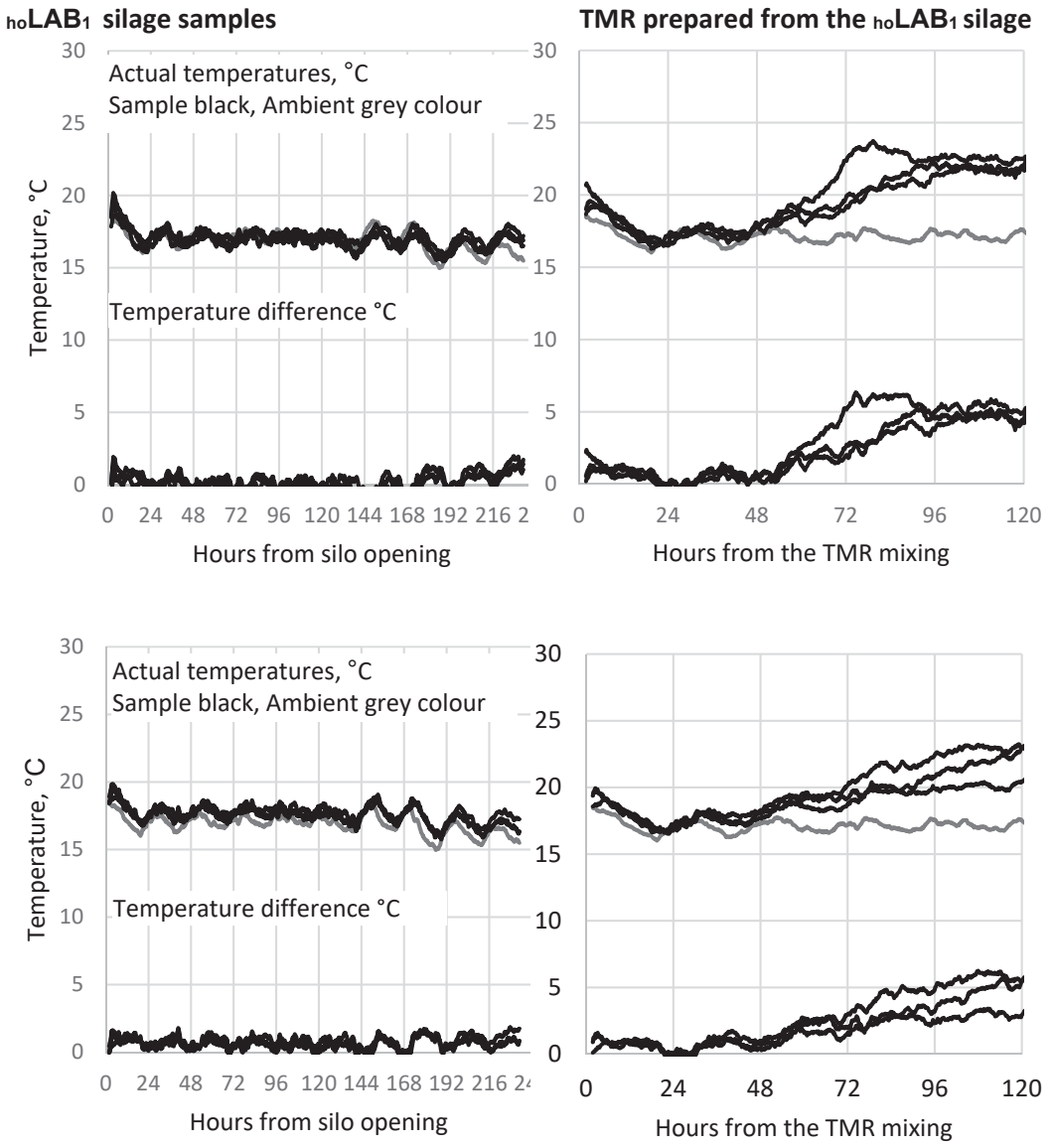



**Figure 6**. Stability of high dry matter silages (above ones) and respective TMR (I). Control silage was made without additive and $_{ho}LAB_1$ silage was made with homofermentative lactic acid bacteria strain.

*Temperature of the feeds prior to the test.* Often it is the case that prior to aerobic stability measurements the feeds are stored in a colder temperature. That may cause errors in the results, if the sample temperatures are variable and below ambient temperature when samples are placed into the insulated boxes (Figure 7). Thus, care should be given to treat all samples in a similar way priopr to initiating the temperature recording.

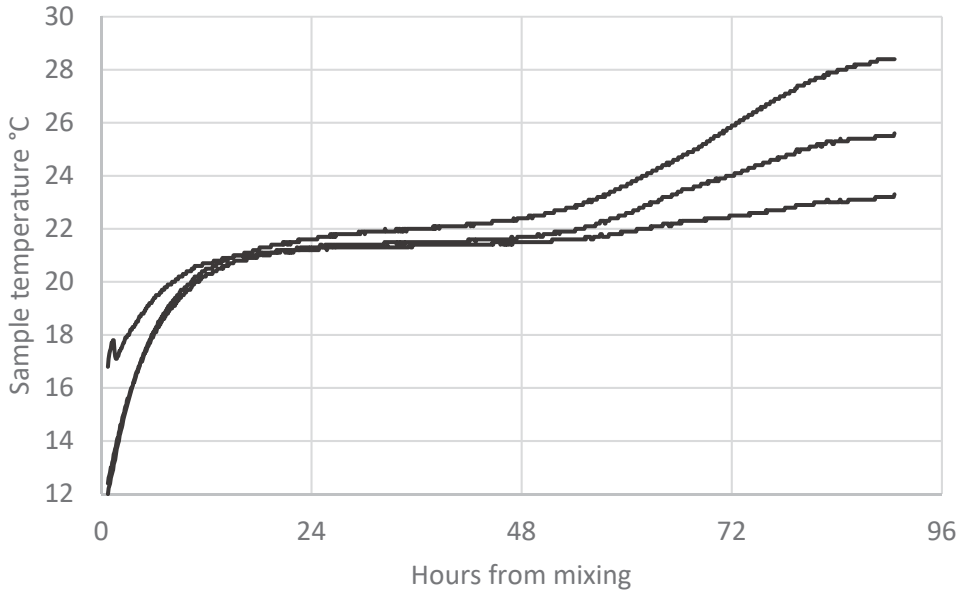



**Figure 7**. Effect of starting temperature on aerobic stability readings (III), temperature curves of the three replicates of TMR 2A (grass silage, rape seed expeller, barley, oats and brewers grains mixed in a mixer wagon) treated with 2 g $kg^{-1}$ liquid preservative.

### 3.1.3. Usefulness of laboratory scale trials

Aerobic stability of the silage or TMR is dependent on microbial species and their numbers, temperature, water activity, nutrient availability, oxygen availability and presence of growth inhibitors (Muck et al. 2003, Kung 2005). Thus, the practical situation on farm level is most often different from the aerobic stability measurements conducted in the laboratory. However, trials conducted at small scale enable researchers to control other factors than those which are to be evaluated. The relative effects of the treatments are expected to be the same at the larger scale, while the absolute length of stability will be different. This means, that small scale trials are useful when comparing additives, but application level of a TMR stabilizer on farm level should be adjusted based on user experiences and the targeted stability at each individual practical case. Furthermore, the application level can also be adjusted when there is changes in ambient temperature or TMR composition.

### 3.1.4. Suggested methodological improvements

To avoid erroneous interpretations of temperature curves, at least the control treatment should show clear heating with the typical exponential temperature increase. In this case,

choice of upper limit for stability would not have a dramatic effect on the resulting aerobic stability (number of hours).

A larger sample size might help to detect a small heat accumulation, but at the same time there is a risk that the large sample is no longer aerobic throughout the follow-up period. Furthermore, other methods such as $CO_2$ measurements could be used when temperature follow-up does not provide reliable results. The results of Franco et al. (2019) demonstrate that $CO_2$ measurements would enable detection of the onset of spoiling earlier with a smaller standard error of the mean even though $CO_2$ was measured only once per day while the temperature was recorded at ten-minute intervals.

As demonstrated by the examples (Figures 1-6), it is always necessary to create pictures of the temperature data to ensure, that the data is interpreted correctly. The resulting stability (hours) should be compared to the curves to ensure right interpretations.

## 3.2. Microbes in TMR

TMR heating is complicated and heterogenous at the level of microbes. The silage components may have been exposed to oxygen already several days before mixing into the TMR. On top of that, several other microbes are added together with the other feed ingredients included in the TMR. Detailed information about succession of microbes in TMR spoilage seems to be nearly nonexistent. The reason for that is both the unique nature of each case combined to the complexity of each case. Detailed information about one case would give only information with only limited usefulness for other cases.

Microbes are not evenly distributed within heterogenous feed like TMR. Different feed ingredients may vary greatly in their microbial composition, and unrepresentative sampling will seriously bias resulting microbial counts. Figure 8 illustrates the yeasts coming from different TMR components. Brewers grains contributed 99 % of yeasts in the TMR, while brewers grains contributed only 11 % of the TMR on fresh matter basis. It is suggested, that microbial counts should be measured separately from each TMR component. That would also help to select an appropriate way to control the TMR heating.

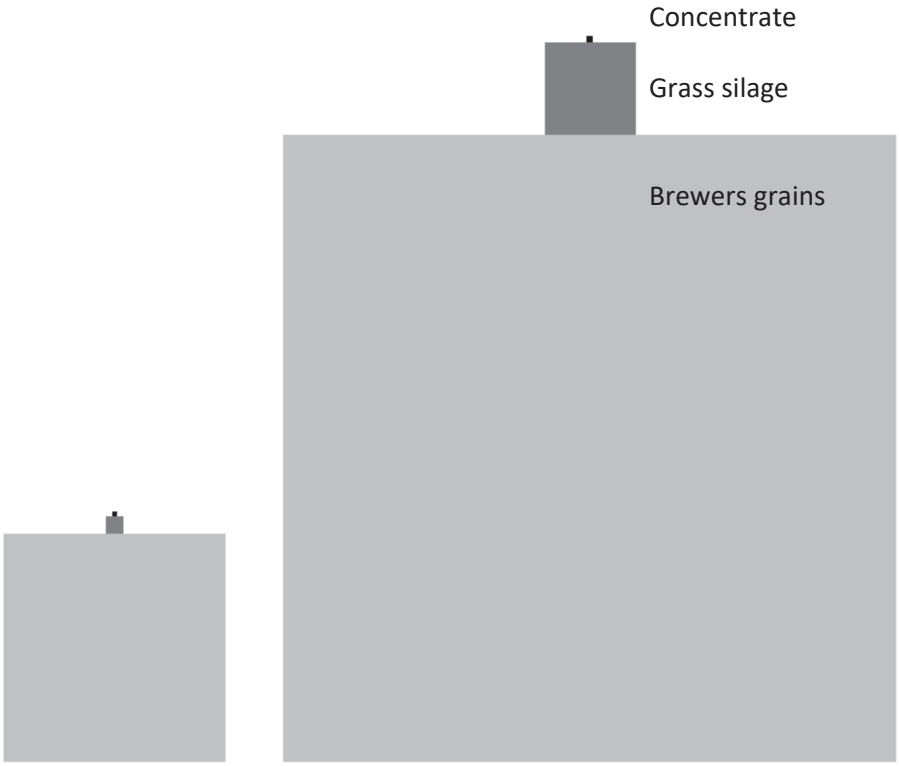



**Figure 8**. Source of yeasts in TMR prepared from the grass silage (LDM $_{ho}$LAB$_1$), pelleted concentrate and brewers grains in proportions of 5:4:1 on DM basis (I). Area of each square is relative to its yeast count. Squares on the left are calculated based on the minimum yeasts counts of each feed while squares on the right are calculated based on the maximum yeasts counts of each feed.

Farmers seldom have the possibility to analyze microbial numbers from feeds. Microbial products, such as fermentation end-products in silages, are commonly analyzed. Also, in some cases also mycotoxins may be analyzed. Heating, bad smell or visually noticeable yeast or mould growth are the typical signs of spoilage that can be detected at farm level.

The TMR ingredients used had high numbers of microbes, which can cause rapid spoiling of the TMR (I,II, III, Figures 9-11). Even dry feed ingredients can harbor high numbers of aerobic spoiling micro-organisms which are dormant as long as the water activity is low enough (Rose et al. 2012).

### 3.2.1. Yeasts

Yeasts are eukaryotic micro-organisms, which normally propagate by budding (Pahlow et al. 2003). They can grow in both anaerobic and aerobic conditions. During ensiling they can ferment sugars to ethanol (Pahlow et al. 2003). Despite remarkable DM loss, energy loss is minimal (2.6 %) when sugars are fermented to ethanol (Rooke & Hatfielf 2003). Ethanol production during ensiling is undesirable, because it consumes sugars without causing a pH drop. Furthermore, ethanol is a volatile organic compound, which can be partially lost during feed out and can contribute to poor air quality in some areas (Hafner et al. 2018). Ethanol in grass silage does not affect milk yield, milk fat or protein yield, but increases milk fat concentration (Huhtanen et al. 2003).

During ensiling, the presence of oxygen enhances survival and growth of yeasts (Jonsson & Pahlow 1984, Lindgren et al. 1985). Lactic acid alone is not able to prevent growth of spoilage yeasts (Savard et al. 2002). Acetic acid (Jonsson and Pahlow 1984), propionic acid (Savard et al. 2002), sorbic acid (Eklund 1983) and sodium benzoate (Bernardes et al. 2015) are all able to reduce yeast viability within the pH range found in silage. The mechanisms are potentially via the undissociated form of the acids (Eklund 1983, Pahlow et al. 2003) or via membrane-activity (Stratford & Anslow 1998). Formic acid may enhance yeast growth during early phases of ensiling but reduce their survival later during ensiling (Driehuis & van Wikselaar 1996). As a consequence formic acid treated silages may show elevated ethanol concentrations, while after a long ensiling period the yeast count is low and aerobic stability is improved in those silages (Weiss et al. 2013, II).

Yeasts have been detected to be responsible for the onset of silage spoilage after silo opening (McDonald et al. 1991). They oxidize lactic acid which results in a pH rise during aerobic spoilage (Pahlow et al. 2003). Yeast counts above 5 $\log_{10}$ cfu $g^{-1}$ are likely to be associated with reduced silage aerobic stability (Wilkinson & Davies 2013).

Kung (2005) detected a negative correlation between aerobic stability and yeast counts in TMR. This is in accordance with the very low (< 3 $\log_{10}$ cfu $g^{-1}$) yeast count and good aerobic stability of the TMR that was prepared without brewers grains (III, 2D). Brewers grains was the feed showing highest yeast counts followed by some silages (Figure 9). In III, all the TMR with low (2D) or moderate (2A and 2B) yeast counts were prepared from grass silage that had a yeast count under the detection limit (< 2 log cfu $g^{-1}$).

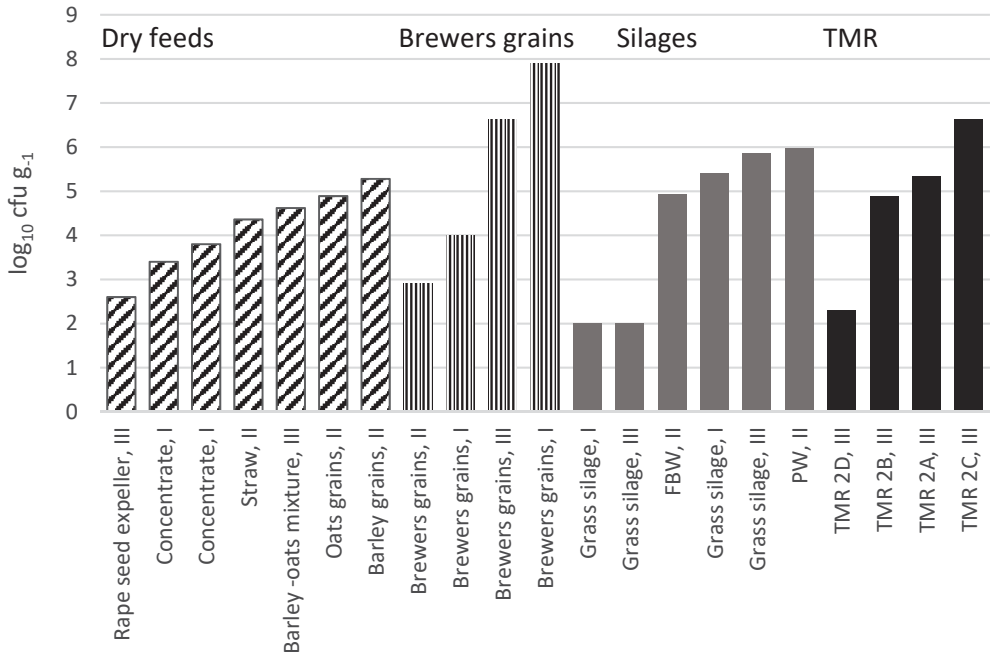



**Figure 9** Yeast counts in the different feed types in I, II and III sorted in ascending order within feed type. From the 18 silages in I only minimum and maximum were included.

### 3.2.2. Aerobic bacteria

In the beginning of aerobic silage spoilage, the pH is too low for many bacteria. However, acetic acid bacteria have been proven to be involved in aerobic spoiling process (Spoelstra et al. 1988). They are obligate aerobic, acid-tolerant bacteria that can oxidize ethanol to acetic acid. Some of them can oxidize both lactic and acetic acids to carbon dioxide and water, thus contributing to a pH rise during aerobic spoilage (Pahlow et al. 2003). When spoilage has initiated and the spoiling process continues, species of *Bacillus* can be detected in high numbers (up to $10^9$ cfu $g^{-1}$, Lindgren et al. 1985, Pahlow et al. 2003). When the silage temperature rises further, high numbers ($10^8$ cfu $g^{-1}$) of Enterobacteria may also occur as detected by Lindgren et al. (1985).

Publications I, II and III include only very limited information about bacterial numbers in the feeds (Figure 10). The total bacteria count was measured from some feeds as an indicator of hygiene level. Total bacteria count is not useful for silages, which anyway typically have high number of LAB.

Aerobic bacteria may also play a significant role in aerobic deterioration of silages (McDonald et al. 1991) as demonstrated by the high count of aerobic bacteria in aerobically deteriorated silage and TMR 2C in IIII. Further, differences were greater between 2A and 2C in aerobic bacteria counts rather than yeast counts, which suggested that aerobic bacteria may have contributed much to deterioration of 2C TMR (III).

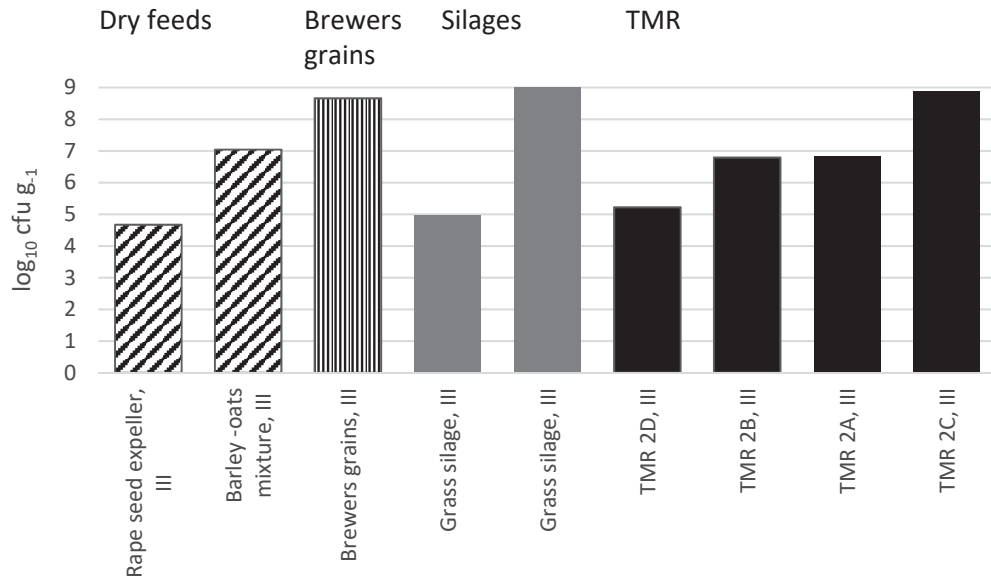



**Figure 10**. Total counts of aerobic bacteria in the different feed types in I, II and III sorted in ascending order within feed type. Total count of aerobic bacteria was not measured in ensiling trials that included lactic acid bacteria treatments.

### 3.2.3. Moulds

Moulds are eukaryotic, usually aerobic micro-organisms (Driehuis & Oude Elferink 2000). Moulds are especially harmful, because many of them can produce mycotoxins. Presence of moulds is often visually detected on surface layers of silages due to poor sealing and/or poor compaction (Pahlow et al. 2003). Mould growth takes place in later stages of silage aerobic spoilage (Driehuis and Oude Elferink 2000). Important mycotoxin-producing moulds associated with aerobic spoilage of silage are *Aspergillus fumigatus, Penicillium roqueforti* and *Byssochlamys nivea,* however there was no direct connection between the mould count and the mycotoxin content of the silage (Driehuis and Oude Elferink 2000).

In grass silages *Penicillium roqueforti* was the most commonly detected group of moulds (Sarlin & Saarisalo 2006), which can also produce mycotoxins (O'Brien et al. 2006). Cogan et al. (2016) did not find mycotoxins in grass silage but did in maize and other silages (mainly whole crop wheat).

Consequences of mycotoxin ingestion by animals can be serious (Koivunen & Huuskonen 2018, Ogunade et al. 2018), so it is a generally accepted practice to totally avoid feeding visually mouldy feed. Separating mouldy spots or the mouldy surface layer from silage may cause substantial amount of additional work and wastage of feed material. When separation is not carefully conducted, mouldy silage will be mixed into TMR

inoculating the whole batch of feed. Other feed components may also contaminate TMR with moulds (Figure 11).

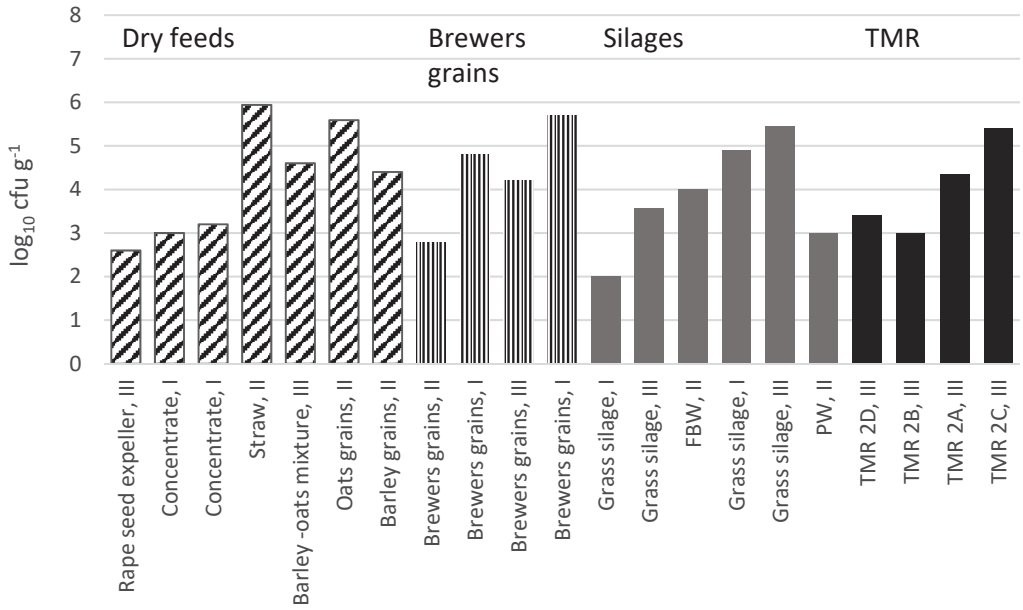



**Figure 11**. Mould counts in the different feed types in I, II and III. Data was sorted in ascending order by yeast count within feed type (Figure 9). From the 18 silages in I only minimum and maximum were included.

## 3.3. Quantifying factors affecting TMR stability

TMR stabilities in trials have varied from 4 hours (I) to over 8 days (Rinne et al. 2018, 2019b). Long stability measured by lack of temperature rise does not mean, that the feed has not gone through any changes. Gerlach et al. (2014 a,b) documented that goats sometimes decreased silage intake before any temperature rise or chemical changes were detected in the silage. In a preference trial, silage DM intake decreased at day 2 or 4 (lucerne silages, Gerlach et al. 2014b), at day 4 (maize silage, Gerlach et al. 2013) or at day 6 or 8 (grass silage, Gerlach et al. 2014a) of aerobic exposure.

Stabilities longer than three days have only limited practical value, because commonly new batches would be mixed more frequently than that. From practical point of view, improvements in TMR stability from less than 24 hours to more than 24 hours are most interesting. Results from I-III suggest, that poor hygiene can be a major factor causing poor TMR stability. Furthermore, inclusion of high-yeast brewers grain together with low DM silage into TMR are also likely to cause heating problems (Figures 12 and 13).

Results suggest, that all attempts to improve TMR stability have a greater effect, when the control sample has a relatively good stability, e.g. the effect of chemical stabilizers was greater when Control TMR had a long stability (III, Rinne et al. 2018). This partially explains, why Kristensen (2019) did not observe many statistically significant differences between different chemical treatments, when the control had a stability of only 8 hours.

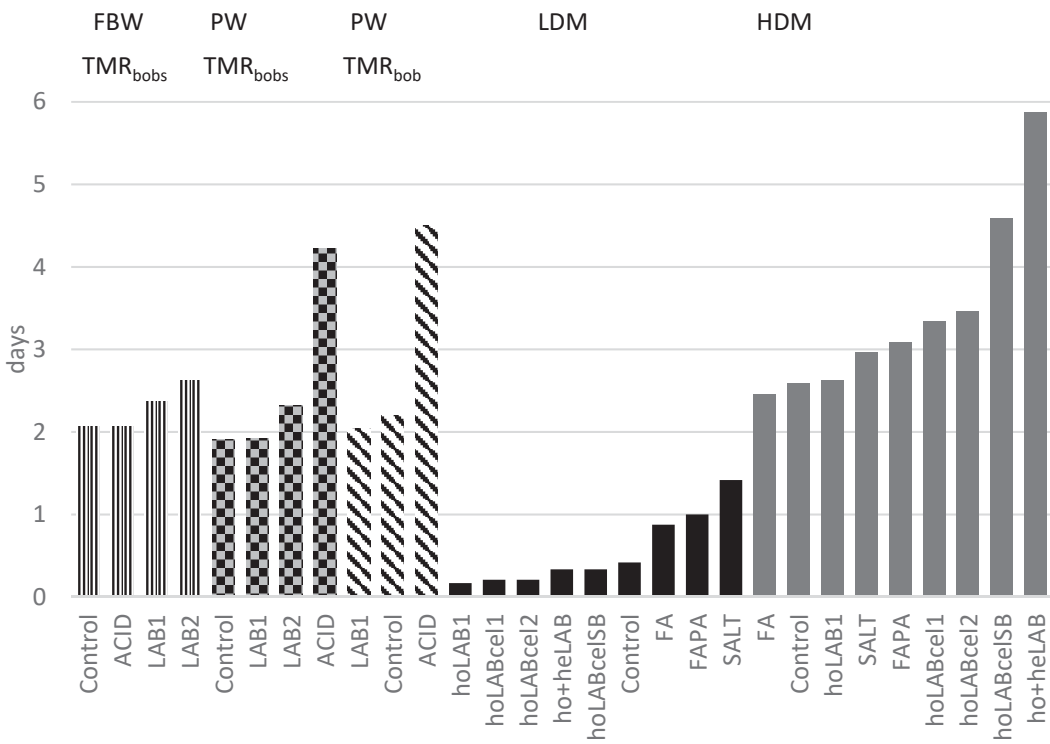



**Figure 12**. Aerobic stability of TMRs in I and II.

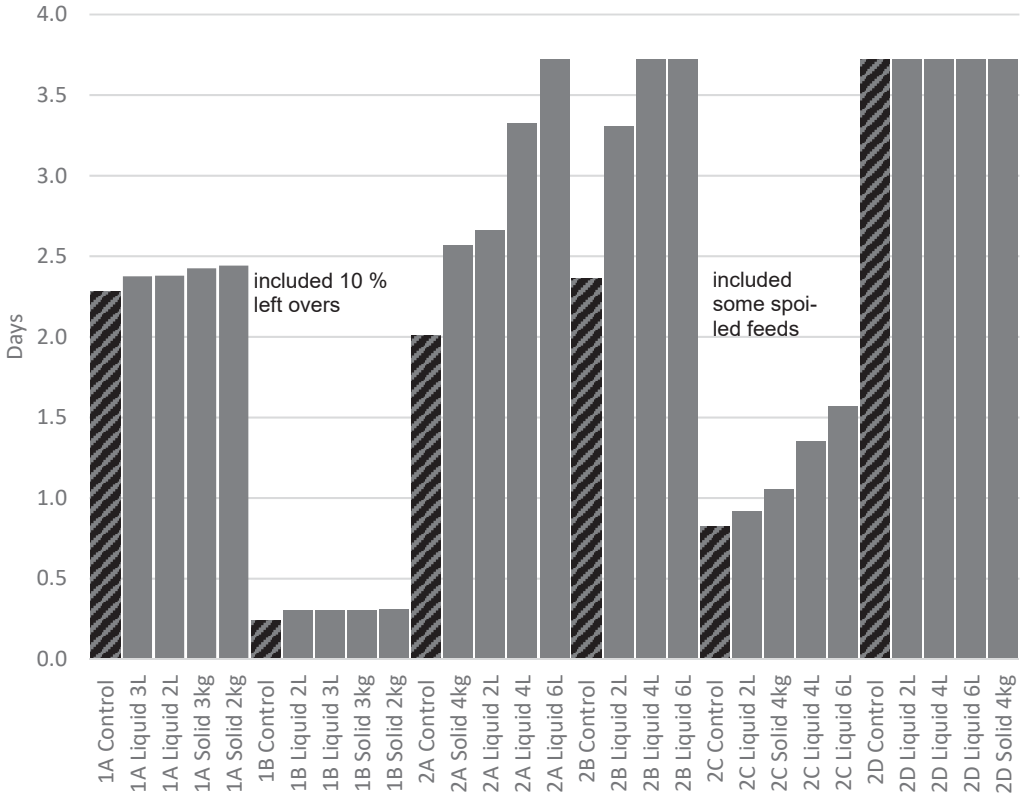



**Figure 13**. Aerobic stability of TMRs in III. Control TMRs without any preservative marked with darker colour.

### 3.3.1. Hygiene

In the legislation (EC 183/2005) feed hygiene means the measures and conditions necessary to control hazards and to ensure fitness for animal consumption of a feed, taking into account its intended use. The regulation includes e.g. hygiene provisions for feed businesses at the level of primary production. The aim is to prevent, eliminate or minimise hazards with the potential to compromise feed safety. Primary products should be protected against contamination and spoilage.

Poor hygiene may ruin TMR stability (III). High numbers of aerobic spoiling microorganisms can be derived from the left overs of old batches or from inclusion of feed that already shows signs of aerobic spoilage. Those contaminants reduced TMR stability by 50 hours (III). The numbers of yeasts, moulds and aerobic bacteria were 55, 257 or 126 times higher in TMR including 26 % (of DM) of aerobically deteriorated silage compared to the TMR prepared from good quality ingredients (Figure 14).

Hygiene can be improved simply by cleaning the mixer more carefully to remove any residues of old batches. Also, cleaning residues from the feed bunk improves hygiene.

Careful removal of any spots of silage showing aerobic spoilage will also improve TMR stability.

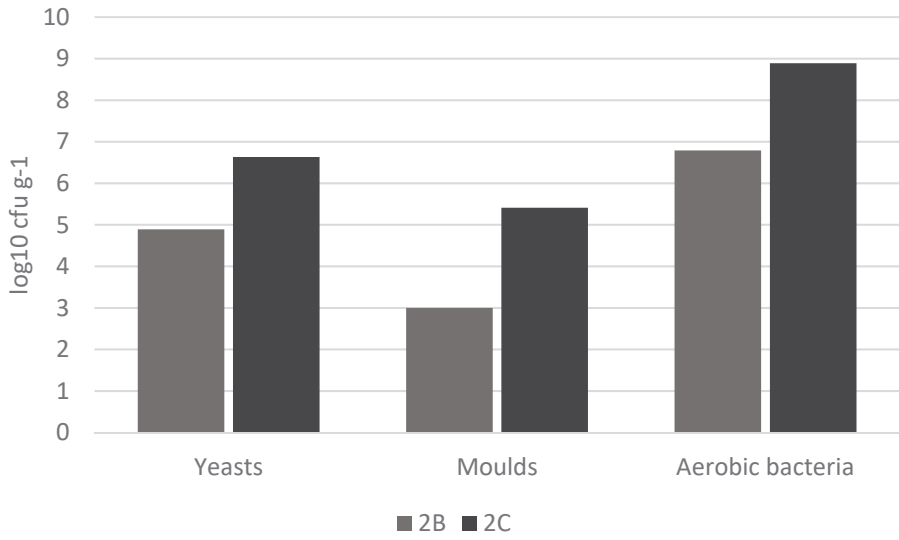



**Figure 14**. Microbial numbers in TMR 2B and inTMR 2C which was otherwise similar, but half of the silage in 2C was aerobically deteriorated (III).

### 3.3.2. Risk feed ingredients for TMR stability

Certain feeds with typically high yeast counts may cause particular problems as TMR components. Practical experiences suggest that during summer time TMR heating problems are exacerbated by the inclusion of brewers grains or straw into the mixture. Results in trial III show, that when brewers grains have high yeast (6.6 $\log_{10}$ cfu $g^{-1}$) content it reduces TMR stability by at least 33 hours. In I, TMR containing brewers grains, LDM silage and pelleted concentrate had stability of less than 35 hours for all the LDM silages. In that trial yeast counts in brewers grains (6.98 $\log_{10}$ cfu $g^{-1}$) was 42 times higher than the highest yeast count measured from grass silages (5.18 $\log_{10}$ cfu $g^{-1}$). However, TMR prepared from HDM silages had a stability of 59 hours or more despite it being prepared using the same brewers grains and pelleted concentrate. Further, inclusion of brewers grains into TMR based on whole crop legume silages did not cause poor stability (II). It is notable, that in trial II brewers grains had low yeast counts (yeast count 2,9 $\log_{10}$ cfu $g^{-1}$). Yeast counts in fresh brewers grains measured by others (3.7– 4.3 $\log_{10}$ cfu $g^{-1}$, Nishino et al., 2003a; Coskuntuna et al. 2010) lie within the range measured in I-III. The high yeast count in brewers grains in I and III is the outcome of the practice to store brewers grains piled on the farm yard without any preservative.

Omitting brewers grains from the mixture could be a tool to improve TMR stability as demonstrated in III. Optionally, an effective approach might be to control the microbes in the brewers grains by applying a preservative, by ensiling brewers grains or by consuming brewers grains more rapidly.

Other byproducts may also pose a risk for TMR stability. Kung (2005) regarded distillers grains as a risk for the TMR stability. In II, the inclusion of straw reduced TMR stability by 6 hours (III) when mixed into legume whole crop based TMR. The small effect of the straw inclusion is understandable based on the straw yeast and mould counts (4.36 and 5.94 $\log_{10}$ cfu $g^{-1}$, respectively) which were on the same level as in the grain components also included into TMR.

Dry grain may contain a total bacteria count of 5.8 - 8.2 $\log_{10}$ cfu $g^{-1}$ and mould count of 2.2 - 2.9 $\log_{10}$ cfu $g^{-1}$ (Laca et al. 2006, Rose et al. 2012). In Finland, harvested grain nearly always has moisture above 140 g $kg^{-1}$ requiring either drying or other preservation methods. After harvesting there is normally some delay until crimping or drying takes place. During that delay the temperature of the grains may increase due to microbial actions. Seppälä et al. (2016) measured total bacteria count 7.6 – 8.4, yeasts 6.1 – 6.3 and moulds 4.6 – 6.0 $\log_{10}$ cfu $g^{-1}$ from the grain prior to crimping.

In Finland, ensiled moist crimped grains are commonly used on ruminant farms. Moist crimped grains may have much higher microbial counts than dry grains. Olstorpe et al. (2010) measured total aerobic bacteria count 8-14, yeast count 1-9 and moulds 1-6 $\log_{10}$ cfu $g^{-1}$ in moist crimped barley during the feed out period. The lowest (< 2 $\log_{10}$ cfu $g^{-1}$) yeast and mould counts were measured in the grains having the highest moisture content (300 g $kg^{-1}$) after a long ensiling period. Microbial load coming from crimped grains can effectively be reduced by using formic and propionic acids when ensiling the crimping grains (Seppälä et al. 2012). Actually, well preserved acid treated crimped grains have lower aerobic microbial numbers than dry grains (Seppälä et al. 2012, 2015a,b). Acid use in crimping also improved aerobic stability of TMR prepared from the crimped grains together with grass silage (Seppälä et al. 2012). Rinne et al. (2019b) detected that additive treatment and fermentation quality of crimped ensiled faba beans may also be reflected into TMR Stability. It is notable that TMR stabilities were long in the trials of Seppälä et al. (90-149 hours, 2012) and Rinne et al. (107-159 hours, 2019b) suggesting that normally other factors than grain treatment have larger effect on TMR stability.

### 3.3.3. Moisture concentration

Moisture concentration of TMR can be manipulated by seraval ways which have different effects on TMR stability. Rinne et al. (2018) showed that adding water can clearly reduce TMR stability (Figure 15). Water activity is one of the main factors contributing to microbial growth. Silage previlting is reflected to TMR moisture concentration. During previlting aerobic microbes proliferate. Prewilting increases DM content restricting fermentation process, decreasing silage density and increasing porosity which all affect microbe

numbers. Thus, effect of silage DM on TMR stability is much more versatile than just increasing moisture of TMR.

The TMR DM content was 330 g $kg^{-1}$ with LDM silage and 590 g $kg^{-1}$ with HDM silage (I). TMR prepared from HDM silages had 69 hours longer stability than TMR from LDM silages. The $_{ho}$LAB treated HDM silages had smaller numbers of yeasts and moulds than LDM silages treated with the same additives. In contrary to $_{ho}$LAB treatments, the Control treatment showed higher yeast counts in HDM silages than in LDM silages.

In practice, increased prewilting may make silage more porous thereby facilitating faster and deeper oxygen penetration during feedout and thus indirectly aerobic stability may be impaired or losses increased (Holmes and Bolsen 2009). The effect of silage porosity was not simulated in trials I and II

TMR prepared from whole crop legume silages had a DM concentration of approximately 280 g $kg^{-1}$, but still their stability was relatively good (over 45 hours). In the case of very wet TMR, plenty of energy is needed to cause detectable heating, which may hide the problem of aerobic spoilage.

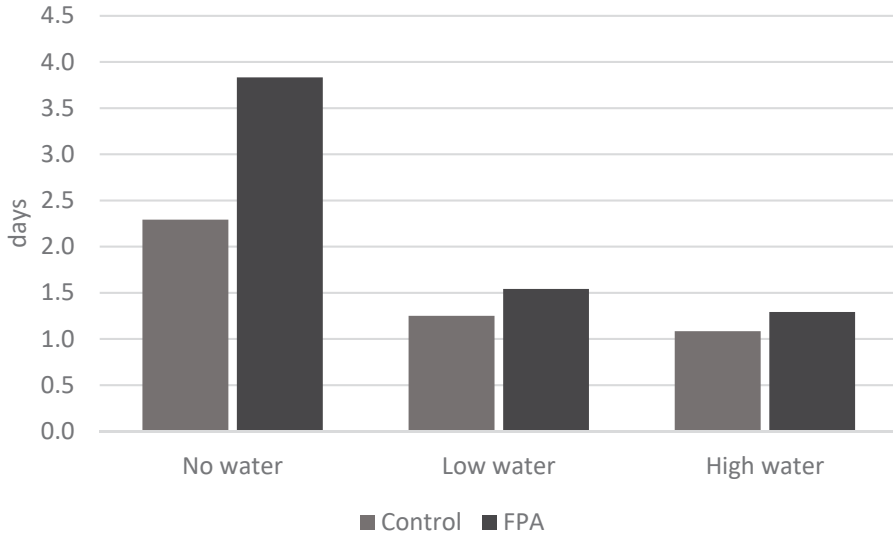



**Figure 15**. Adding water into TMR can clearly reduce TMR stability as well as eliminate the stabilizing effect of added preservative (Data adopted from Rinne et al. 2018). TMR feed ingredients were grass silage, dry rolled barley and oats, rapeseed meal and mineral mixture. FPA TMR included a blend of formic and propionic acids added into TMR at the time of mixing while Control TMR was mixed without a preservative.

### 3.3.4. Chemical TMR stabilizers

Adding chemical additives into TMR that is showing vigorous heating may give only a limited improvement (III, Rinne et al. 2018, Kristensen 2019, Figure 16) or sometimes a remarkable improvement (Jaakkola et al. 2017) into stability. The poor response to the use

of stabilizers in the first trial in III can partly be explained by the fact that the temperature was recorded only twice per day, and stability was estimated mathematically between the time points, which was not sensitive enough. Otherwise both results of III and results of others (Nuβbaum 2005, Rinne et al. 2018, Figure 17) suggest that a better response for chemical stabilizers can be expected, when the control treatment has good stability.

This was further demonstrated mathematically (Figures 18-19) using a simple exponential growth model. It was assumed, that the termination of aerobic stability is linearly linked to microbe count and the end of stability was set to $10^7$ cfu $g^{-1}$. When initial microbe count was low ($2.3 \cdot 10^4$ cfu $g^{-1}$) nine population duplications are needed to achieve the limit. With high initial count ($2.3 \cdot 10^6$ cfu $g^{-1}$) after three population duplications the limit is reached. So, in the case of high initial count of microbes the preservative must be strong enough to virtually stop the microbial activity totally or othervise the benefit will be negligible.

When the trial target is to detect significant differences, the trial should be conducted using a TMR that is not heating too vigorously. By this approach the errors caused by intrinsic properties of measuring system will be in smaller role compared to the phenomenon in focus. On the other hand, the measured responses will be much greater (e.g. more than 40 hours) than what will be seen in practice on farms using the stabilizers, where it is more realistic to gain improvement by some hours, which however can be enough at farm level. It is notable, that the same spoiling species should be present in in the laboratory model than in the farm level to justify the interpretations.

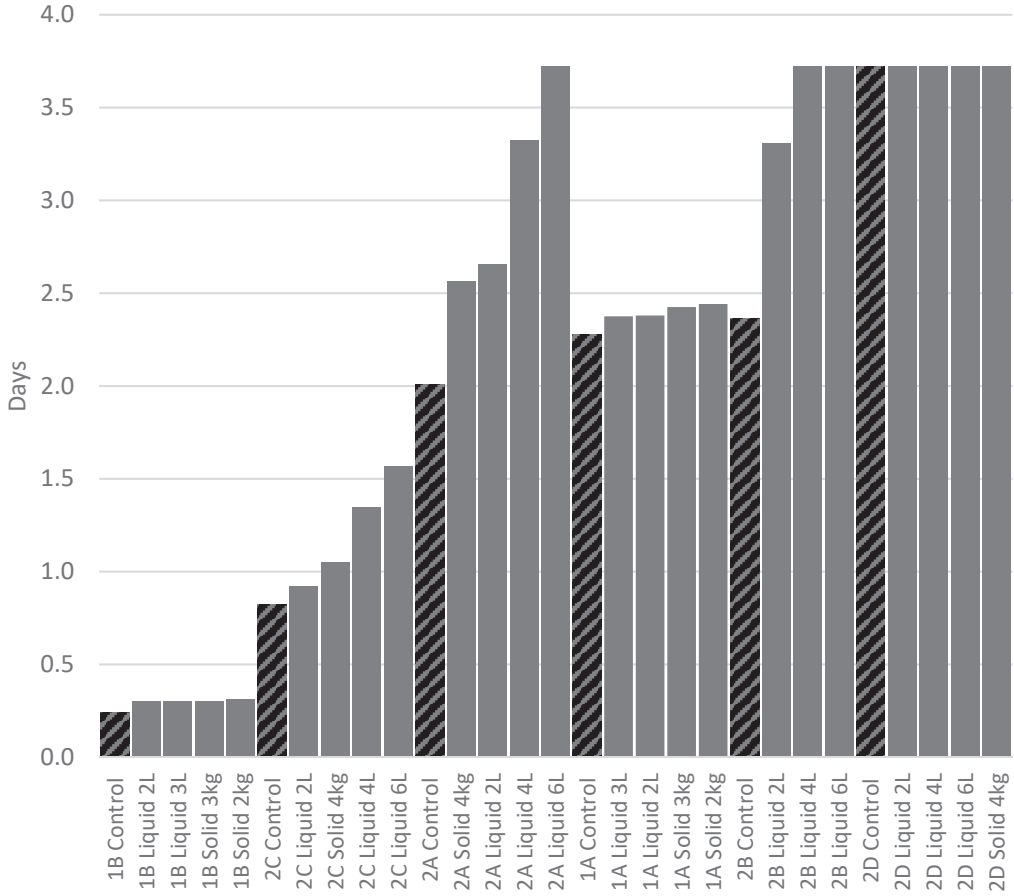



**Figure 16**. Aerobic stability of TMRs in trial III (Same picture as Figure 13 arranged differently). The effect of TMR stabilizers on TMR stability. Control TMRs without any preservative marked with darker colour.

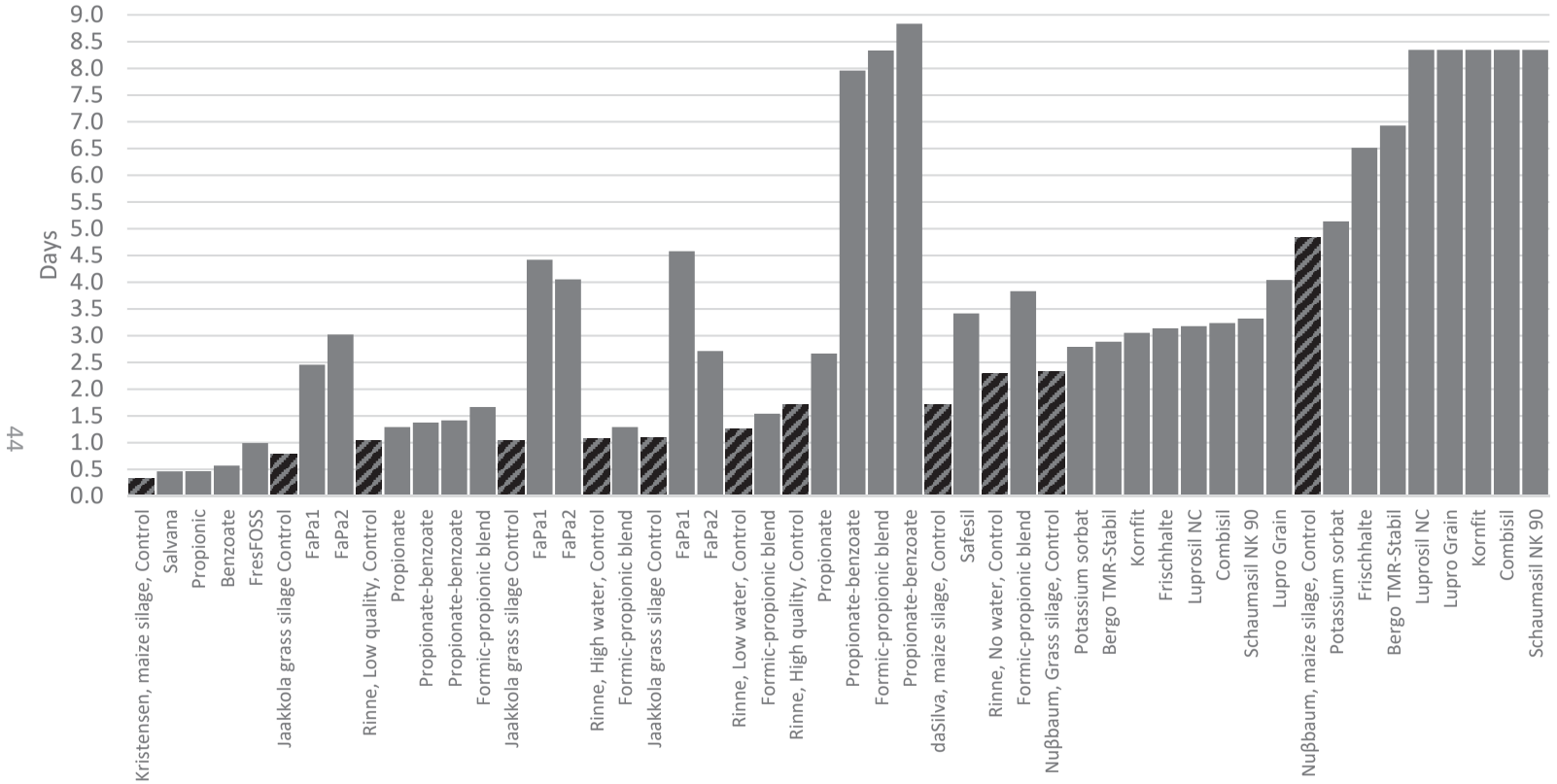
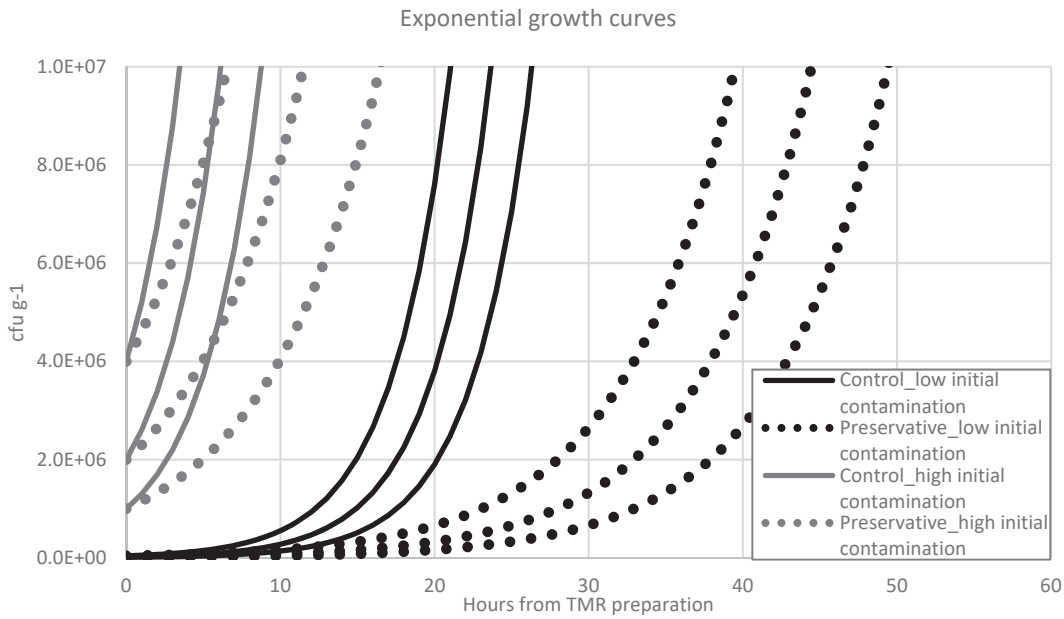
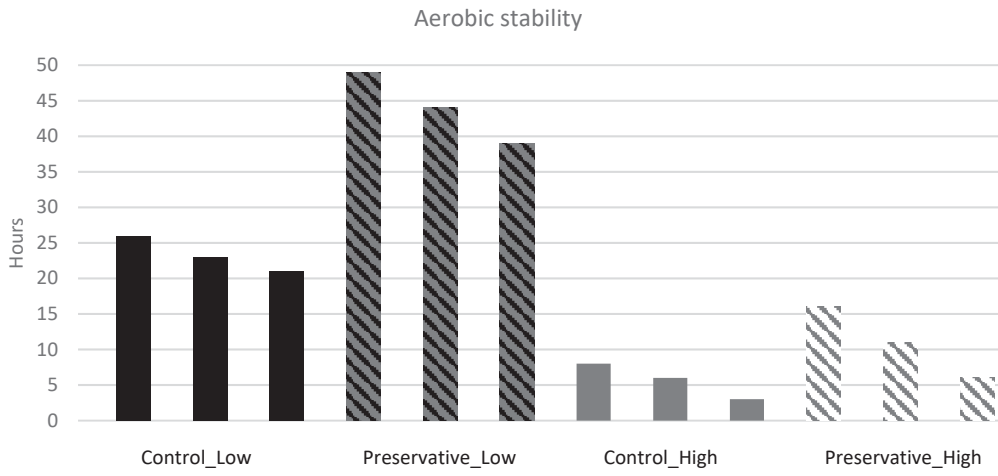



**Figure 17**. Effect of TMR stabilizers on TMR stability. Control TMRs without any preservative marked with darker colour. Results arranged in ascending order of the stability of Control TMR (Nuβbaum 2005, Jaakkola et al. 2017, DaSilva 2018, Rinne et al. 2018, Kristensen 2019, exact compositions of the treatments were missing in many of the publications).




**Figures 18-19**. An exponential growth model was used to demonstrate the effects of initial contamination and growth rate on resulting aerobic stability. Used model was $x_t = x_0(1+r)^t$, where $x_0$ is the microbial contamination (cfu $g^{-1}$) at time 0, r is growth rate and time (hours) goes on in discrete intervals. Low initial contamination (Low) was either $1 \cdot 10^4$, $2 \cdot 10^4$ or $4 \cdot 10^4$ cfu $g^{-1}$; high initial contamination (High) was either $1 \cdot 10^6$, $2 \cdot 10^6$ or $4 \cdot 10^6$ cfu $g^{-1}$. Growth rate was 0.3 (Control) or 0.15 (Preservative) simulating a case where preservative is slowing the growth of microbes. Aerobic stability was the time until x reached $10^7$ cfu $g^{-1}$. In this model Preservative doubled aerobic stability on both Low and High initial contamination levels. The simulated effect of Preservative was highly significant (p=0.003, ANOVA) in case of Low initial contamination while in case of High initial contamination there was no significant effect.

### 3.3.5. Silage fermentation quality

Silage stability is presumed to be linearly linked to TMR stability (Kung 2005). There was one clear exception to this principle, namely LDM Control silage having stability of 13.8 days when TMR prepared from that silage was stable only for 10 hours. Without that exception, the coefficient of regression ($R^2$) between LDM silages and TMR was 0.947, and for the whole LDM data it was 0.637. The $R^2$ for $TMR_{bobs}$ including both PW and FBW was 0.983 (Figure 20).

The LDM silages had on average 12-times higher stability than the respective TMR. Legume whole crop silages had stability 1.4 times higher than the respective TMR. The explanation could be the difference in the microbiological quality of the other ingredients, especially brewers grains (yeast count 7.9 vs 2.9 $\log_{10}$ cfu $g^{-1}$).

In the case of LDM TMR, the majority of the yeasts originated from the brewers grains. However, the shortest TMR stability was measured from those LDM TMR that had the highest yeast counts in respective LDM silages as well as elevated mould counts. Results suggest that the yeasts from brewers grains did not fully override the effect of silage yeasts. That is further supported by the fact, that the highest stability measured from TMRbobs was made from silage having the lowest yeast count (PW ACID). The effect of the choice of silage additive treatment on TMR stability was 13 hours (FBW), 30 hours (LDM), 57 hours (PW) or 82 hours (HDM). In the other publications (Figure 21) the effect of silage additive has been at maximum 7 hours (Jaakkola et al. 2017), 25 hours (Taylor et al. 2002), 30 hours (DaSilva 2018) or 32 hours (Kung et al. 2003b).

Silage aerobic stability can be managed in several ways during the ensiling process (Holmes & Bolsen 2009, Wilkinson & Davies 2013, Kung 2014, Borreani et al. 2018). Rapid field wilting with minimal mechanical treatment is likely to result in relatively low counts of yeasts in harvested grass material (Jonsson et al. 1990). Rapid silo filling, enough packing weight and plastic covering weighted down with tyres or gravel bags are ways to minimize oxygen availability during early phases of ensiling (Kung 2014). High density and low porosity assist to minimize the rate of oxygen ingress into silage mass during feed-out. Rate of silage removal should exceed depth of air penetration into the silo (Wilkinson & Davies 2013, Borreani et al. 2018). By the good harvesting and silo management practices together with an appropriate silage additive (I) it is possible to make a grass silage that has low yeast count followed by a good aerobic stability (Mäki 1997).

Several reviews have addressed the effects of silage additives on silage aerobic stability (Wilkinson & Davies 2013, Knický & Spörndly 2014, Kung 2014, Borreani et al. 2018, Muck et al. 2018). Silage additives can be classified as i) stimulators of fermentation, e.g. homofermenative LAB, ii) inhibitors of fermentation, e.g. formic acid, iii) inhibitors of aerobic spoilage e.g. benzoate, sorbate, and heterofermentative *Lactobacillus buchneri* and iv) nutrients and absorbents, while some additives fall into multiple categories (Kung et al. 2003).

An extremely dominant homolactic acid fermentation caused by microbial inoculation provides silages that have minimal amount of organic acids with antifungal properties

(Kung 2014). Those types of silages may have clearly shorter aerobic stability than silages without additive, especially when the Control silage made without additives contains relatively high amounts of acetic acid (I, LDM). In the review of Oliveira et al. (2017) it was concluded that LAB inoculation (homofermentative or facultatively heterofermentative LAB strains) increased silage yeast count without an effect on aerobic stability. However, from 130 publications 86 reported yeasts counts and only 40 reported aerobic stability. Also, in trial II the effect of LAB treatments on stability of whole crops were variable. Results presented in I suggest that homofermentative LAB strains were able to drop yeasts counts in HDM silages while the effect was opposite in LDM silages.

Acetic acid produced by heterofermentative LAB has often improved the aerobic stability of silage (Driehuis et al. 1999). Concentration of at least 8 g undissociated acetic acid $kg^{-1}$ fresh weight is suggested to be the threshold for a low risk of aerobic instability (Wilkinson & Davies 2013). When the inoculum included a heterofermentative strain, the aerobic stability of the LDM silage and HDM TMR was improved compared to homofermentative strains only (I).

Salts of sorbic acid and benzoic acids are often included into commercial silage additives to improve aerobic stability (Nadeau et al. 2012, Auerbach & Nadeau 2013, Knický & Spörndly 2014, da Silva 2018), or they can be combined with a homofermentative LAB inoculant (Rammer et al. 1999, Owen 2002, White et al. 2002) to combine benefits of both types of additives (Wilkinson & Davies 2013). The inclusion of sodium benzoate into the treatment $_{ho}$LABcelSB was too small to make a statistically significant improvement, although numerical improvements in stability were seen compared to homofermentative LAB treatments, but not compared to Control (I). SALT treatment (I) made only some minor changes in silage fermentation quality and was not able to change silage aerobic stability compared to Control treatment. However, the stability of LDM TMR was improved by 24 hours when SALT was used as silage additive. It is notable that Control LDM and HDM silages had such good stability that any improvements would be very difficult to achieve. Knický & Spörndly (2014) suggested a trial arrangement (loose packing, air inlets opened for 8 hours 7 and 14 days before silo opening) that dropped stability of Control treatment from 148 to 22 hours and made it possible to show the potential of silage additive to improve silage aerobic stability.

Formic acid-based additives have been able to improve silage stability after a three months ensiling period (Rinne et al. 2016). Sometimes the effect is linked to the elevated ethanol concentrations in those silages (Weiss 2016), as was the case in II with PW ACID silage. However, FBW ACID silage had poor stability despite the acid treatment containing 20 % propionic acid, which should have improved efficiency against yeasts. In the trial of Rinne et al. (2016) the same additive resulted in a greater improvement in aerobic stability of grass silage than formic acid alone without increasing ethanol concentration.

Generally, all the factors affecting silage (and TMR) aerobic stability can have numerous interactions. Due to the complexity of silage ecosystem, Weiss et al. (2016) concluded that it seems unlikely that it will ever be possible to precisely predict the outcome of silage fermentations.

While silage and TMR should be aerobically stable, they should at the same time provide nutrients in such a form that production responses are not sacrificed. For example, high amounts of acetic acid will improve stability while at the same time silage intake (Huhtanen et al. 2007) and expected production response will be reduced (Huhtanen et al. 2003) and DM losses during the ensiling period increase.

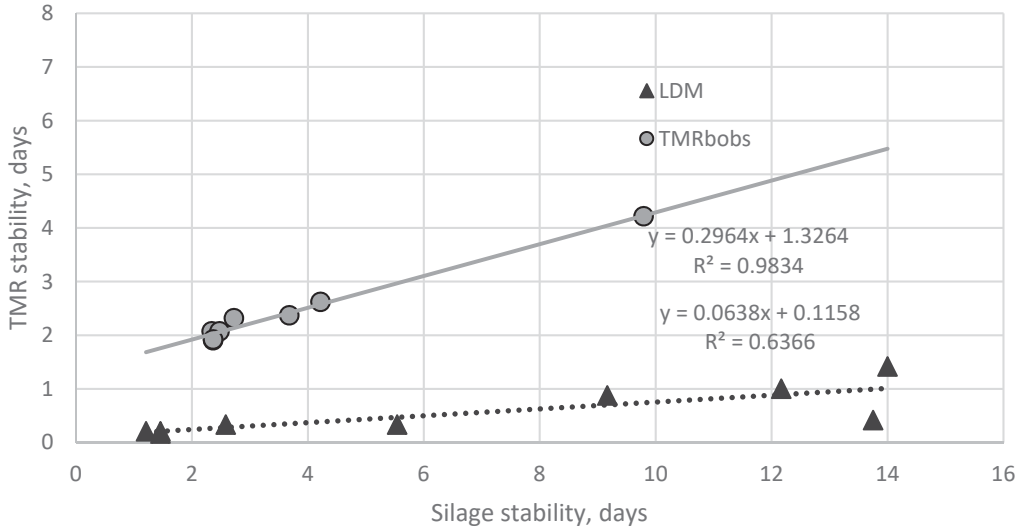



**Figure 20**. TMR aerobic stability was linearly dependent on the aerobic stability of the silage used as the main component in the TMR (I, II). LDM = low dry matter silages (I), $TMR_{bobs}$ = TMR including whole crop silage, barley, oats, brewers grains and straw.

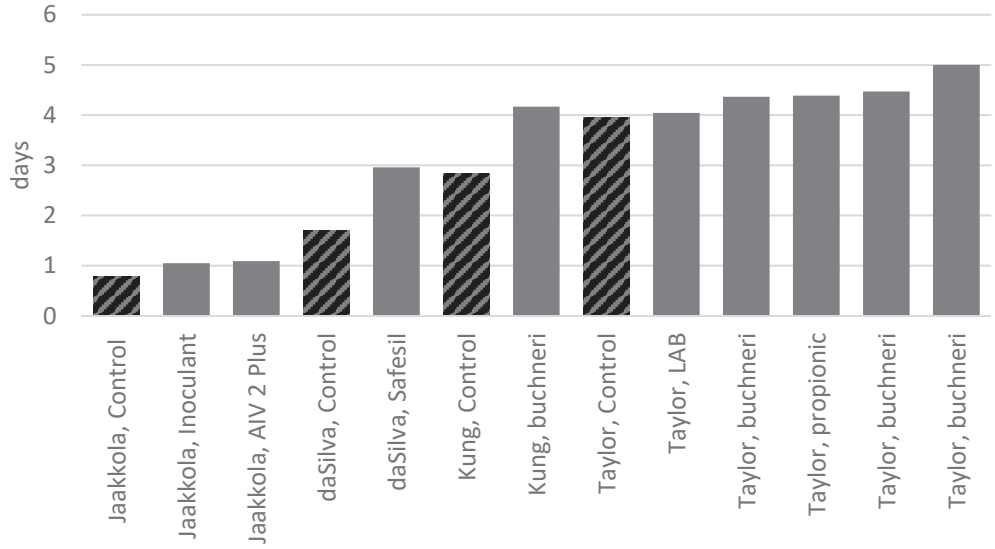



**Figure 21**. Effect of silage additive on the stability of the TMR prepared using that silage. The Control silage without additive marked with darker colour (Taylor et al. 2002, Kung et al. 2003b, Jaakkola et al. 2017, DaSilva 2018)

# 4. Conclusion and practical applications of the results

## 4.1. Concluding remarks

1. Silage aerobic stability is most often directly reflected to TMR stability within crop type and DM class. An exception was the non-additive treated LDM (stable silage vs. easily heating TMR). Data did not include silages with high butyric acid concentrations.
2. Silage additives can manipulate silage and TMR aerobic stability. Within additive treated silages there was a strong correlation between silage and TMR stability.
3. If silages are mixed into TMR immediately after silo opening, low silage DM provides microbes plenty of moisture for rapid growth. Wet feeds have however high specific heat capacity which can result in a situation where detected temperature rise is small.
4. If TMR is composed of very high dry matter ingredients, low water availability is slowing down the growth of spoiling microbes. However, silage prewilting includes other factors risking silage aerobic stability, e.g. longer aerobic phase prior to ensiling and high silage porosity. Thus, in practical conditions high number of aerobic microbes in prewilted silage entering TMR may overrun the effect of low moisture.
5. Chemical TMR preservatives can improve TMR stability. Better response is expected when TMR has low initial number of spoiling microbes.

Several actions supported by the research conducted in this thesis can be applied at farm level to prevent aerobic spoilage of TMR. Actions can be divided to those that can be taken at the time of TMR mixing, while others such as fermentation quality of the silage depend on decisions made earlier.

1) Poor hygiene in TMR preparation should be eradicated such as residues of old batches or feed that already show spoilage.
2) If by-products such as brewers grains with risk of containing high amounts of aerobic microbes are used in the TMR, leaving them out of the recipe may alleviate problems. In that case, it is recommendable to pay attention to better preservation of such components to improve their hygienic quality.
3) Water is often added to TMR to decrease the sorting of it. In case of heating problems unnecessary water addition should be avoided, because it increases water activity in the TMR and promotes microbial growth.
4) Use of a proper silage additive can improve stability after silo opening and subsequently also TMR stability.
5) Chemical stabilizers added into TMR may improve stability. The application level needs to be adjusted at the farm level based on experience.

## 4.2. Further research

To minimize feed and production losses, farmers need tools to evaluate the shelf life and microbiological safety of their feeds in a cost-efficient manner. Currently there is lack of those tools at farm level. More applied research is needed to assist farmers to avoid losses caused by aerobic microbes.

It is more important to control the spoiling micro-organisms in the feed ingredients than in the TMR. Further, it is easier to conduct research and draw conclusions at the level of single ingredients than when the feeds are mixed into TMR.

However, when exploring microbial quality or aerobic stability of cattle feeds, their effect on TMR quality should be considered whenever it is possible. At present TMR heating problems are quite common and the changing climate may extend their presence to cover the whole year.

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