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Investigation of aerobic stability in extruded silages

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Abstract

In dairy farming, feed cost represents the highest single expense. To maintain a high milk production, feed intake must be maintained, and forage is the major feed for dairy cows in many parts of the world. However, due to constraint on feed intake, forage alone cannot meet the demand of a high producing dairy cow because of its large particle size and high fibre content. Feed processing and preservation aiming at particle size reduction has shown promising results in increasing intake. Extrusion is a mechanical process where screws shear and mix lignocellulosic material for particle size reduction and cell wall disintegration. During extrusion temperature rise in the processed biomass. Rise in temperature and mixing are potential risk factors for reduced aerobic stability in silage. It is well known that aerobic deterioration is an important source of feed waste in silage related products. It is therefore necessary to study and gain insight in how to avoid aerobic deterioration in silages. The aim of the present study was therefore to investigate the effect of extrusion on aerobic stability.

Silage samples were collected from a bunker silos at Lövsta, (SLU research station). A mixture of Timothy grass/red clover and whole crop barley (WCB) were processed using a Bio-extruder in two Trials. In Trial 1, silage of good hygienic quality used while in Trial 2 silage of poorer hygienic quality was used. A study was conducted using the aerobic stability test unit at the forage lab, SLU. The unit was connected to a computer for recording temperature every two hours until temperature in aerated silage had increased by +5^{o} C above ambient temperature. This experiment employed a factorial design with two factors to consider; types of silage and treatment of silage. In Trial 1 there were two types of silages (Timothy grass/red clover and WCB) collected from the centre of the silo, and three treatments (extruded silage that was kept warm after extrusion, extruded silage that was cooled off and untreated silage as control). In Trial 2, the same types of silages as in Trial 1 was used but collected from the periphery of the silo and for the grass/clover even from a different silo. In Trial 2, the same treatments as in Trial 1 were used, but a fourth treatment was added (extruded silage that was kept warm with inclusion of silage additive). Samples were collected and processed on 4 consecutive days in each trial resulting in 24 samples in Trial 1 and 32 samples in Trial 2. These samples were incubated in the aerobic stability unit and temperature of each sample was recorded continously over 7 days.

After incubation, the fermentation residues were used to produce juice for pH measurement.

In Trial 1, all incubated silages displayed a similar pattern in the development of temperature over time. That means temperature of the aerated silages did not increased by +5° C until measurement stopped at 164 hours. In Trial 2, silages displayed different patterns in the increase in temperature over time (hours). Generally, in Trial 2 it took less time for temperature in the silage to rise by +5° C. This difference between the trials indicates that the silage in Trial 1 was of better quality (more stable) compared to Trial 2.

In Trial 1, results showed that the effects of silage type on temperature and pH before aerobic stability test were significant ($P<0.001$) meaning that temperature was higher in WCB and pH lower, compared with grass/clover silage. The treatment effects were significant on temperature ($p<0.001$) with increased temperature after extrusion. The interaction effect between treatments and silage type was observed to be significant only on temperature ($p = 0.01$) with a greater difference in temperature before and after extrusion in grass/clover silage.

In Trial 2, the results showed that the effect of silage type was significant on pH before aerobic stability test (higher for WCB, $p = 0.007$) and after aerobic stability test (higher for grass/clover, $p = 0.017$), as well as in number of yeast CFU (greater for WCB, $p = 0.003$) and moulds CFU (greater for grass/clover. $p = 0.009$). Significant treatment effect was observed for yeast CFU (reduced after treatment, $p = 0.01$). The interaction effect between treatments and silage type was significant only on temperature ($p = 0.015$) with a greater increase in WCB compared to grass/clover.

The findings of this study have shown that the process of extrusion with different silage treatments did not have a significant effect on the aerobic stability of the silage types. However, pH, temperature, microbes (yeasts and moulds) of the extruded silage were found to be important parameters determining silage quality.

Keywords: Silage, extrusion, aerobic stability, additive

Preface

It has been a great privilege to spend two years discussing and thinking about animal nutrition at Swedish University of Agricultural sciences (SLU). Unquestionably, I have gained sufficient knowledge and skills in multidisplinary subjects from SLU and broadened my capacity in conducting research.

The accomplishment of coursework and MSc would not have been possible without Swedish Institute (SI) financial support and invaluable contribution from different colleagues and I would like to thank them all for their unconditional support and inspiration.

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Lastly, I would like to dedicate this work to my late parents Landoard and Mecktilida Kalikawe who encouraged me in my academic carrier. I hope they would have been proud to see me achieving my MSc abroad. May their souls rest in eternal peace

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Abbreviations

CFU	Colony forming unit
CP	Crude Protein
DM	Dry matter
DMI	Dry matter intake
FPS	Forage particle size
GLM	General linear model
LAB	Lactic acid bacteria
LB	Lignocellulosic biomass
MC	Moisture content
ME	Metabolizable energy
Mixture	Timothy Grass /red clover
NDF	Neutral detergent fibre
OM	Organic matter
PVC	Polyvinyl chloride
SLU	Sveriges lantbruksuniversitet
VFA	Volatile fatty acids
WCB	Whole crop barley
WSC	Water soluble carbohydrate

1 Introduction

The demand for milk, meat and their by-products as source of animal protein is increasing worldwide due to drastic increase in human population (Delgado, 2003; Duniere *et al*., 2013). To meet increasing demand for animal protein, deliberate efforts toward improving livestock productivity are inevitable. Feed cost represents the highest single expense in intensive dairy farms whereby both quality and quantity are thought to play a key role (Duniere *et al*., 2013). Forage is the major feed for dairy cows in various parts of the world. For example, forages contribute 60% of nutrient requirements of Swedish dairy cows; where ley is the dominating crop (Knicky, 2005). However, to ensure all year round supply of feed to dairy cows, feed preservation is essential and silage making is the most important feed preservation method in countries like Sweden (Kasmaei, 2016).

Silage is the material produced from a forage crop in an oxygen free environment, aiming at preserving it by natural fermentation. This ensures food security all the year round in case of seasonal availability of grazing resources due to weather challenges (McDonald *et al*., 2011). However, if oxygen gains access to stored or feed-out materials, aerobic microbial activities take place. This might cause deterioration to an extent that the feed becomes inedible. Aerobic deterioration results in feed losses and possibly toxin production, which might lead to feed shortage and increased feed cost (Duniere et al., 2013). The deterioration can be described as the loss of aerobic stability of silage which is measured by the time (hours) the silage remains stable before its temperature rises by at least 2° C above the ambient temperature. This happens as a result of microbial activities that follow increased pH of the biomass (McDonald *et al*., 2011).

By processing forage to reduce particle size, feed intake may be improved (Duque *et al*., 2017). Thus, particle size reduction can have a substantial effect on animal performance. This has created an interest for a more extensive forage processing than can be accomplished by chopping alone (Nasrollahi *et al*. 2015). In the 1950s, pelleting of forage came into practice to cause extensive particle size reduction. This was to improve forage feeding value and increase the harvest window (Behnke, 1996).

However, the extrusion process leads to a rise in temperature of the extruded silage, making the substrate for microbes responsible for silage deterioration more easily available. Therefore, the present study was conducted to investigate if extruded silage would reduce aerobic stability of silage. The study also gives an insight to understand the effect of additive added on the warm extruded silage in relation to aerobic stability.

2 Literature review

2.1 Cow`s feed requirements and restrictions

Forage constitutes a major part of dairy cow rations. As a feed source, forage is vital for the cow to maintain a normal rumen function (Van Soest *et al*., 1991a). Thus, successful preservation of forage is necessary for a good hygienic quality and to keep nutrient losses at a minimum (Kasmaei, 2016). Dairy cows as ruminants are endowed with microbes in the rumen. The microbes facilitate utilization of energy from the cellulosic carbohydrate and protein to volatile fatty acids (VFA) that in turn are utilized by the cow as the source of metabolized energy (ME). Also, a smaller amount of isobutyrate, valerate and isovalerate are produced depending on the feed source (Van Soest *et al*., 1991b).

Unlike concentrates, forages are rich in fibre with large particle size that promotes chewing and rumination. Chewing reduces particle size which is necessary for passage through the rumen (Chamberlain & Wilkinson, 1996). Chewing and rumination are crucial processes because they stimulate saliva production which buffers the rumen fluids. In doing so, they help to maintain the pH values of the rumen fluid above 6.2 which should be ideal for fibre degradation (Márquez *et al*., 2009). It follows that, feeding high proportion of readily fermentable carbohydrates (concentrates i.e. less fibres) will decrease rumen pH since less fibrous feeds tend to reduce chewing, rumination and saliva production; and so increase production of VFA. Mertens (1997) reported that physical effectiveness or roughage values of a feed and fibre requirement of dairy cows are key consideration in the cow`s ration. This is important because it helps to reduce metabolic disorders like sub-acute and acute rumen acidosis. So, maintaining milk fat production and optimizing ruminal fermentation are vital. The latter are determined by particle size and inherent characteristics of neutral detergent fibre (NDF) that also affect chewing activity, ruminal pH and milk production (Jung & Allen, 1995).

2.2 Forage plant

In spite of intensive concentrate feeding in ruminant animal production, forage continues to represent the single most important component as feed resources for ruminants (Jung & Allen, 1995). However, cell wall concentration limits the intake potential and energy availability of forage crops in high-yielding dairy cows and lignin is the key element that limits cell wall digestibility (Jung & Allen, 1995). In most cases, grasses and clover form a mix that complement each other because of their differences in chemical composition. Their content and degradability of NDF at different times determine forage quality at various harvest occasions (Rinne & Nykänen, 2008).

2.2.1 Timothy and red clover mixture

Timothy (*Phleum pratense* L) is a type of grass that is highly adapted to cold weather. This feature makes it an effective grass in the temperate countries like those found in Northern Europe (Frame, 2011). Likewise, red clover (*Trifolium pratense*) is also an important forage species that in most cases is mixed with timothy grass in the Scandinavian countries (Rinne & Nykänen 2008). In that region, a mixture of timothy grass and red clover forms a popular feed source for dairy cows due to chemical composition of these varieties, e.g. crude protein (CP) and NDF, which is basically due to different blooming time and leaf development. Therefore, a good proportion of these species in the sward increases the possibility of producing a high-quality forage at different harvesting periods (Rinne & Nykänen, 2008). However, different patterns of maturation in these forage species have an effect on animal performance (Troelsen & Campbell, 1969). The NDF content in timothy grass is high and tend to increase with maturity and this contributes to reduction of readily degradable constituents, potential degradability of organic matter (OM) and NDF (Hetta *et al*., 2004). Thus, maturation, i.e. from early to later harvest date, has an influence on the degradation parameters in timothy grass and red clover that require an important consideration to attain a desirable forage digestibility and hence animal performance.

2.2.2 Whole crop barley

Like several other cereal crops, whole crop barley (WCB) is used as a forage for dairy cows that can provide several advantages to the dairy sector (Hargreaves *et al*., 2009). It can offer a source of supplementary feed with a potential degradability and appreciable amount of starch (Adesogan *et al*., 1999). WCB can be grown in regions where maize for silage is inadequately grown owing to climatic challenges; for example in places that are very cold like northern part of Sweden (Hill & Leaver, 1999). Hill & Leaver (1999) observed that for the WCB to be important, it must have moderate to

high yield of harvestable dry matter (DM) per hectare. Their study evaluated stage of harvest at a high intake potential (early cut) which could then be preserved as silage and possibly hay to ensure feed security all the year round especially in areas which are limited by climatic conditions such as extreme drought and winter. This can be explained by the fact that, fodder crop of exceptional quality can only be available at a certain time of the year.

2.3 Forage physical treatment and digestibility

In order to have a good estimate of the animal's dietary energy supplies, animal`s body weight, physiological status including daily milk production, stage of pregnancy and stage of lactation are the key factors one should consider so to predict performance (Volden, 2011). Voluntary intake of forage is a critical determinant of animal's performance. Cell wall affects intake by contributing to ruminal fill which is a function of digestion and passage rates. Likewise, indigestible fraction i.e. lignin (that makes forage to not be a sole source of energy to high-yielding dairy cows) affects intake (Jung & Allen, 1995). Hence, concentrate feed is needed to supply the high-yielding cow with enough energy and protein to meet the requirement (Beauchemin *et al.*, 2003). Cereals represent the most important source of concentrate. Its limitation is the rapid fermentation in the rumen which can have a negative effect on rumen function and increase the risk of metabolic disorders that can lead to poor performance (Krause *et al.*, 2002).

Moreover, to have a major effect on animal performance, forage processing needs to be more extensive than can be accomplished by chopping alone. Minson (1963) found that pelleting causes an extensive particle size reduction and it came in practice in the 1950s to improve forage feeding value. However, despite of it being positively affecting animal production through increased intake, often depressed digestibility was observed. Minson (1963) also reported that improved feed conversion efficiency and increased meat production were observed with pelleted rations caused by an increased feed intake. Using lactating Holstein cows fitted with ruminal and duodenal fistula, Rode *et al*. (1985) observed an increased total protein flow to the duodenum when hay was pelleted. Using model simulations Mertens and Ely (1979) found that intake was expected to increase by 45% in a 500 kg steer when fed pelleted hay as compared to long hay. Effects of pelleting was studied extensively until 1980s but after that, information is scarce, presumably due to less interest and knowledge gap in pelleted forage from the industry.

2.3.1 Effects of chopping on forage intake and utilization

Rumen passage rate determines forage feed intake in an ad-lib feeding system. Longer particle size tends to cause slow passage rate through the rumen and hence contributing to rumen fill (Jung & Allen, 1995). To pass on from the rumen, feed particles need to be reduced in size and density needs to be increased. Particle size reduction in cow is mainly a function of mastication during ingestion and rumination. Lignification of fibre fraction and the surface area being exposed to microbes are the main factors that determine the digestion rate of feed materials in the rumen; and the density of digested materials increases with time (Bailey *et al*., 1990)

Rumen passage rate and hence feed intake is dependent on the particle size reduction. Chopping is commonly used in silage making and in practice chopping length is between 10 and 20 mm. Rustas *et al*. (2010) found that a particle size reduction to 20 mm decreased the eating time but did not noticeably improve intake in cattle. The reason for this could be probably that during ingestive mastication of the cow, feed particles get reduced to a similar extent as during chopping (Bailey *et al*. 1990). Chopping to shorter particle size can affect animal performance due to the fact that it has positive effects on voluntary intake (Jung & Allen, 1995). On the other hand, Beauchemin *et al*. (2003) found no effect of particle size on dry matter intake (DMI) and eating time, but on chewing and rumination time, which were observed to be linked to the particle size rather than the proportion of hay and silage. They came up with such findings after studying how different proportions of silage and hay i.e. 50:50 and 25:75 in alfalfa-based diet containing 60% forage affected animal performance. In this case, hay was either chopped or ground. The findings showed that rumination and chewing decreased when feeding ground hay when compared to feeding chopped hay. The ruminal pH was higher for cows fed with less silage ratio (25:75) compared to high silage ratio (50:50). Similarly, the cows fed ground hay had low pH compared with the cows fed chopped hay. The milk yield and milk composition exhibited no difference between ground or chopped hay.

Likewise, a study by Nasrollahi *et al*. (2015) noted an increased DMI by decreased particle size of the forage, which can be explained by less fill and increased passage rate through the rumen. Nevertheless, higher passage rate through the rumen due to decreased forage particle size caused a lower digestibility of NDF. This can be explained by the reduced chance for the microbes to act on them, which is compensated by the increased intake. Nasrollahi *et al*. (2015) using a meta-analysis approach of 45 papers, reported forage particle size to have an influence on feed intake and milk production of dairy cattle. Nevertheless, its impact depends on level of feeding, source, and preservation of forage in the diet.

Moreover, Nasrollahi *et al*. (2016) reported an increased proportion of propionate in a diet with reduced particle size, that implies a different fermentation pattern

and probably less methane production. Production of propionate reduces methane production by suppressing methane generating bacteria and reducing the availability of hydrogen (H) as it is a substrate for propionic synthesis (Russell, 1998).

2.3.2 Extrusion process and its effect on fibre

Extrusion is a technology that was first discovered to be used for metal conformations but soon expanded to many other applications in several fields including food and feed processing (Duque *et al*., 2017). Being a common technique used to improve the value of concentrate feeds, it has not been applied to process forage as animal feed. In a study by Damborg *et al*. (2018), where protein was extracted from clovers, lucerne and perennial ryegrass by a twin-screw press, it was found that CP concentration of the pulp was similar to the CP content of the original plant material. Pulp is the fibre-rich residues from processing the plant. Reduction in particle size leads to increased passage rate which results in less time for microbial digestion at the rumen. Pulp has a larger proportion of rumen escape protein which may result in a more efficient CP utilization in the intestine following endogenous enzyme digestion (Damborg *et al*., 2018).

Bruins & Sanders (2012) and Damborg *et al*. (2018) reported that processing forage for biorefinery will catalyse the transition from fossil-based to bio-based economy both ecologically and economically. They recommended the fibre-rich pulp to be used as protein supplement for monogastric animals. The extrusion process would provide an opportunity for reducing feed-food competition for cereals which are also important for humans especially in developing countries in which cereals act as a staple food. This is because extrusion process increases forage intake and a substantial increase in energy supply hence reducing need for cereals in feeding animals.

2.4 Energy considerations

Mechanical and thermal energy are the two forms of energy that ensure that the extruder operates. Thermal energy helps to reach and maintain operation temperature while mechanical energy of the motor rotates the screws (Duque *et al*., 2017). The mechanical energy demanded to drive the screws is high and thermal energy is a result of the friction during the processing of lignocellulosic biomass (LB). The study by Cha *et al*. (2015) discovered a means of LB processing using low temperatures with a promising result. This appears to be a motivation to farmers and other stakeholders because it uses less thermal energy and hence it would be more cost effective. Likewise, Karunanithy & Muthukumarappan (2012), when studying the moisture contents

of the selected feed stocks, found that fill and viscosity in the extruder, screw compression ratio, screw speed and barrel temperature were the main factors that influenced mechanical energy consumption. Interestingly, they found the torque to be inversely proportional to the screw speed in the sense that mechanical energy demand is lower at a high speed of the screw and vice versa.

2.4.1 Effects of extrusion on biomass temperature

The extrusion process leads to elevation of temperature of processed biomass and at very high temperatures, extrusion may even cause partial charring of the biomass (Silva *et al*., 2013).

Duque *et al*. (2017) noted that the key effects of extrusion of LB were reduction of the particle size, increasing specific surface area, changes in the crystallinity of fibres and the biomass structure. Leu & Zhu (2013) categorized mechanical effect on biomass in two classes namely class I and II. Class I effect is considered to be more superficial referring only to cutting, breaking, separating the fibre bundles and just mildly increase the fibre external surface. Class II effect causes entire cell wall disruption and reduces the size of fibres to very tiny particles by breaking up the cross-linked micro-fibrils, including the internal fibrillation. Chen *et al*. (2014), studying methane production from extrusion as pre-treatment of rice straws, found that the cellulose of rice straw was disrupted after extrusion pre-treatment, a phenomenon that can be explained by higher degradation of cellulose and hemicellulose. They also claimed that the extrusion process had nothing to do with chemical compositions of the LB; instead, it only involves physical changes that include water holding capacity, specific porosity and specific surface area. The effects of extrusion are facilitated by different parts of the extruder that form a cross-sectional profile forcing materials through a die of a desired cross section upon being subjected to an expansion when it exits the die (Zheng & Rehmann, 2014). The cell structure of biomass that is passed through the extruder is disintegrated under elevated temperature and pressure, creating increased surface area for enzymes to act on them.

2.5 Crops for silage

Silage is the feed material that is produced in an oxygen-free environment by controlled fermentation of a large variety of crops that are considered to have a moderate moisture content (Kasmaei, 2016). The process of making silage is called ensiling. The crops to be considered for preservation as silage need to have a significant amount of fermentable substrate in the form of water-soluble carbohydrate (WSC) (McDonald *et al*., 1991). Also, low buffering capacity to ease pH drop, dry matter (DM) content

above 200 g kg^{-1} and a physical structure that can be easily compacted in the silo after harvesting are desirable (McDonald *et al*., 2011; McDonald *et al*., 1991). However, several crops do not achieve these requirements hence requiring pre-treatments in the fields that include wilting, fine chopping, and/or the use of additives (Weinberg *et al*., 1996).

2.6 The process of silage aerobic deterioration

To produce silage, one needs to fully understand the factors underlying the process that determines fermentation and storage properties and take precaution thereafter. Spoilage of silage is undesirable and includes growth of detrimental microorganisms, loss of nutrients, production of harmful and toxic substances that may be harmful to livestock and reduce digestibility of the feed through maillard reaction (Ruxton & Gibson, 1993). Silage exposure to oxygen leads to oxidation processes due to the increased activity of yeasts and moulds which results in increased temperature hence heating of the silage. Ensiling conditions strongly influence the fermentation characteristics and thus the nutritional quality of the ensiled forage. Also, silage stability upon opening of the silo is important in determining the quality and value of the silage. When exposed to air many factors determine the sensitivity of the silage for its deterioration. Important factors include air, ambient temperature and substrate availability (Ashbell *et al*., 2002). Aerobic microorganisms, mainly yeast and moulds, cause deterioration of the silage (McDonald *et al*., 2011).

Yeasts and moulds are responsible for aerobic deterioration of silage whereby yeasts initiate deterioration by consuming lactic acid (Tabacco *et al*., 2011). Upon gaining access to oxygen and energy as their substrate, yeasts grow and thrive well in silage leading to loss of nutrients before the material is ensiled. Similarly, ingress of air during storage or after opening the silo during feed out allows yeasts to thrive and deteriorate silage (Guo *et al*., 2014; McDonald *et al*., 2011).

Yeasts or acetic acid bacteria increase in numbers during aerobic exposure and degrade sugars and lactate that increase pH. The aerobic respiration of these microorganisms produces heat which makes silages to warm up, consequently leading to mould growth (mycotoxins), which forces farmers to discard most of the heated silage (Wang *et al*., 2019; McDonald *et al*., 1991).

Spoilage creates feed shortage and hence more expenses to farmers to feed their animals. The most common means of inhibiting the growth of these undesirable microorganisms is to promote lactic acid fermentation which is facilitated by facultative anaerobic LAB present on harvested crops. Their main role is to ferment naturally occurring sugars to a blend of acids mainly lactic acid that lowers pH to a level that

inhibits undesirable bacteria (Wang *et al*., 2019; Liu *et al*., 2014). However, this inhibition does not depend on pH fall alone, but also moisture content and temperature. The wetter the material, the lower will be the critical pH value (McDonald *et al*., 2011; McDonald *et al*., 1991). It has been established that provided that the silo remains sealed and free from rain penetration, achievement of pH value of approximately 4.0 will preserve the silage satisfactorily and hence minimise losses of nutrients (McDonald *et al*., 1991). Initial LAB population and availability of substrate on the ensiled crop is an important factor towards inhibition of undesirable bacteria. This is highly influenced by physical processing that involves chopping and mincing. In finely chopped materials with the particle length of <25 mm, plant sap is quickly liberated, and LAB growth is stimulated (Guo *et al*., 2014; McDonald *et al*., 2011, McDonald *et al*., 1991).

2.7 Physical and management factors affecting silage aerobic stability

Aerobic stability of silage is defined as the amount of time a silage remains stable (does not spoil) after it is exposed to air (oxygen). Specifically, it is the amount of time it would take for the silage temperature to exceed ambient temperature by 2° C, after the silage has been exposed to air (O'Kiely, 1993). Generally, the longer the silage remains stable after being exposed to air, the better. A realistic practical target for silage aerobic stability is 168 hours (7 days) including time in the feed trough (Wilkinson & Davies, 2013).

The aerobic stability is an important factor as it ensures that well-preserved nutrients are provided to the animal with minimal amounts of yeasts and moulds. A number of factors affect aerobic stability of silage. Ingress of air is considered as the major factor affecting the aerobic stability of silage during feed-out, as it allows growth and activity of yeasts and moulds which lead to silage spoilage (Wilkinson & Davies, 2013; Woolford, 1990). Silage density and porosity are key physical factors that affect the rate of ingress of oxygen into the silage mass during the feed-out period (Holmes & Bolsen, 2009).

As the rate of ingress of air determines aerobic stability, rate of removal of silage from the exposed feed-out face should exceed the rate of ingress of air. During opening of the silos and feed-out process, heating is mostly associated to yeasts and moulds activities. Basically, silage spoilage is related to heating which is a result of prolonged exposure of the silage to air. Therefore, measures aiming at minimizing air exposure to silage are of paramount importance for the production of stable silage.

2.8 Effects of additives on silage aerobic stability

Additives are preservatives added to the harvested crop essentially to ensure an efficient fermentation process and improve aerobic stability of forages. Also, additives are added, in order to enhance the aerobic stability (shelf life) of such crops by reducing oxygen and increasing acidity (Knicky, 2005).

Silage additives are categorized into five key categories namely fermentation stimulants, fermentation inhibitors, aerobic deterioration inhibitors, absorbents added to especially low DM forages and nutrients that are added to crops at time of ensiling (McDonald *et al*., 2011, 1991). Fermentation stimulants which include fermentable carbohydrate sugar sources like sucrose, molasses, glucose; enzymes; and inoculants such as LAB promote the development of desirable bacteria that stimulate fermentation (Canibe *et al.,* 2014; McDonald *et al*., 2011; McDonald *et al*., 1991). Acids and organic acid salts that include formic, acetic, acrylic, and lactic acids partially or completely prevent microbial growth in silages (McDonald *et al*., 2011).

Stimulants help in the growth of the LAB and the production of lactic acid, which leads to increased rate of decline of pH. By lowering pH, stimulants improve aerobic stability of silage. Stimulants include fermentable carbohydrate sugar sources like sucrose, molasses, glucose; bacterial inoculants and enzymes.

Inhibitors help to slow down unwanted silage degradation, by reducing yeast and mould growth or reducing the breakdown of plant proteins. Inhibitors are such as propionic acid, non-protein nitrogen and acids (formic acid, formaldehyde, sulphuric Acid).

2.9 Economic and environmental perspective

Greenhouse gas emissions produced during feed production and preservations contribute to climate changes (Kaparaju & Rintala, 2011). Activities that lead to greenhouse gas emissions include mechanical cultivation and use of synthetic fertilizers in an effort to improve soil fertility for crop growth. This leads to carbon dioxide emissions and nitrogen leakages that promote global warming and greenhouse effect (Hernandez-Rivera *et al*., 2019). Likewise, effluents of wastewater produced during ensiling of crops that have a high moisture content contribute to environmental pollution. This can be minimized by harvesting crops at optimal stage of maturity or wilting (Duniere *et al*., 2013). For instance, in corn silage, at optimal stage of maturity a DM of 30-40% can be expected. Grasses and legumes that have high moisture content require wilting in the fields. Encouraging a diversity of perennial grasses and legumes that exhibit good interaction, legumes ensures nitrogen fixation in the soil and this

property helps to reduce the risk of nitrogen leakage which are due to fertilization (Knicky, 2005).

An increased performance of newly received feedlot cattle was noted, and it was found to be due to pre-treated (extruded) silage that led to protein escaping the ruminal microbial digestion (Fluharty and Loerch, 1995). This altered the fermentation pattern of the feed that resulted to high profile amino acids for growth and productive needs of the animal which is a key for profitable livestock rearing. This is in agreement with Broderick *et al.* (1999) who noted an increased digestibility and milk yield from cows fed macerated (processed) forage when compared with those fed unprocessed forage. Likewise, Patel *et al.* (2017) recommended the use of high quality silage in the diet of dairy cows because, dairy farming is based on input-output relationship. Allowing about 70% of high-quality silage in the cow`s diet, profitability can be guaranteed.

2.10 Aim of the experiment

To investigate if the process of extrusion of silage at the time the silo is open affects the aerobic stability.

2.10.1 Main objective

To study how extrusion of silage from ley and from whole crop barley affects aerobic stability at the following conditions:

- extrusion followed by letting silage cool down immediately after;
- extrusion followed by keeping the silage warm;
- extrusion followed by keeping the silage warm combined with treatment of the silage with an additive.

3 Materials and methods

3.1 Materials

In this study, two types of silage were used: mixture of timothy grass and red clover and whole crop silage barley (WCB).

3.2 Methodology

3.2.1 Sample collection

Two separate trials were conducted three weeks apart; and in each trial, sampling and processing was done four days in a row. Sampling was done at Lövsta, Swedish Livestock Research Centre. Samples were taken from WCB and Timothy grass/red clover bunker silos that were open for feed out.

In each day of Trial 1, silage sub-samples were carefully collected from five to six different spots at the centre of the bunker silos. The sub-samples were then thoroughly mixed to ensure homogeneity. Thereafter, approximately 25 kg of the composite sample were placed in a plastic bag ready for the extrusion process.

In Trial 2, the same procedure was used. However, in contrast to Trial 1, samples were collected from the sides/peripheral parts of the bunker silos to get more of the unstable/contaminated silage. Also, as opposed to Trial 1, samples were collected from a different Timothy grass/red clover silage bunker silo. The reason for this was that the previously sampled silo had been used up and a new had been opened. The same WCB bunker silo was sampled for both trials.

In both trials during each of the sampling day, samples of approximately 2 kg from each silage type (timothy grass-clover mixture and WCB) were collected from the

bunker silos for microbial (yeasts and moulds) analysis. The samples were put in labelled plastic bags and were stored in a refrigerator until analysis.

3.2.2 Sample processing, transport, and storage

Using a digital thermometer, temperature was recorded before extrusion from both silage types. Temperature was measured from different spots of the silage and the average was taken. Before extrusion, a control (untreated) sample was taken from each silage type, and was placed in a plastic bag. Then, the silage was extruded using the Bio-extruder (MSZ-B15e LEHMANN Maschinenbau GmbH) **(**Figure 1&2**)**. Immediately after extrusion, the temperature of the extruded silage was measured. A sample of approximately 2 kg of warm extruded silage was placed in a plastic bag and was kept in an insulated box to maintain its temperature during transportation to the laboratory. The remaining extruded silage was carefully spread out on the plastic sheet and was left to cool down for 5 minutes. Thereafter, a sample of approximately 2 kg from the cooled extruded silage was collected and was put in a in plastic bag. The samples were subsequently transported to the forage lab at Sveriges lantbruksuniversitet (SLU) for further analysis.

3.2.3 Treatment application

The experiment was conducted according to a factorial (2 x 3; Trial 1) design; involving types of silage (timothy grass /red clover & WCB) and treatment (warm extruded silage, cold extruded silage and control silage) as factors. In Trial 1, samples were collected over four consecutive days. In Trial 2, the same types of silages and treatments were used but with an addition of a fourth treatment (warm extruded silage with an additive) hence a 2 x 4 factorial design. Silage additive (ProMyr™ TMR Performance) was composed of propionic acid (50-60%), hexanoic acid (15-25%), Sodium formate (1-5%), 1,2,3 propanetriol, glycerol (1-5%) and Glycerol propionates (15-25%). Four millilitres (4 mls) of additive was added into 1 kg of silage and was thoroughly mixed to ensure homogeneity. The total number of samples to be tested for aerobic stability (AST) were 24 (6 samples per day for four days) and 32 (8 samples per day for four days) in Trial 1 and 2 respectively.

Figure 1. Bio-extruder MSZ-B15e LEH-MANN Maschinenbau GmbH with extruded silage in the black basin

Figure 2. The bio-extruder's screws

Figure 3. Control and processed silage, from left to right control whole crop barley, processed whole crop barley, control timothy grass and red clover mixture, processed timothy grass and red clover mixture

3.2.4 Aerobic stability test

At the laboratory, for both trials and at each sampling day, two predetermined/defined amounts of samples (DLG filling weight list, 2013), for each of the experimental conditions were measured, to be placed in the aerobic stability unit for 24-hours and 7-days incubation. The aerobic stability was defined as the time (days) until temperature in aerated silage had increased +5° C.

For Trial 1, 300g and 150g for each of 3 WCB samples; and 334g and 150g for each of the 3 Timothy grass/red clover sample were measured, for 7-days and 24-hours incubation, respectively. For Trial 2, 300g and 150g for each of the 4 WCB samples; and 550g and 220g for each of the 4 Timothy grass/red clover mixture samples were measured, for 7-days and 24-hours incubation, respectively.

Samples were loosely and aseptically placed in well sterilized polyvinyl chloride pipes (PVC pipes) with the volume of approximately 1300 cm^3. The pipe had a piece of geotextile (a material in which air can easily pass) attached at the bottom of the pipe by a rubber band to allow the air to flow through the sample. Samples were kept at two different incubation durations in the aerobic stability unit (Figure 4) i.e., 24 hours and 7 days. Immediately after filling, the PVC pipes were placed in a stylofoam block with temperature sensors connected to a data logger for temperature registration at 2-hour intervals.

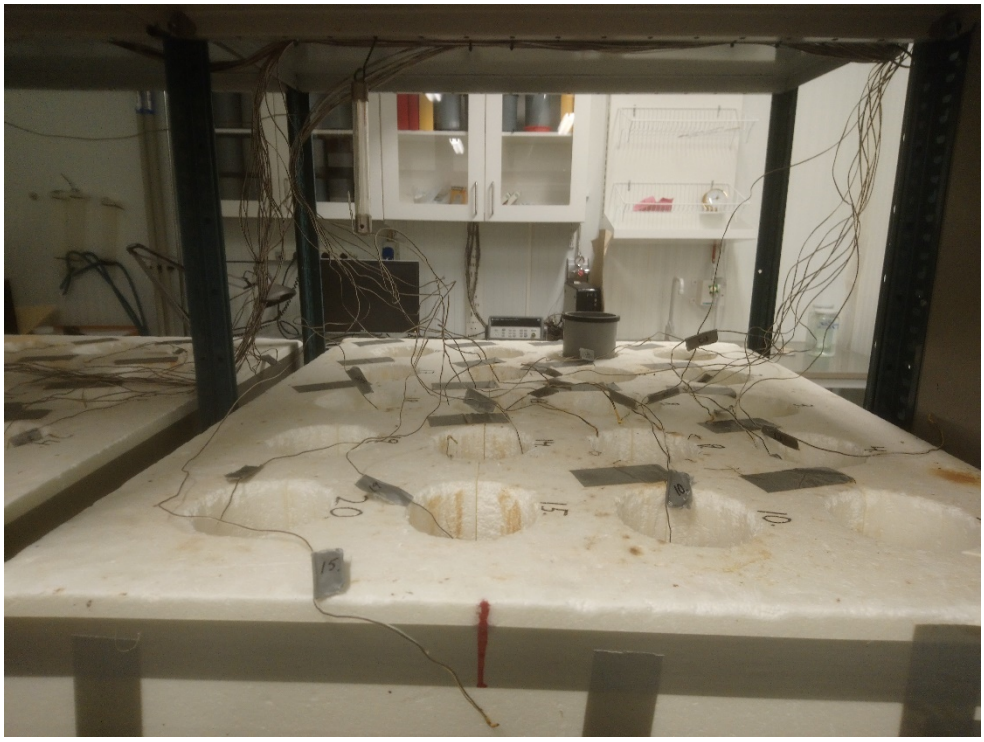

Figure 4. Aerobic stability unit connected with a data logger for temperature registration.

3.2.5 Dry matter (DM) determination

DM was determined on both silage types at each sampling day. Samples were weighed and placed in an oven for at least 18 hours at 60° C, after which they were weighed for DM calculation.

3.2.6 pH determination

In both trials, for determination of pH, samples of 300 g from each of the silage types were placed in plastic bags, before and after the aerobic stability test. An equal amount of distilled water was added and the mixture was kept for 30 minutes in the freezer. Then, an appreciable amount of liquid was squeezed into a container through a pierced angle of the bag for subsequent pH measurement. Using the Metrohm 654 pH-meter (Figure 5) pH was measured on all the samples.

Figure 5. Metrohm 654 pH meter for pH measurement

3.2.7 Cultivation, identification and enumeration of yeast and mould species

For microbial analysis, samples collected at day 1 and 2 were mixed; and those collected at day 3 and 4 were also mixed. Then, thirty grams (30 g) of each of the mixed samples were put in stomacher bags followed by addition of 270 g of Ringer's solution (Merck, Darmstald, Germany). The mixtures were thoroughly mixed by the stomacher machine for 2 minutes. Thereafter, 1 ml of each solution was diluted with 9 ml of Ringer's solution, followed by stepwise tenfold dilutions resulting in dilution series of 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5} of concentrations. Then 0.1 ml of fluid sample was placed on malt extract agar (MEA) plates (Merck, Darmstald, Germany) and was evenly spread out with an inoculation spreader. The inoculated plates were incubated for 3 and 5 days for yeasts and moulds, respectively. After incubation, identification of

yeasts and mould species was done, and their colony forming units (CFU) were counted and recorded. During counting, when numbers of CFU were massive, the petri dish was divided into four quarters, and then counting was done on one quarter, and the obtained number was multiplied by four (4) to obtain the estimated total number.

3.2.8 Statistical Analysis.

Results from the experiments were analysed by the software Minitab 18 (Minitab, Inc., state college, PA, USA). Using analysis of variance (ANOVA, general linear model), the effect of silage type (grass-clover and WCB), different treatments (cold, warm, warm with additive and control) and the interaction between them were computed. The effects were measured on dry matter (DM) content, temperature, aerobic stability (hours), pH both before and after aerobic stability test and yeast and mould CFU. Mean values and standard deviations were computed. Differences in means were considered significant at $P < 0.05$.

For the ANOVA model used was:-

$$y_{ij} = \mu + s_i + t_j + st_{ij} + error_{ij} \qquad \text{Equation 1}$$

where μ is the overall mean, s_i is the effect of the i^{th} silage type, , t_j the effect of the j^{th} treatment) st_{ij} is the interaction between i^{th} silage type and j^{th} treatment and $error_{ij}$ is the error term.

4 Results

4.1 Analysis of variance (ANOVA) results in Trial 1

The effects of the silage type and treatments are shown in Table 1. The results show that the effect of silage type on DM content, temperature and pH before aerobic stability test were significant (P<0.001. The treatment effects were significant for temperature (P<0.001) only. The interaction effect between treatments and silage type was observed to be significant only for temperature (P = 0.01).

Table 1. Effects of treatment on silage characteristics and aerobic stability in Trial 1.

Silage	Treatment	DM	Temp	A.S.	pH		Yeasts	Moulds
		%	˚C	h	before	after	log cfu/g FM	
Mixture	Control	41	2	164	4.3	4.2	1.7	<1.7
	Extruded cold	40	nm	164	4.2	4.2	1.8	<1.7
	Extruded warm	na	26	164	na	4.2	2.2	<1.7
WCB	Control	47	8	164	4.1	4.1	1.8	<1.7
	Extruded cold	49	nm	164	4.1	4.1	2.0	<1.7
	Extruded warm	na	28	164	na	4.1	2.7	<1.7
SED		0.6	1.0	1.9	0.04	0.08	0.86	nc
p value - silage		<0,001	<0.001	1	<0.001	0.18	0.64	nc
p value - treatment		0.19	<0.001	1	0.44	0.88	0.54	nc
P value - interaction		0.15	0.01	0.39	0.34	0.57	0.95	nc

Abbreviations: DM - dry matter; AS - aerobic stability, hours until a rise in temperature of 5° C; cfu - colony forming units; FM - fresh matter; WCB - whole crop barley silage; na - not

analysed; nm - not measured; nc - not calculated; SED - standard error of the difference

4.2 Analysis of variance (ANOVA) results in Trial 2

In this trial, we considered silage type, treatments and their interaction as variances. The effects of the silage type and treatments are shown in Table 2. The results showed that the effect of silage type was significant on DM content (P<0.001), temperature (P<0.001), pH before aerobic stability test (P = 0.007) and after aerobic stability test (P = 0.017), as well as in number of yeast CFU (P = 0.003) and mould CFU (P = 0.009) (Table 2). Significant treatment effects were observed only on temperature (P<0.001) and yeast CFU (P = 0.01). The interaction effect between treatments and silage type was significant only on temperature at P = 0.015.

Table 2: Trial 2 summary of Dry matter (DM) content, Temperature, Aerobic Stability A.S., pH both before and after A.S test, Yeast and Mould colony forming units.

Silage	Treatment	DM	Temp	A.S.	pH		Yeasts	Moulds
		%	˚C	h	before	after	log cfu/g FM	
Mix-ture	Control	23	9	44	4.3	7.3	5.5	4.3
	Extruded cold	24	20	50	4.2	8.0	4.9	5.0
	Extruded warm	na	nm	48	4.2	8.0	4.9	4.7
	Extruded warm + additive	na	nm	75	4.1	6.9	3.3	4.3
WCB	Control	46	11	64	4.5	6.7	7.3	1.7
	Extruded cold	47	25	70	4.5	6.9	5.8	2.5
	Extruded warm	na	nm	52	4.5	6.9	5.9	2.4
	Extruded warm + additive	na	nm	94	4.4	5.3	5.1	2.9
SED		1.0	0.7	25.8	0.15	0.85	0.63	1.23
p value - silage		<0.001	<0.001	0.28	0.007	0.017	0.003	0.009
p value - treatment		0.087	<0.001	0.26	0.67	0.11	0.01	0.82
p value - interaction		0.58	0.015	0.96	0.93	0.88	0.67	0.9

Abbreviations: DM - dry matter; AS - aerobic stability, hours until a rise in temperature of 5° C; cfu - colony forming units; FM - fresh matter; WCB - whole crop barley silage; na - not analysed; nm - not measured; nc - not calculated; SED - standard error of the difference.

5 Discussion

5.1 Aerobic stability test

The aerobic stability of the silage is an important factor for making sure that the forage biomass provides the required nutrients to the animal with negligible amount of moulds and toxins that can jeopardize health of both animals and humans (Wilkinson & Davies, 2013). Temperature, DM content, pH, and yeast as well as mould numbers are important parameters for the heating of silage which are determined by ingress of air in silos and the concentration of fermentation products such as acetic acid and butyric acids (Ohyama *et al*., 1980).

In the present study, silage taken from bunker silos was extensively processed using a bio-extruder to investigate the effect of extrusion on aerobic stability. The results of Trial 1 have shown that the two silage types were relatively stable with no significant difference between them. For all incubated samples 164 hours elapsed before the temperature rose by +5° C. This indicates a relatively stable silage. However, it should be noted that 164 h was the limit of the test and hence the threshold temperature was not reached

As opposed to Trial 1, the results of Trial 2 showed that the aerobic stability of the two silage types were relatively low, but as in Trial 1 there was no difference between them. This is probably as a result of the location in the bunker silos from which the silage was collected (periphery/sides) and the manner the samples were taken (from the surface of the silos) during Trial 2. The silage was expected to be relatively unstable due to a higher exposure to air. The presence of oxygen initiates the activity of undesirable microbes like yeasts and moulds. These microbes grow on residue substrates and fermentation products leading to production of heat and an increase in pH. These changes make the silages aerobically unstable resulting in low silage quality.

In Trial 1, samples were taken from the centre of the bunker silos to avoid collecting exposed silage. A study by Chen & Weinberg (2009) showed that a silage sample taken from the centre of the bunker silos and from the areas adjacent to the wall of the silo were judged to be of good quality.

The results of this study have shown that there was no significant difference in aerobic stability between the silage types. However, from the results of Trial 2, although not significantly, aerobic stability was relatively higher in WCB as compared to the grass/clover mixture. This might have been caused by low buffering capacity of the WCB which facilitates pH drop. This enhances ensiling process and stabilizes the resulting silage (Hargreaves *et al*., 2009).

5.2 Dry matter content

In the present study, the effect of silage type on DM content was significant. The DM content of WCB was significantly higher than that of the mixture. This could indicate that WCB can be expected to be more stable than the mixture, although not seen in this project. DM content, which is an indication of water availability in the silage has been reported to be an important factor determining microbial activity in silages (McDonald *et al*., 1991). For instance, Clostridia are believed to be more sensitive to reduced water availability (Knicky, 2005).

5.3 Microbial activity

In Trial 1, yeast counts were small and no moulds were observed, indicating good quality silages and potentially stable. However, in Trial 2, yeast and mould growth were considerable, indicating an unstable silage. This was probably due to that the silage was more exposed to oxygen and hence aerobic deterioration. In Trial 2, sample collection was from the peripheral parts of the bunker silos that are considered somehow contaminated owing to oxygen penetration. This is in agreement with Knicky (2005) who noted that moulds often grow in the peripheral parts of the silos because of higher possibility of air ingress in the silos

At silo opening and feed-out, the environment condition of the silo changes from anaerobic to aerobic creating favourable condition for aerobic micro-organisms. This favours yeasts, that were previously made dormant, to become active and multiply. This process leads to silage deterioration (Woolford, 1990).

WCB had significantly higher numbers of yeast CFUs as compared to the mixture. This can be related to greater porosity of the WCB which enables more oxygen penetration into the silage leading to higher microbial activity and an increase in pH.

However, contrasting results were observed with mould counts which were significantly higher in the mixture as compared to the WCB. This could be attributed to the higher moisture content of the grass/clover mixture, as indicated by the lower dry matter content which was about half of that of the WCB. Mould growth is enhanced by relatively higher moisture contents (McDonald *et al*., 1991).

Previous studies have shown that yeasts are the primary and most important undesirable micro-organisms that initiate aerobic deterioration of silages (Knicky, 2005; McDonald *et al*., 1991). Wilkinson & Davies (2013) reported that yeasts and moulds are key contributors of aerobic stability loss of silages. The authors suggested increased risk of massive population of yeasts and moulds to be associated with crop characteristics at harvest. Also, Knicky (2005) and Pahlow *et al*. (2003) noted that yeasts and moulds were responsible for the losses that are due to aerobic deterioration that mainly occur during feeding when the silage is exposed to oxygen. Therefore, it is important to reduce the area that is exposed to air during the feed-out period to minimize the deterioration of silage which is due to yeasts and moulds (McDonald *et al*., 2011).

As pointed out earlier, yeast and moulds often start the deterioration process by raising pH. Consequently, the nutritive value and hygienic quality of the silage is reduced due to depressed aerobic stability owing to proliferation of large population of micro-organisms such as obligate and facultative aerobic ones (such as Mycobacteria, Escherichia and Clostridia) (Kasmaei, 2016). Their activities are responsible for aerobic deterioration and lowering aerobic stability of silage by raising its pH.

5.4 Silage pH

In the present study, silage type was shown to have a significant effect on pH before the aerobic stability test in Trial 1. Likewise, silage type was shown to have a significant effect on pH both before and after aerobic stability test in Trial 2. In Trial 1, pH before and after aerobic stability test ranged between 4.1-4.3 indicating low pH of the silage. Increase in pH is associated with an increased activity of undesirable micro-organisms resulting in degradation of sugars and lactate (Wang *et al*., 2019).

Successful preservation of forages through which losses of nutrients are minimal and expected hygienic quality remains high, depends on the level of pH drop during ensiling process. This is determined by the amount of WSC of ensiled crop (Kasmaei, 2016). This helps to reduce pH of the forage biomass which enables the pH tolerant LAB to dominate the process and hence suppress colonization and proliferation of epiphytic microbes responsible for silage deterioration. This is facilitated by the acidic environment of the silage that creates unfavourable condition for most of undesirable silage micro-organisms (Kasmaei, 2016). This is probably the explanation to that low yeast counts were observed in Trial 1 and moulds were under detection limit. On the

other hand, in Trial 2, pH values before aerobic stability test ranged between 4.1-4.5 and were higher for WCB than in Trial 1. These findings showed that the silage was unstable and this can be explained by Kasmaei (2016) who noted that spoilage of ensiled materials is often likely to occur at feed-out and this spoilage is due to presence of lactate-assimilating yeasts.

5.5 Effects of additive

In the present study, in Trial 2, silage additive was added in warm extruded silage to investigate its effect on the activity of microbes under the assumption that it would improve storage stability. Silage additive had a significant effect on pH before and after aerobic stability test. For yeast counts, the silage with additives had lower counts compared to the control for both silage types in Trial 2. It follows that, the additive had an impact on the reduction of pH that determines yeast counts in silages. These findings for yeast counts are in agreement with Borreani *et al*. (2018) who reported that it is possible to delay aerobic deterioration in aerobic condition by inhibiting yeast growth and multiplications by applying appropriate silage additive and levels.

5.6 The effect of temperature on aerobic stability

Rapid heating of the silage and hence aerobic stability depends on depth of air penetration and rate of unloading from the silo during feed-out (Knicky, 2005). It follows that, the effect of extrusion on aerobic stability with warm extruded silage could reduce the time (hours) for the silage to heat up. This is under the assumption that temperature has an effect on microbial activity and hence aerobic stability. In addition, temperature rise in the extruder could have an influence on the availability of the substrate to microbes. However, despite the observed rise in temperature, there was no effect of extrusion on aerobic stability in this study.

Microbes that dominate fermentation during ensiling process and the speed of fermentation depend on the temperature of the crop (Borreani *et al*., 2018). In tropical climate, high temperature poses challenges in the production of silages because it encourages the growth of epiphytic micro-organisms such as enterobacteria and clostridia (Kim & Adesogan, 2006). The temperature history of the silage during storage affect its aerobic stability and is an important benchmark in determining the differences between silages and different ways by which silages are preserved. Some research results showed that an optimum temperature of about 30° C supports the increase in yeast counts that determine deterioration of silage biomass (Ashbell *et al*., 2002).

Furthermore, McDonald *et al*. (1991) reported temperature ranges for yeast growth to be varying from 5° C to 50° C and an optimal growth of many species occur at 30° C. These findings are related with the current study results whereby in both trials, treatment had a significant effect on temperature and the interaction between silage type and treatment showed a significant effect on temperature in both trials (Table 1 and 2). Hence, the extrusion led to a significant increase in temperature in both silage types and trials. However, despite the increase in temperature, extrusion had no effect on aerobic stability of the silages.

6 Conclusion

The findings of this study have shown that the process of extrusion with different silage treatments did not have a significant effect on the aerobic stability of the silage types. The results showed that extrusion led to a significant increase in temperature in both silage types and trials. In both trials, temperature after extrusion was higher in WCB as compared to grass/clover silage. However, extrusion led to a greater difference in temperature before and after extrusion for grass/clover silage in Trial 1, but a greater difference for WCB in Trial 2.

As for pH, WCB had a lower pH before aerobic stability test compared with grass/clover silage in Trial 1. In Trial 2, pH before the aerobic stability test was higher for WCB, while as pH after the aerobic stability test was higher for grass/clover silage. The number of yeast CFU was greater for WCB, but for moulds the number of CFU was greater for grass/clover silage. Therefore, further research is needed to investigate if extrusion can have an influence on aerobic stability of silage.

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