

**FERMENTATION, STABILITY AND  
DEGRADABILITY OF WHOLE-CROP OAT SILAGE  
ENSILED WITH A COMMERCIAL INOCULANT**

by

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degree of Master of Science in Agriculture*

at

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Date: March 2010

## Declaration

By submitting this thesis electronically, I declare that the entirety of the work contained therein is my own, original work and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

Date : 19 February 2010



## Abstract

Title : Fermentation, stability and degradability of whole-crop oat silage ensiled with a commercial inoculant

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South Africa is well-known for periodic dry periods and uncertain rainfall. Ensiling of crops is a method of preserving forage and ensures feed availability during periods when the supply of good quality forage is low. Cereal-based silages, especially in the Western Cape, South Africa, represent a significant proportion of feed consumed by ruminant animals, particularly high-productyion dairy cattle. However, farmers are still concerned about the technical challenges of ensiling cereal crops. Previous research done on lactic acid bacteria (LAB) inoculants used on cereal based silage has indicated a potential for improving silage fermentation, stability and degradability, thus enhancing feed conversion and production by ruminants.

Two experiments were conducted to determine the effects of inoculating whole-crop oat silage with Lalsil® Cereal Lactobacilli (*Lactobacillus buchneri* (NCIMB 40788) and *Pediococcus acidilactici* (CNCM MA 18/5M)) LAB on

- (1) silage fermentation,
- (2) aerobic stability and
- (3) nutritional value of silage ensiled under
  - a. micro-silos conditions and
  - b. in a bunker under outdoor conditions of a Mediterranean summer.

Oats (*Avena sativa*, cv SSH 405) were planted on 60 ha under dryland conditions at Elsenburg in the Western Cape province, South Africa. Whole-crop oats were harvested at the soft dough stage and length of the chopped material was 9 mm (Day of ensiling – Day 0).

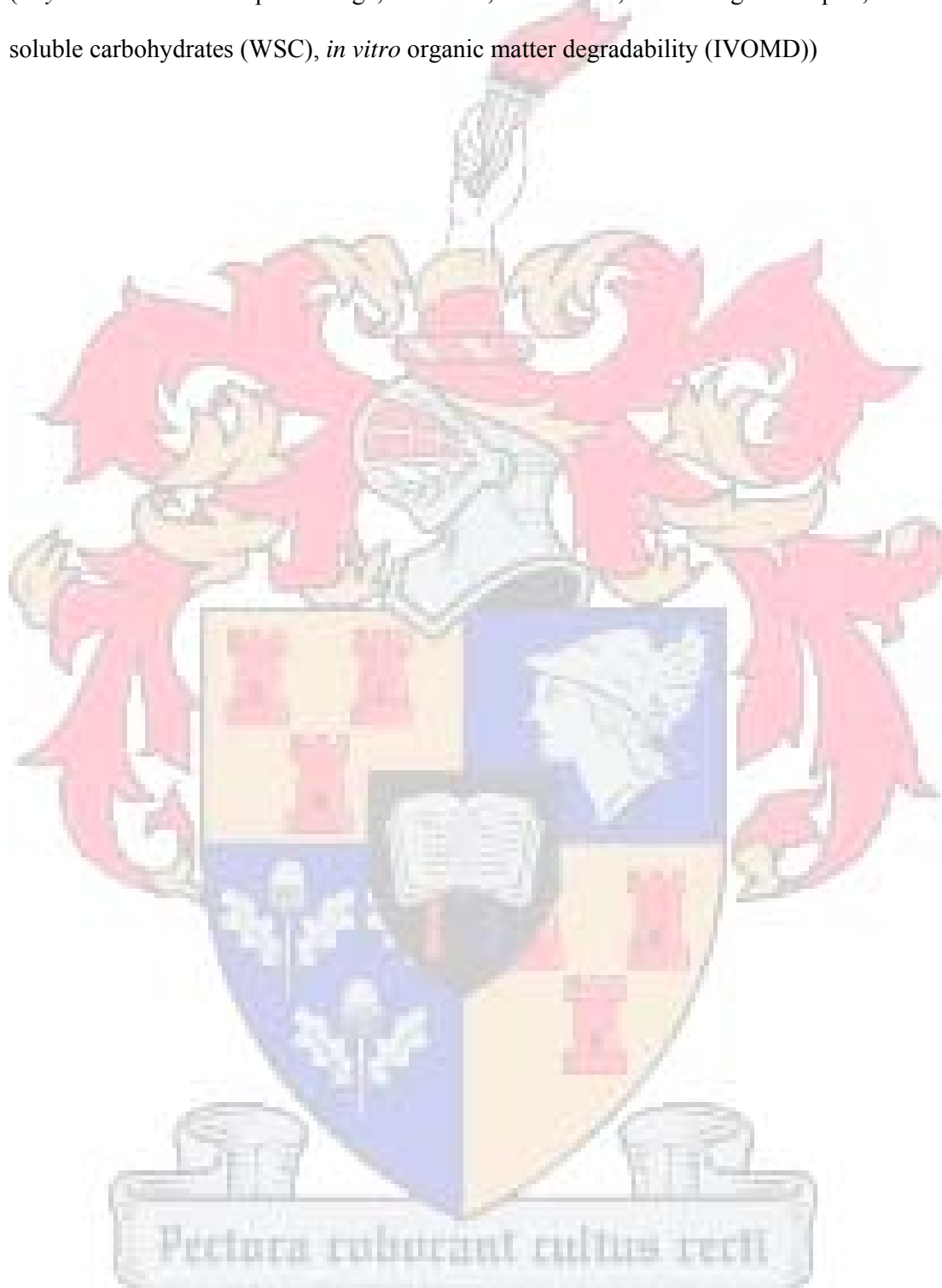
Chopped whole-crop oats were sampled, mixed thoroughly and divided into two portions. The Inoculant (Lalsil® Cereal) was applied to one portion to provide  $5.79 \times 10^9$  colony forming units (CFU) of LAB per gram of fresh material. In the first experiment twenty-four glass silos (1.5 L glass jars) (WECK, GmbH u.Co., Wehr-Ofligen, W. Germany) were filled for each of the control and inoculant treatments. The glass silos were stored in a dark room in the laboratory at ambient temperature. Three glass silos were opened for each treatment on days 1, 2, 4, 8, 15, 30, 60 and 102 post-ensiling to determine fermentation dynamics.

A parallel study was done with the same chopped whole-crop oats using the buried bag technique in a bunker silo. Whole-crop oats were ensiled in six net bags per treatment buried in a bunker filled with the same untreated whole crop oats. Bags, attached with nylon lines (3 m lengths) for easy retrieval were buried at 1m and 2 m depths in the same bunker. The net bags in the bunker were retrieved after 186 days of ensiling.

Dry matter (DM), organic matter (OM), neutral detergent fibre (NDF), crude protein (CP), lactic acid levels, pH, water soluble carbohydrates (WSC) and *in vitro* organic matter degradability (IVOMD) for both studies were determined. Silage of both experiments was exposed to aerobic conditions for ten days to determine aerobic stability. It is concluded that the inoculant Lalsil® Cereal had the effect of reducing the rate of consumption of WSC during the anaerobic phase and aerobic exposure for both

experiments. Silage spoilage due to yeasts and moulds was however more evident with the inoculated silage due the presence of sugars in the micro-silos experiment.

(*Key words:* Whole-crop oat silage, inoculant, micro-silos, buried bag techniques, water soluble carbohydrates (WSC), *in vitro* organic matter degradability (IVOMD))



## Opsomming

Titel : Die fermentasie en stabiliteit van hawer kuilvoer ingekuul met 'n kommersiële inokulant

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Suid-Afrika is bekend vir droë periodes en wisselvallige reënval. Die inkuiling van gewasse is 'n goeie manier om ruvoer te voorsien in tye van droogtes of tekorte. Kleingraan kuilvoer is veral bekend in die Wes-Kaap, Suid-Afrika en maak 'n groot deel uit van die melkkoei se rantsoen. Landbouers is nog steeds bekommerd oor die tegniese aspekte wanneer dit kom by die inkuil van gewasse. Vorige navorsing het getoon dat die gebruik van 'n melksuurbakteriese inokulant saam met die inkuiling van gewasse moontlik die potensiaal het om fermentasie, stabiliteit en degradering te verbeter en sodoende voeromset te verbeter.

Twee eksperimente is uitgevoer om die effek van die inkuiling van hawerkuilvoer met Lalsil® Cereal Lactobacilli (*Lactobacillus buchneri* (NCIMB 40788) en *Pediococcus acidilactici* (CNCM MA 18/5M)) LAB te bepaal op

- (1) kuilvoer fermentasie,
- (2) aërobiese stabiliteit en
- (3) nutriëntwaarde van die kuilvoer ingekuul in
  - a. mikrosilo's en
  - b. in 'n bunker in die buitelig.

Hawer (*Avena sativa*, cv SSH 405) is op 60 ha droë land geplant op Elsenburg in die Wes-Kaap, Suid-Afrika. Die hawer is ingekuul tydens die sagte deeg stadium en die gekapte materiaal was ongeveer 9 mm lank.

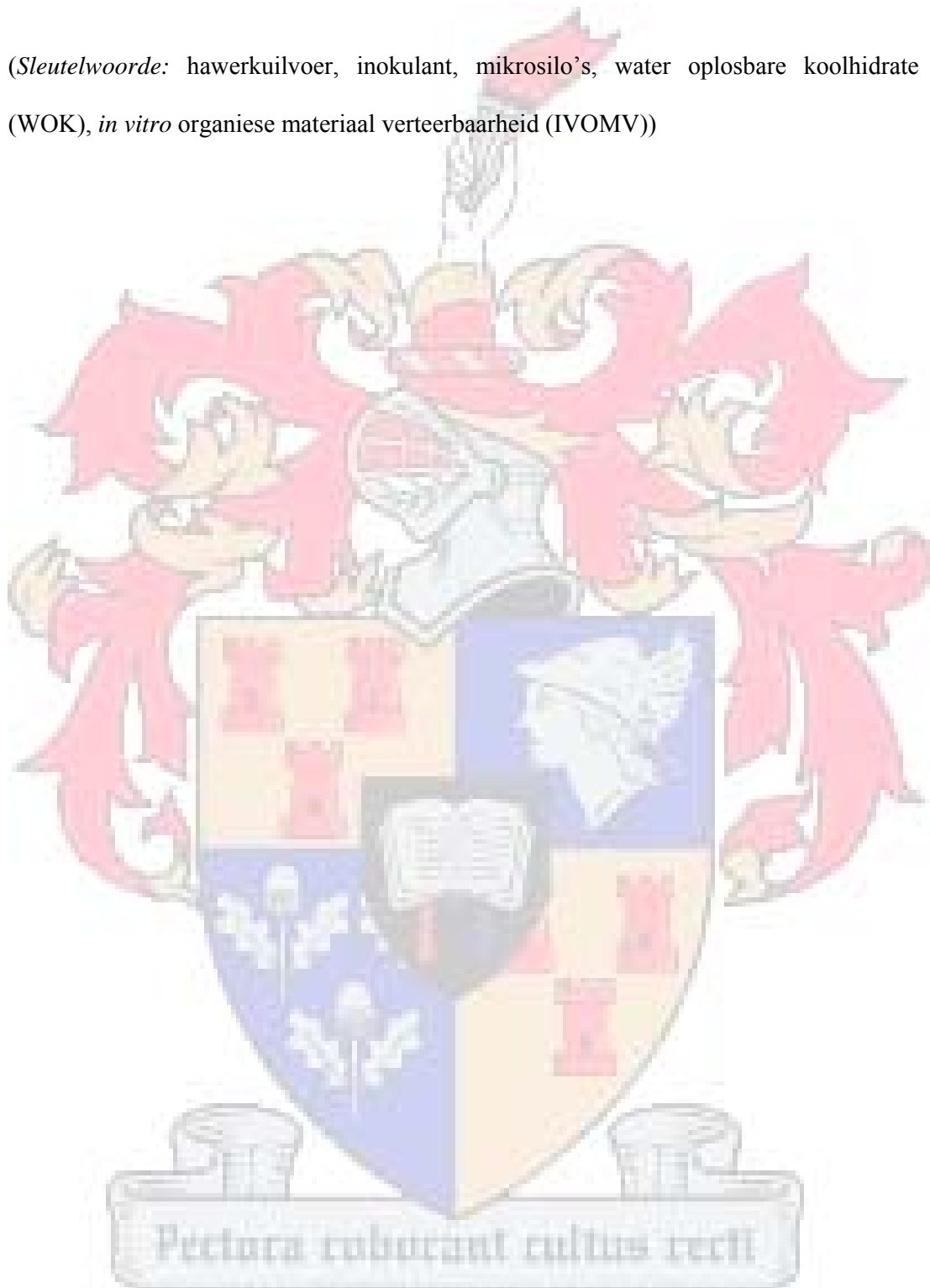
Gekapte materiaal was deeglik gemeng en in twee gedeel. Die inokulant (Lalsil® Cereal) is op die een gedeelte gesproei om  $5.79 \times 10^9$  kolonie-vormende eenhede (KVE) melksuurbakterieë per gram vars materiaal te voorsien. Tydens die eerste eksperiment is 24 mikrosilo's (1.5 L glas silo) (WECK, GmbH u.Co., Wehr-Ofligen, W. Duitsland) vir elke behandeling vol kuilvoer gemaak. Hierdie mikrosilo's is gestoor in 'n donker kamer teen kamertemperatuur. Drie mikrosilo's is per behandeling oopgemaak op dag 1, 4, 8, 15, 30, 60 en 102 na inkuiling om die fermentasie-dinamika te bepaal.

'n Parallele studie is gedoen met dieselfde materiaal ingekuul in netsakke binne die bunker. Die materiaal was ingekuul in ses netsakke vir elke behandeling. Nylon toue (3 m) is aan die sakke vasgemaak om die uithaal daarvan op latere stadium te vergemaklik. Hierdie sakke is ingekuul op verskillende dieptes, 1 m en 2 m in dieselfde bunker. Die sakke is na 186 dae weer uitgehaal.

Droë materiaal (DM), organiese materiaal (OM), neutraal bestande vesel (NBV), ruproteïen (RP), melksuurvlakke, pH, water oplosbare koolhidrate (WOK) en *in vitro* organiese materiaal verteerbaarheid (IVOMV) vir beide studies is bepaal. Kuilvoer van beide eksperimente is ook blootgestel aan aërobiese toestande vir 10 dae aan aërobiese toestande blootgestel om aërobiese stabiliteit te bepaal. Daar is bepaal dat die inokulant Lalsil® Cereal het die tempo van WOK verbruik verminder gedurende die anaërobiese fase sowel as die aërobiese fase vir beide eksperimente. Kuilvoer wat bederf het as

gevolg van giste en swamme was meer sigbaar by die inokulant behandelde kuilvoer as gevolg van die teenwoordigheid van suikers in die mikrosilo's.

(*Sleutelwoorde*: hawerkuilvoer, inokulant, mikrosilo's, water oplosbare koolhidrate (WOK), *in vitro* organiese materiaal verteerbaarheid (IVOMV))





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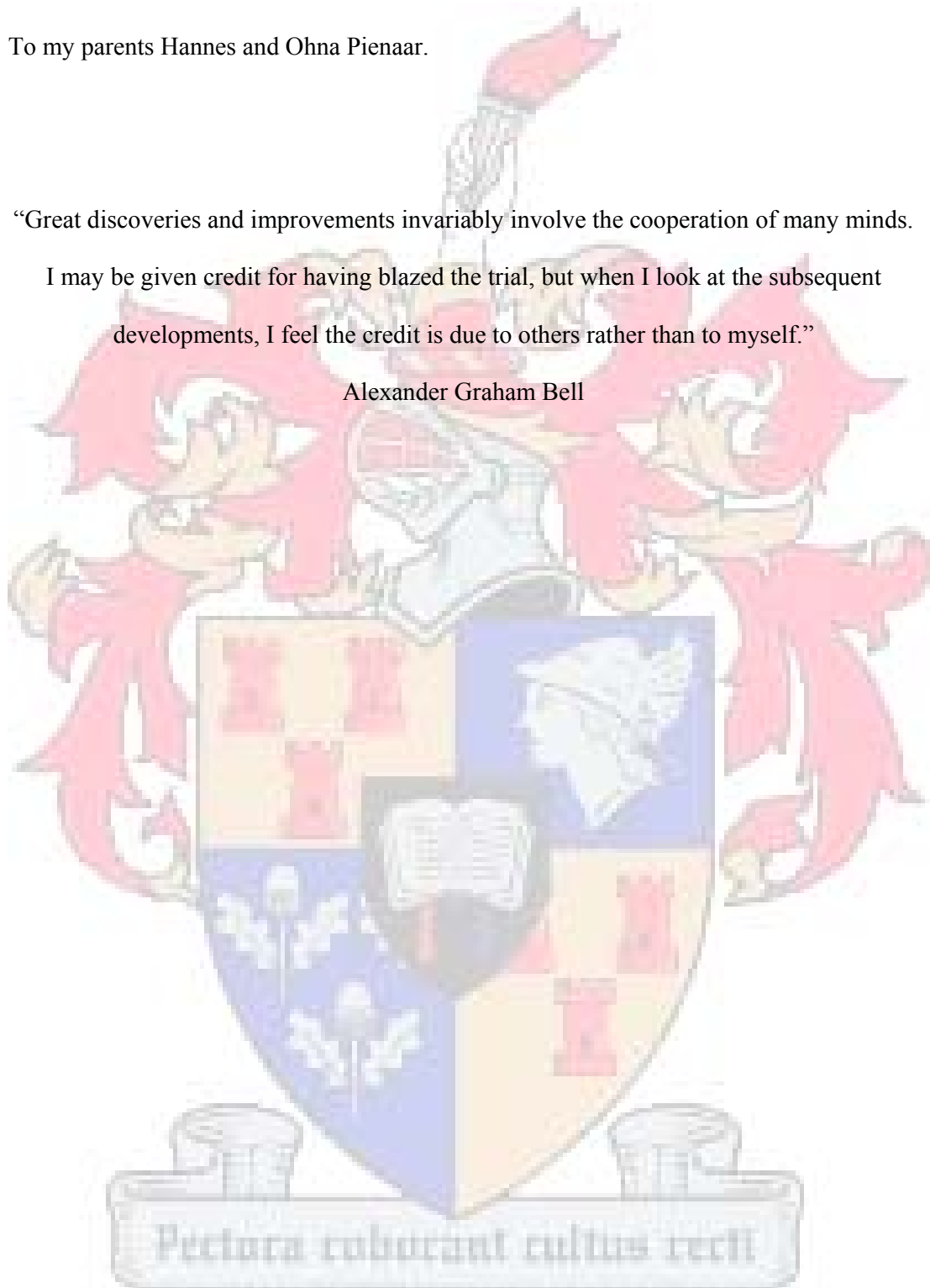
## Dedication

To my parents Hannes and Ohna Pienaar.

“Great discoveries and improvements invariably involve the cooperation of many minds.

I may be given credit for having blazed the trail, but when I look at the subsequent developments, I feel the credit is due to others rather than to myself.”

Alexander Graham Bell



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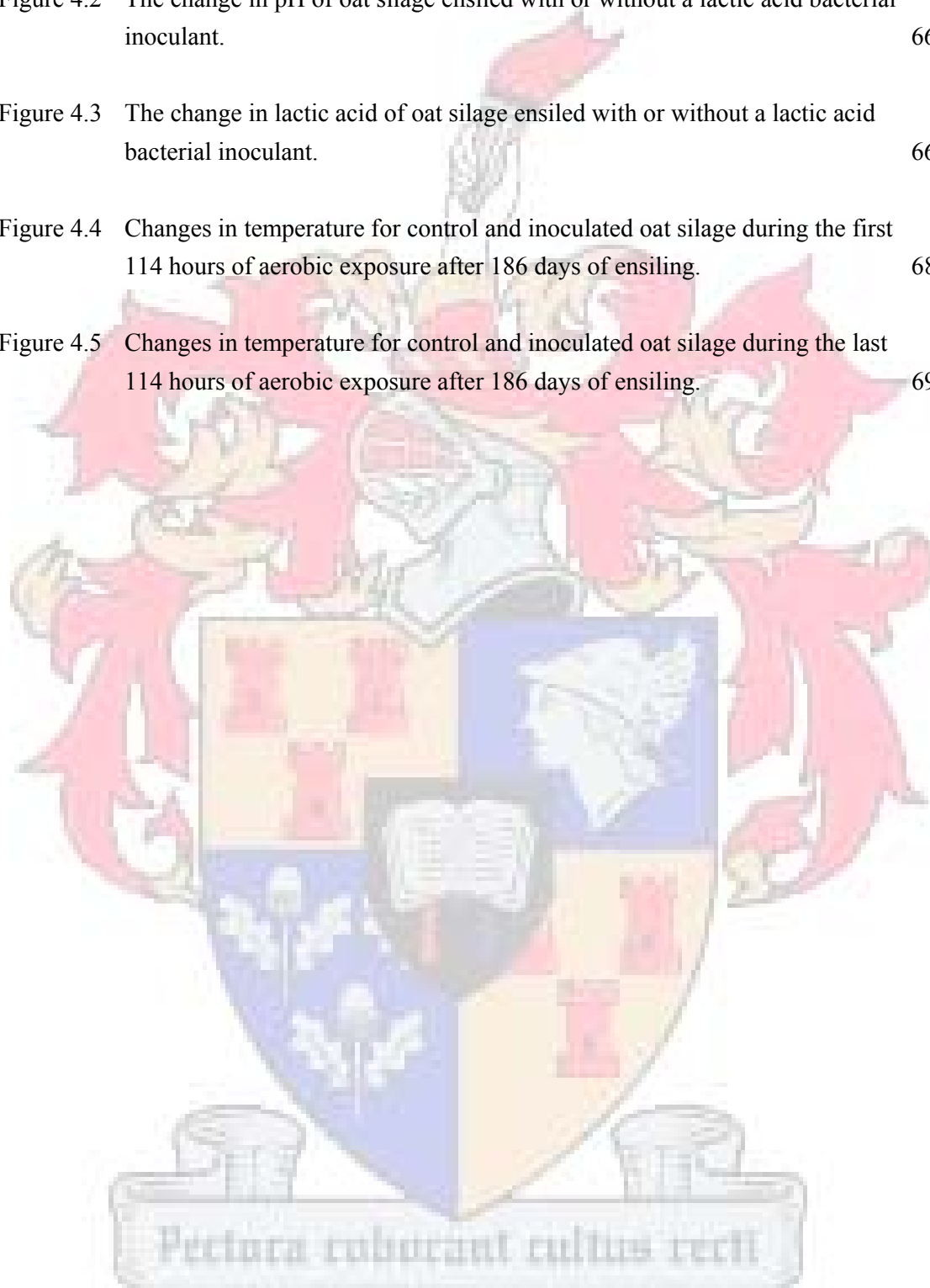
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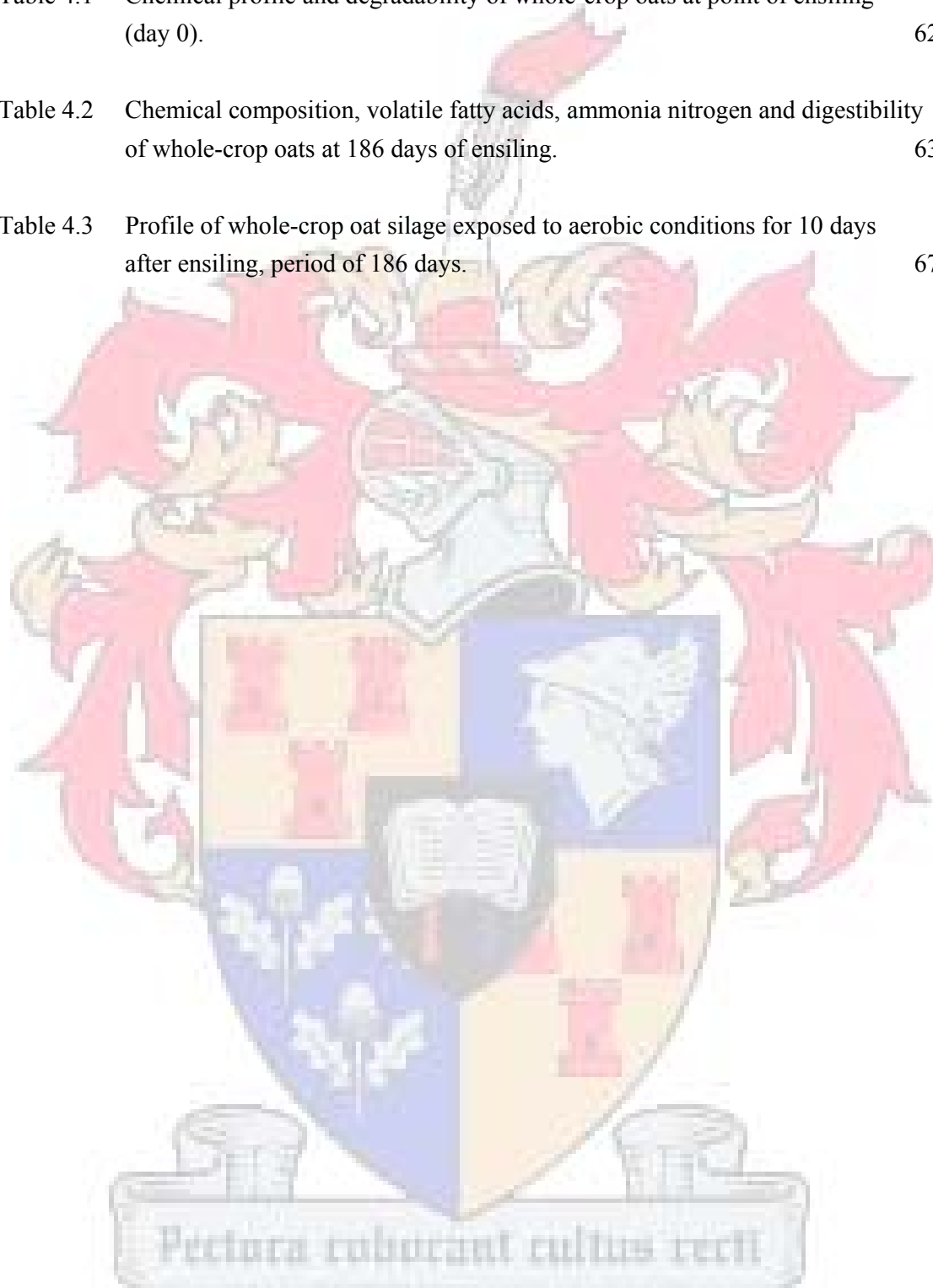


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# CHAPTER 1

## INTRODUCTION

Forage conservation by ensiling ensures feed availability during periods when supply of good quality forage is low (McDonald *et al.*, 1991). McDonald *et al.* (2002) defined the process of forage fermentation as ensiling. Cereal-based silages represent a significant proportion of feed consumed by ruminant animals, particularly high-production dairy cattle. Farmers are however, concerned about technical challenges of ensiling cereal crops (Wilkinson, 2005).

Silage is made from a large variety of cereals such as maize (*Zea mays*), oats (*Avena sativa*), and barley (*Hordeum vulgare*); legumes (lucerne; *Medicago sativa*) and tropical grasses such as Napier grass (*Penisetum purpureum*) and sugar cane (*Saccharum officinarum*). Maize is the most common cereal crop conserved as silage and large areas are cultivated under maize for this purpose in many parts of the world. Maize is relatively high in dry matter (DM) content, has a low buffering capacity and contains relatively high levels of water soluble carbohydrates (WSC) for satisfactory fermentation to lactic acid (McDonald *et al.*, 1991). However, the increase in utilization of maize as biofuel particularly in developed countries and also in South Africa necessitates a shift toward use of alternative crops such as oats (Van den berg & Rademakers, 2007). Oats are utilised as silage especially in temperate and Mediterranean climate zones.

Crops preserved as silage should have relatively high amounts of WSC, a low buffering capacity and a DM content above 200 g/kg; a pH of about 4.0 is optimum to form stable silages (McDonald *et al.*, 1991). External factors such as additives are also essential in enhancing crop fermentation. Several additives have been developed over the past years for promoting and stabilizing ensiled crops. Silage additives can be classified according to two main types: (1) fermentation stimulants, such as sugars, inoculants and enzymes, which encourage growth of lactic acid producing bacteria; and (2) fermentation inhibitors, such as acids and formalin, which partially

or completely inhibit microbial growth (McDonald *et al.*, 2002). Efficiency of inoculants is however affected by levels and different types of microflora present on the crop at the time of ensiling. If the number of lactic acid bacteria (LAB) present on a crop before ensiling is low, fermentation of WSC may be poor resulting in poor silage (Meeske *et al.*, 2002).

Filya *et al.* (2002) found that whole-crop wheat ensiled with LAB inoculants (*Lactobacillus plantarum* + *Enterococcus faecium* and *Lactobacillus pentosus*), had lower amounts of carbon dioxide (CO<sub>2</sub>) in the wilted silages, 6.1 g/kg DM and 1.1 g/kg DM, respectively, compared with uninoculated silage that resulted in about 9.2 g/kg DM. Lower CO<sub>2</sub> production is an indicator of reduced carbohydrate breakdown. Meeske *et al.* (2002) found that whole-crop oats ensiled with an inoculant containing *Lactobacillus plantarum*, *Streptococcus faecium* and *Pediococcus acidilactici* increased feed intake by 0.6 kg DM/cow/day, and milk production increased by 6 %.

Inoculants such as Lalsil® Cereal containing *Lactobacillus buchneri* and *P. acidilactici* are still being tested for their use on whole-crop oats and other cereals. The aim of this study was therefore to determine the effects of a Lalsil® Cereal containing inoculant on fermentation of whole-crop oats in the bunker and in micro-silos on

- (1) fermentation characteristics,
- (2) aerobic stability and
- (3) nutritional value of the silage.



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## CHAPTER 2

### LITERATURE REVIEW

#### 2.1 Introduction

Silage is important forage and a source of energy for ruminants reared under intensive management conditions. It is one of the oldest methods of preserving forage and could be defined as follows: it is material produced by controlled fermentation of a crop of high moisture content (McDonald *et al.*, 2002). However, silage spoilage is a major source of concern resulting in significant forage and energy loss (McCullough, 1978).

Preservation is important in achieving consistency in feed supply in those seasons when forage availability is a major source of concern. Almost any crop can be preserved as silage, but the popular crops are grasses, legumes and whole cereals, especially wheat and maize (McDonald *et al.*, 2002). Crops that make excellent silage have relatively high levels of WSC, relatively low buffering capacity and DM content above 200 g kg<sup>-1</sup>. According to McDonald *et al.* (1991) crops for ensiling should have a physical structure that will allow for effortless compaction.

In Northern Europe, grass has been ensiled in Sweden and in the Baltic provinces of Russia since the beginning of the eighteenth century, while beet tops and leaves were ensiled in Northern Germany at the beginning of the nineteenth century.

Maize silage is an excellent source of energy and contains 40% to 50% grain (DM basis). Dairy cows (low producers, dry cows and heifers) fed maize silage *ad libitum* can get too fat and this could lead to ketosis or low milk production the following lactation. Maize silage should not exceed 55% of the diet (DM basis), especially for lactating cows (Staples, 2003)

## 2.2 Forage conservation systems

Although there is notable variation in forage conservation systems, the primary methods involve either the harvest of dry hay or silage. To produce dry hay, the crop is mowed and dried in the field to a moisture level that allows stable storage, normally 150-200 g kg<sup>-1</sup> moisture. Hay at this moisture can be stored for many months. Higher-moisture forage, 500-850 g kg<sup>-1</sup> can be stored as silage (Barnes *et al.*, 1995).

Each forage conservation method offers advantages and disadvantages (Boeke *et al.*, 1991):

### 2.2.1 Advantages of haymaking

- Transportation costs of hay are significantly lower because most of the moisture has been removed.
- High-quality hay enhances a desirable DM intake by animals and a better growth potential.
- Seasonal surpluses of hay in production can be conserved for utilization during periods of feed shortage.

### 2.2.2 Disadvantages of haymaking

- Tractor operations result in soil compaction and reduce water penetration and can thus reduce future pasture production. It may be necessary to periodically break the soil crust to facilitate water and fertiliser penetration.
- The energy value of hay may be too low and may necessitate the supplementation with extra feed.
- Hay does not keep for an indefinite period of time whereas silage can be stored for more than a year.
- Hay can easily be destroyed by fire.

- Haymaking requires optimum weather conditions without any rain which could precipitate the development of moulds.
- Feeding losses can be as high as 30% if proper “feeding out” facilities are not provided.

The advantages and disadvantages of silage (Engelbrecht, 1999):

### ***2.2.3 Advantages of silage***

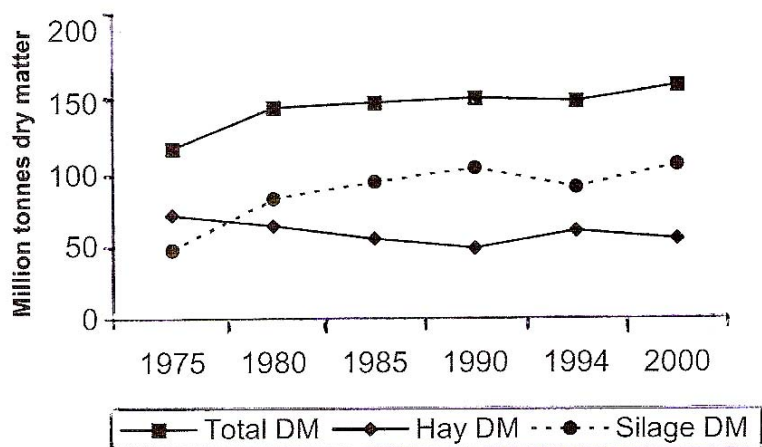
- Ensiling of crops decrease loss of material during periods when there is an oversupply of forage.
- Good quality feed is available during periods of forage scarcity.
- Well-covered silage is not susceptible to spoilage due to variable weather conditions.

### ***2.2.4 Disadvantages of silage***

- Poor management and knowledge of silage could lead to major losses of the crop.
- Marketing of silage is limited and transportation is difficult due to bulkiness.
- Fresh silage is required daily otherwise secondary fermentation would result in crop spoilage.

The estimated global production of silage is 250 million tonnes of DM per year (Wilkins *et al.*, 1999). Estimated figures of silage and hay DM produced in Western Europe are shown in Figure 2.1. There was a steady increase in silage production since 1975. Production of hay however declined slightly. Since 1994, the production of hay and silage has remained more or less stable. The total amount of silage made in Western Europe in 2000, in terms of fresh weight, was about 500 million tonnes (Wilkinson, 2005). Utilization of maize silage is high in South Africa, especially in the provinces where the geography allows the production of maize, namely the Free State, North West, Gauteng, Mpumalanga and Limpopo.





**Figure 2.1** Estimated production values of silage and hay DM in Western Europe: 1975 to 2000 (Wilkinson, 2005).

### 2.3 Principles of ensiling

The main objective of ensiling is to achieve anaerobic conditions as soon as possible, thereby providing an environment under which natural fermentation can take place. Compacting the material and sealing the silo prevents re-entry of air. Air that is left in the forage is quickly removed by crop respiratory enzymes. Oxygen causes aerobic spoilage as a result of respiration and material decays to worthless, toxic products (McDonald *et al.*, 1991). Finer chopping of harvested plant material is one of the strategies for improving compaction and fermentation of silages. This improves palatability and intake of silage (Apolant & Chesnutt, 1985).

The second objective is to discourage undesirable micro-organisms such as clostridia and enterobacteria. Clostridia are found on harvested forage and soil in the form of spores and increase rapidly under anaerobic conditions producing butyric acid. Clostridia cause proteolysis resulting in low quality silage. Enterobacteria are non-spore-forming, facultative anaerobes, which ferment sugars to acetic acid and have the ability to degrade amino acids (McDonald *et al.*, 1991). Growth of these undesirable micro-organisms is inhibited by lactic acid fermentation. The pH at which



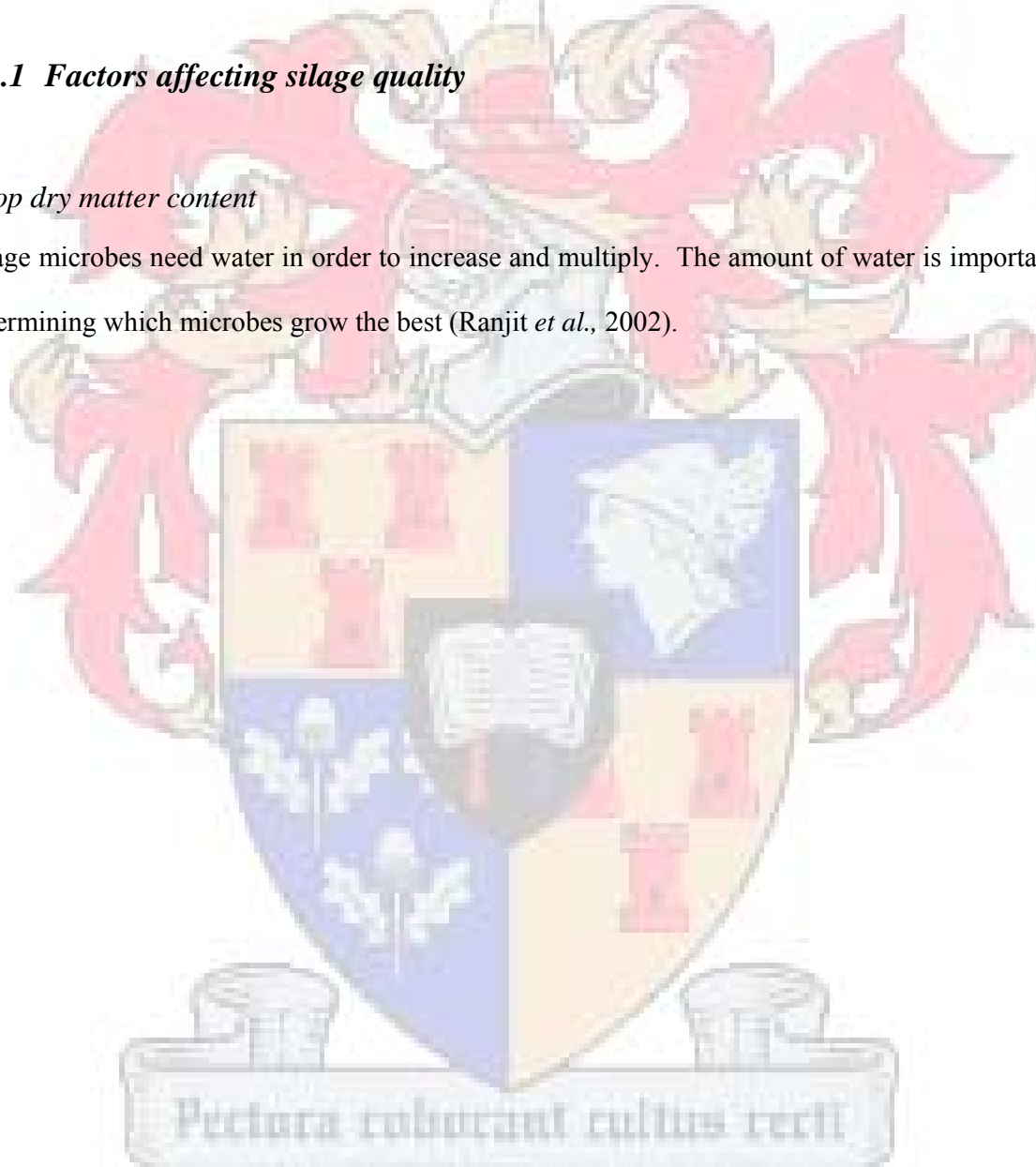
growth of clostridia and enterobacteria is inhibited depends on the moisture content and temperature. The wetter the material, the lower the critical pH drops. The ideal pH at which most of the acid tolerant clostridia will be inhibited is at a pH just below 5 (Jonsson, 1991).

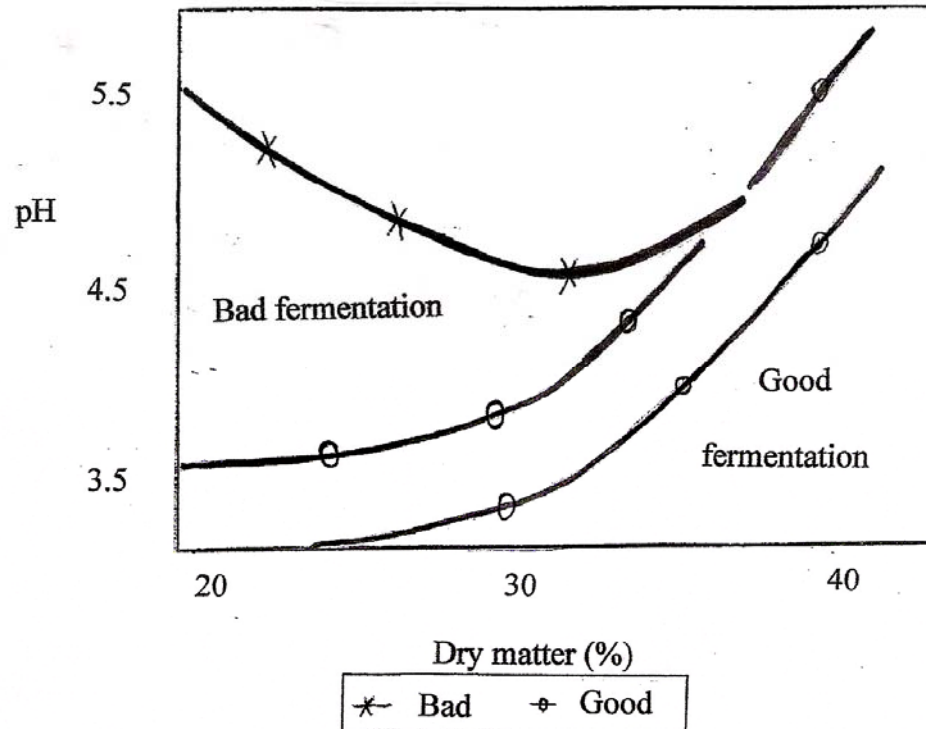
Growth of clostridia can be inhibited by reducing the moisture content by wilting prior to ensiling. Lactic acid bacteria have a high tolerance to low moisture conditions and are able to dominate the fermentation of high DM crops (McDonald *et al.*, 1991).

### ***2.3.1 Factors affecting silage quality***

#### *Crop dry matter content*

Silage microbes need water in order to increase and multiply. The amount of water is important in determining which microbes grow the best (Ranjit *et al.*, 2002).





**Figure 2.2** Moisture and silage fermentation (Ranjit *et al.*, 2002).

Dry matter less than 25% and low pH (<4.5) would result in bad quality silage. Dry matter, preferably less than 40% and an average pH of 5.5, would result in good quality silage. Undesirable bacteria favour wetter conditions in the silo. Therefore silage with high DM content decreases the risk of poor quality fermentation. The sugar content of the crop is relatively high if it is harvested at high DM content (Wilkinson, 2005). High DM content could be achieved by delaying harvest until the crop is relatively mature, and leaving the crop to wilt before harvesting. Results of DM concentration on silage fermentation can be seen in Table 2.1 for grass ensiled without additive (Wilkinson, 2005).

Silage with high DM content does not pack well and thus it is therefore difficult to exclude all of the oxygen from the forage mass. As the DM content increases, growth of LAB is limited and the rate and of fermentation is reduced (acidification occurs at a slower rate and the amount of total acid produced is less). It is better to wilt forages with a DM content above 30% to 35% prior

to ensiling (Wilkinson, 2005). The pH needed for preservation depends on the DM value of the silage and can be determined as follows (ED d'H d' Yvoy & Meeske, 1999):

$$\text{pH} = 0.00359 \times \text{DM (g kg}^{-1}\text{)} + 3.44$$

$$\text{DM} = 15\% \quad \text{pH} = 3.98$$

$$\text{DM} = 45\% \quad \text{pH} = 5.06$$

**Table 2.1** Effect of dry matter content on fermentation (McDonald, 1976).

	Unwilted	Wilted	
		1 day	2 days
Dry matter (g/kg fresh weight)	159	336	469
pH	3.7	4.1	4.9
Ammonia nitrogen (g/kg total N)	69	59	43
Water soluble carbohydrates (WSC) (g/kg DM)	17	117	164
Lactic acid (g/kg DM)	121	54	17
Acetic acid (g/kg DM)	36	21	12
Butyric acid (g/kg DM)	0	0	0
Lactic acid (g/kg total acids)	770	720	590

There are several effects of increasing the DM content of the crop on the composition of the silage:

- Limited fermentation.
- Decline in the proportion of fermentation acids present as lactic acid.
- Enhancement of the quantity of residual sugars in the silage.

Crops that are greatly wilted (>500 g DM/kg fresh weight) are susceptible to moulds. Crops exposed to wilting over extended periods lose WSC and have low digestibility (Wilkinson, 2005).



### 2.3.2 Effect of sugar content

Sugars in herbage are mainly glucose and fructose that are fermented to energy (McCullough, 1978).

If the sugar (WSC) concentration of a crop is quite high, the chances are fair of achieving excellent fermentation and a well-preserved product. This is particularly true if the crop is harvested with a short period of field wilting (Wilkinson, 2005).

The above statement is illustrated in Table 2.2 for two different crops harvested at the same DM and ensiled without an additive.

**Table 2.2** Typical dry matter and water soluble carbohydrate concentration of different crops (Wilkinson, 2005).

Crop	DM*	Water soluble carbohydrates
	(g/kg FW)	(g/kg FW)
Maize ( <i>Zea mays</i> )	300	70
Italian ryegrass ( <i>Lolium multiflorum</i> )	220	50
Perennial ryegrass ( <i>Lolium perenne</i> )	200	35
Tall fescue ( <i>Festuca arundinacea</i> )	190	20
Cocksfoot ( <i>Dactylis glomerata</i> )	170	20
Red Clover ( <i>Trifolium pratense</i> )	130	15
White clover ( <i>Trifolium repens</i> )	120	10
Lucerne ( <i>Medicago sativa</i> )	150	9

\* Unwilted, dry weather, FW = Fresh weight

Water soluble carbohydrates are the primary fermentation substrate. In temperate grass forages, glucose, fructose, sucrose and fructans are the primary WSC (Downing *et al.*, 2008).

### ***2.3.3 Silage pH***

A crucial aspect of silage fermentation is acidification. The initial decrease in pH produced by primary fermentation depends on the extent to which plant cells are ruptured by the chopping process, and on the buffering capacity or resistance to the acidification of the crop. If the decline in pH drop is not enough (pH 3-4) to prevent the development of coliform and clostridial bacteria, the pH could rise again. A rise in pH reflects the fermentation of lactic acid to weaker acids such as acetic and butyric acid. Extensive degradation of proteins and amino acids to amines, amides and ammonia may also occur. In such situations of extensive protein degradation, fermentation acids are present as ammonium salts to a level similar to that at the outset (Wilkinson, 2005). For example, the buffering content of the forage could have an effect on the silage fermentation. Alfalfa has a high buffering capacity in comparison to maize, thus it takes more acid production to lower the pH in alfalfa than in maize silage resulting in alfalfa being more difficult to ensile.

Plant material in the field can range from a pH of about 5 to 6 and decrease to a pH of 3.6 to 4.5

## **2.4 Chemistry of silage fermentation**

### ***2.4.1 Cutting and early stages of ensiling***

Ensiling occurs in two different phases namely: (1) the aerobic phase and (2) the anaerobic phase. Respiration takes place during the aerobic phase until all the available oxygen is consumed. Respiration is the oxidative degradation of organic compounds such as carbohydrates to yield usable energy as shown in the equation below (McDonald *et al.*, 2002).

The equation of Respiration:



After forage chopping, plant respiration continues for several hours and plant enzymes (e.g. proteases) are active until all the available oxygen is depleted. Rapid removal of oxygen is vital because it prevents the growth of unwanted aerobic bacteria, yeast and moulds that compete with beneficial bacteria for substrate. If oxygen is not rapidly removed, high temperatures and prolonged heating ensue (Kung, 2001).

Carbohydrates are the major respiratory source in particular hexose sugars, which undergo glycolysis and subsequent oxidation via the tricarboxylic acid cycle to  $\text{CO}_2$  and water. In the harvested plant, biosynthetic reactions are restricted and almost all the energy in the hexose is converted into heat. In the plant this heat energy would disappear into the atmosphere, but in the silo or bunker the heat is retained in the mass of herbage, causing an increase in temperature. The loss of soluble carbohydrates through respiration is a wasteful process and could result in a depletion of substrate that may adversely affect subsequent fermentation (McDonald *et al.*, 2002). The length of respiration plays an important role: the longer the period of respiration, the more WSC are consumed, resulting in a rise in temperature in the bunker (Zietsman, 1978).

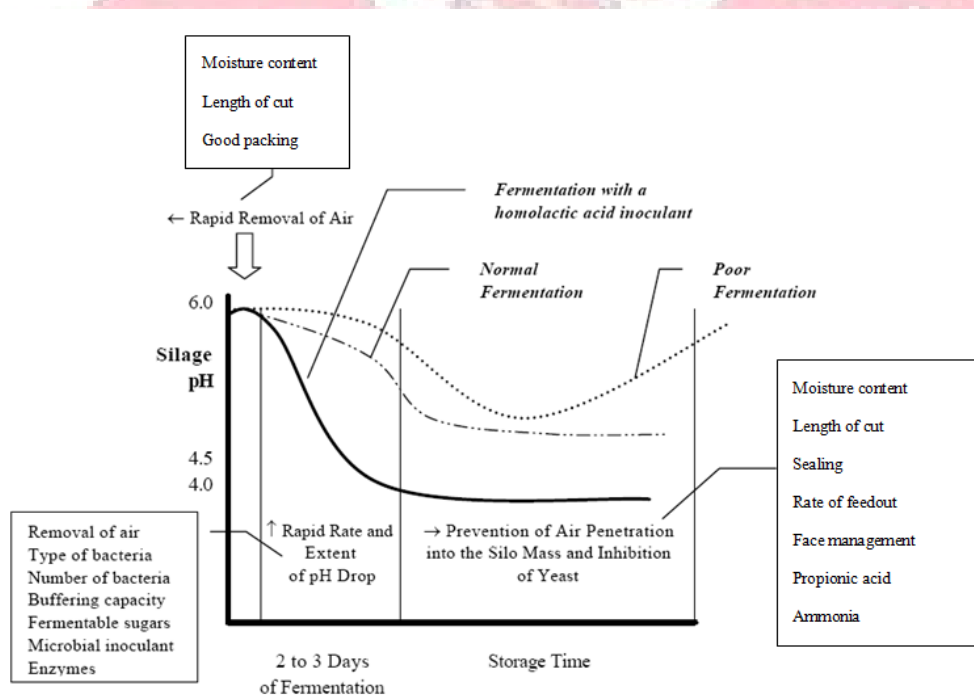
Oxygen can be eliminated by wilting the plant material to the recommended DM for the specific crop – maize silage at 35% DM, alfalfa at 35-45% DM, grasses at 35-45% DM and small grains at 30 to 40% DM (Kung, 1998), chopping forage to a correct length (about 9.5 to 12.7 mm (Kung, 1998), quick packing and good compacting, even distribution of forage in the storage structure, and immediate sealing of the silo (Kung, 2001).

#### ***2.4.2 Silage fermentation***

This is the anaerobic phase during which organic compounds and sugars are broken down to short-chain volatile fatty acids, mainly lactic acid, butyric acid and acetic acid. Lactic acid bacteria use WSC to produce lactic acid which is the primary acid responsible for decreasing the pH in silage.

A quick decrease in pH value will help to limit the breakdown of protein in the bunker by inactivating plant proteases and inhibiting the growth of undesirable anaerobic micro-organisms such as enterobacteria and clostridia (Kung, 2001).

Good silage will remain stable and will not change in composition or heat once the air is eliminated and it has achieved a low pH. However, the primary micro-organisms that cause aerobic spoilage and heating are yeasts and not moulds. When yeasts are exposed to oxygen, they metabolize lactic acid and this causes the pH of silage to increase hence allowing other bacteria to grow and further spoil the mass. Figure 2.3 is an illustration of the silage fermentation process.



**Figure 2.3** The three major events that make good silage and factors that can affect the silage fermentation process (Kung, 2001).

Undesirable bacteria (clostridia) tend to thrive in wet silage and can result in excessive protein degradation, DM losses and production of toxins. One more fact that may affect the ensiling process is the amount of WSC present for good fermentation to take place.

The biochemistry processes are shown in Table 2.3. Table 2.4 shows the end-products of silage fermentation while Table 2.5 illustrates variations in the amount of end-productions.



**Table 2.3** Fermentation pathways in ensilage (McDonald *et al.*, 2002).**Lactic acid bacteria***Homofermentative:*

Glucose  $\rightarrow$  2 Lactic acid

Fructose  $\rightarrow$  2 Lactic acid

Pentose  $\rightarrow$  Lactic acid + Acetic acid

*Heterofermentative:*

Glucose  $\rightarrow$  Lactic acid + Ethanol + CO<sub>2</sub>

3 Fructose  $\rightarrow$  Lactic acid + 2 Mannitol + Acetic acid + CO<sub>2</sub>

Pentose  $\rightarrow$  Lactic acid + Acetic acid

**Clostridia***Saccharolytic:*

2 Lactic acid  $\rightarrow$  Butyric acid + 2 CO<sub>2</sub> + 2 H<sub>2</sub>

*Proteolytic**Deamination*

Glutamic acid  $\rightarrow$  Acetic acid + Pyruvic acid + NH<sub>3</sub>

Lysine  $\rightarrow$  Acetic acid + Butyric acid + 2 NH<sub>3</sub>

*Decarboxylation*

Arginine  $\rightarrow$  Putrescine + CO<sub>2</sub>

Glutamic acid  $\rightarrow$   $\gamma$ -Aminobutyric acid + CO<sub>2</sub>

Histidine  $\rightarrow$  Histamine + CO<sub>2</sub>

Lysine  $\rightarrow$  Cadaverine + CO<sub>2</sub>

*Oxidation/ reduction (Stickland)*

Alanine + 2 Glycine  $\rightarrow$  3 Acetic acid + 3 NH<sub>3</sub> + CO<sub>2</sub>

**Enterobacteria**

Glucose  $\rightarrow$  Acetic acid + Ethanol + 2 CO<sub>2</sub> + 2H<sub>2</sub>





**Table 2.4** Common end-products of silage fermentation (McDonald *et al.*, 1991).

Item	Positive or Negative	Actions
pH	+	Low pH inhibits bacterial activity.
Lactic acid	+	Inhibits bacterial activity by lowering pH.
Acetic acid	-	Associated with undesirable fermentations.
	+	Inhibits yeasts responsible for aerobic spoilage.
Butyric acid	-	Associated with protein degradation, toxin formation, and large losses of DM and energy.
Ethanol	-	Indicator of undesirable yeast fermentation and high DM losses
Ammonia	-	High levels indicate excessive protein breakdown
Acid Detergent Insoluble Nitrogen (ADIN)	-	High levels indicate heat-damaged protein and low energy content.

**Table 2.5** Amounts of common fermentation end-products in various silages (McDonald *et al.*, 1991).

Item	Alfalfa Silage 30-35% DM	Alfalfa Silage 45-55% DM	Grass Silage 25-35% DM	Maize Silage 35-40% DM	High Moisture Maize 70-73% DM
pH	4.3 - 4.5	4.7 - 5.0	4.3 - 4.7	3.7 - 4.2	4.0 - 4.5
Lactic acid %	7 - 8	2 - 4	6 - 10	4 - 7	0.5 - 2.0
Acetic acid %	2 - 3	0.5 - 2.0	1 - 3	1 - 3	< 0.5
Propionic acid %	< 0.5	< 0.1	< 0.1	< 0.1	< 0.1
Butyric acid %	< 0.5	0	< 0.5	0	0
Ethanol %	0.5 - 1.0	0.5	0.5 - 1.0	1 - 3	0.2 - 2.0
Ammonia-N, % of CP	10 - 15	<12	8 - 12	5 - 7	< 10

## 2.5 Micro-organisms

### 2.5.1 Lactic acid bacteria

Lactic acid bacteria are crucial for good and stable silage production. There are several types of lactic acid producing bacteria; these are shown in Table 2.6. Lactic acid bacteria can be classified according to two different types, namely homofermentative and heterofermentative. These differ in their products of fermentation and their efficiency as producers of lactate. Even though LAB seem to coexist with plants, their role on the plant surface is still unknown (McDonald *et al.*, 1991).

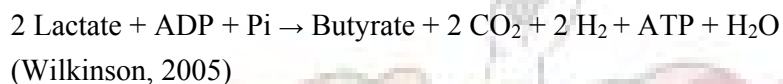
**Table 2.6** Some lactic acid bacteria of importance during ensiling (Adapted from McDonald *et al.*, 1991).

Genus	Glucose fermentation	Morphology	Species
Lactobacillus	Homofermentative	Rod	<i>L. acidophilus</i> <i>L. casei</i> <i>L. coryniformis</i> <i>L. curvatus</i> <i>L. plantarum</i> <i>L. salivarius</i>
	Heterofermentative	Rod	<i>L. brevis</i> <i>L. buchneri</i> <i>L. fermentum</i> <i>L. viridescens</i>
Pediococcus	Homofermentative	Coccus	<i>P. acidilactici</i> <i>P. damnosus</i> ( <i>cerevisiae</i> ) <i>P. pentosaceus</i>
Enterococcus	Homofermentative	Coccus	<i>E. faecalis</i> <i>E. faecium</i>
Lactococcus	Homofermentative	Coccus	<i>L. Lactis</i>
Streptococcus	Homofermentative	Coccus	<i>S. bovis</i>
Leuconostoc	Heterofermentative	Coccus	<i>L. mesenteroides</i>

\*About 15 to 20% of the total lactic acid is the L (+) isomer.

### 2.5.2 Clostridia

Clostridia (*Clostridium butyricum* and *Clostridium tyrobutyricum*) bacteria are found on forage. These bacteria use forage carbohydrates, proteins and lactic acid as their energy source and ferment it to butyric acid resulting in a rise of pH. Butyric acid is an indicator of rotten or putrefied silage.



Clostridia bacteria are promoted in situations where there are insufficient forage carbohydrate levels (for instance when it rains while the forage is wilting) or an extended respiration period due to poor packing and seepage as a result of extreme forage moisture (Wilkinson, 2005). High humidity during wilting and poor silage packing promotes Clostridia growth.

**Table 2.7** Clostridia of importance during ensiling (McDonald *et al.*, 1991).

Lactate fermenters	Amino acid fermenters	Others
<i>C. butyricum</i>	<i>C. bifermentans</i>	<i>C. perfringens</i>
<i>C. paraputrificum</i>	<i>C. sporogenes</i>	<i>C. sphenoides</i>
<i>C. tyrobutyricum</i>		

### 2.5.3 Yeasts and moulds

Yeasts are present in silages, and although relatively inactive during ensilage, can become very dynamic under aerobic conditions, following the opening of the silo or removal of the silage (Wilkinson, 2005).

Pectora roburant cultus recti

**Table 2.8** Yeasts found during ensiling (McDonald *et al.*, 1991).

	Fermentation		Assimilation	Crop
	Glucose sugars		DL - lactate	
<i>Candida</i>				
<i>Albican</i>	+	(M)	+/-	Grass
<i>Bimundalis</i>	(+)		+	Grass
<i>Famata</i>	+/-		+	Maize
<i>Holmii</i>	+	(S,G)	+/-	Maize
<i>Krusei</i>	+		+	Grass/maize
<i>Lambica</i>	+		+	Grass/maize
<i>Melinii</i>	-		+	Grass/maize
<i>Silvicola</i>	+	(G)	-	lucerne/wheat

#### 2.5.4 Fermentation of proteins

Rapid proteolysis takes place after harvesting the crop. After a few days of wilting the protein content could decline as much as 50%. The amount of protein degradation varies depending on plant species, DM content and temperature. When the material is ensiled, proteolysis is prolonged but the activity declines as the pH value lowers. Products of proteolysis are amino acids and peptides of different chain lengths. Additional breakdown of amino acid occurs as a result of plant enzyme activity, but this is considered to be limited (McDonald *et al.*, 2002).

## 2.6 Silage additives

Additives are important in enhancing fermentation of silage material. They promote growth of lactic acid producing bacteria (*Lactobacilli*) and ultimately reduce DM losses during storage and improve the feeding value of silage (Bolsen & Heidker, 1985). The effects of additives vary depending on the type of additive and the crop-specific nature thereof. However, good management of the ensiling process is crucial in quality control.

The concept of adding a microbial inoculant to silage was to add fast growing LAB in order to dominate the fermentation process resulting in higher quality and palatable silage. Some of the

familiar homolactic acid bacteria used in silage inoculants include the following species: *L. plantarum*, *Lactobacillus acidophilus*, *P. acidilactici*, *Pediococcus pentacaceus* and *E. faecium*. Most of the time microbial inoculants contain one or more of these bacteria mentioned above and have been selected for their ability to dominate fermentation. The motivation for multiple organisms comes from the potential of synergistic actions, such as the faster growth rate in *Enterococcus* > *Pediococcus* > *Lactobacillus*. Another illustration is that *Pediococcus* strains are more tolerant of high DM conditions than are *Lactobacillus* and have a wider range of optimal temperature and pH for the growth. Frequent and experimental microbes that have been studied as silage inoculants are listed in Table 2.9 (Kung, 2001).

**Table 2.9** Some of the more common bacteria used as silage inoculants and some reasons for their use (Kung, 2001).

Organisms	Type of organism	General Reasons for Addition	Primary End-products
<i>Lactobacillus plantarum</i>	Lactic acid bacteria Homolactic	-rapid production of lactic acid relatively acid tolerant	Lactic acid
<i>Pediococcus acidilactici, cerevisae</i>	Lactic acid bacteria Homolactic	-rapid production of lactic acid -faster growing than <i>Lactobacillus</i> -some strains show good growth at cooler temperatures -some strains have good osmotolerance	Lactic acid
<i>Enterococcus faecium</i>	Lactic acid bacteria Homolactic	-rapid production of lactic acid -faster growing than <i>Lactobacillus</i>	Lactic acid
<i>Propionibacterium shermanii, jensenii</i>	Propionibacteria	-production of antifungal compounds	Propionic and acetic acids CO <sub>2</sub>
<i>Lactobacillus buchneri</i>	Lactic acid bacteria Heterolactic	-production of antifungal compounds	Lactic and acetic acids Propanediol CO <sub>2</sub>

Lalsil® Cereal contains both *L. buchneri* and *P. acidilactici*, promoting better fermentation. Silage additives are normally classified in five different categories. The first two categories of Table 2.10, namely fermentation stimulants and fermentation inhibitors, are concerned with fermentation control and act either by encouraging lactic acid fermentation or by inhibiting microbial growth

partially or completely. The third group's aim is mainly to control the deterioration of silage upon exposure of oxygen. The nutrients category is added to crops at the time of ensiling in order to improve the nutritional value of the silage. The second last group (absorbents) is added to low DM crops to reduce loss of nutrients and pollution of water courses by runoff (McDonald *et al.*, 1991)

**Table 2.10** Classification of silage additives (McDonald *et al.*,1991).

Fermentation stimulants		Fermentation inhibitors		Aerobic deterioration inhibitors	Nutrients*	Absorbents
Bacterial Cultures	Carbohydrate sources*	Acids	Others			
Lactic acid Bacteria	Glucose Sucrose Molasses Cereals Whey Beet pulp Citrus pulp Potatoes Cell Wall degrading enzymes	Mineral acids Formic acid Acetic acid Lactic acid Benzoic acid Acrylic acid Glycollic acid Sulphamic acid Citric acid Sorbic acid	Formaldehyde Paraformaldehyde Glutaraldehyde Sodium nitrite Sulphur dioxide Sodium metabisulphite Ammonium bisulphate Sodium Chloride Antibiotics Carbon dioxide Carbon bisulphide Hexamethylenetetramine Bronopol Sodium hydroxide	Lactic acid bacteria Propionic acid Caproic acid Sorbic acid Pimaricin Ammonia	Urea Ammonia Biuret Minerals	Barley Straw Sugar beet pulp Polymers Bentonite

\*Most substances listed under carbohydrate sources can also be listed under nutrients.

According to Weinberg *et al.* (2007) inoculants are used as silage additives to help with preservation efficiency and because they utilize WSC efficiently.

### 2.6.1 Silage additives on the market in South Africa

In South Africa the two main companies selling additives are Alltech (Pty) Ltd and Vitam International. Some of Vitam International's products are Lalsil Fresh, Lalsil Dry and Lalsil Cana.

Alltech has one inoculant on the market in South Africa namely Sill-All 4X4. These companies sell their products directly to users.

## **2.7 Impact of additives on animal production**

### **2.7.1 Silage in dairy cattle production**

The effectiveness of silage inoculants and preservation have been assessed through measurement of feed intake, live weight gain, feed efficiency and milk production. The outcomes varied however, with some notable improvements observed in some studies. Improvements of these parameters ranged from 5 to 11% (Muck, 1993; Kung *et al.*, 2003). In many cases where an inoculant was used, the idea was to have an expected effect on animal performance, but in some case studies it did not have a significant effect (Weinberg *et al.*, 2007).

In South Africa a study was done to determine the effect of adding an enzyme containing lactic acid bacterial inoculant to big round bale silage on intake, milk production and the milk composition of Jersey cows. The outcome was that milk production of cows fed inoculated silage was higher than the cows receiving the control silage. DM intakes were 4.5% and 4.9% of live weight for the control and inoculated silage diets. The adding of the inoculant had a lowering effect on the milk urea nitrogen (MUN) content of the milk produced (Meeske *et al.*, 2002).

According to Gordon (1989) and Kung *et al.* (1993), small cereal grain crops and alfalfa have responded well to a microbial inoculant with homofermentative LAB. Maize with high moisture content has also been improved with homofermentative LAB. However, homofermentative LAB microbial inoculation of maize silage has resulted in less reliable results. For example, of 14 published (peer-reviewed) studies in North America where maize silage was treated with homofermentative LAB, improvements in animal performance were found in only three instances and changes in fermentation end-products were small (Kung, 2001).



Bolsen *et al.* (1992) reported on 19 surveys carried out at the University of Kansas State where maize silage inoculated with homofermentative LAB had 1.3% unit higher DM recovery, supported 1.8% more efficient gain and produced 1.6 kg more gain per ton of crop ensiled with beef cattle. In some case studies (Gordon, 1989; Kung *et al.*, 1993), increased animal responses have been observed with inoculation, even though there was little effect on the end-products of fermentation.

## 2.8 Conclusion

Forage conservation by ensiling ensures feed availability during periods when supply of good quality forage is low (McDonald *et al.*, 1991). Cereal-based silages represent a significant proportion of feed consumed by ruminant animals, particularly high production dairy cattle in South Africa.

The above review therefore points to a need for continued evaluation of silage inoculants. The environment, crops and microbes are constantly changing due to internal and external pressures. Hence the aim of the present study was to determine the effect of a new silage inoculant, Lalsil® Cereal on the fermentation dynamics, aerobic stability and nutritional value of whole-crop oat silage grown in a Mediterranean climate.





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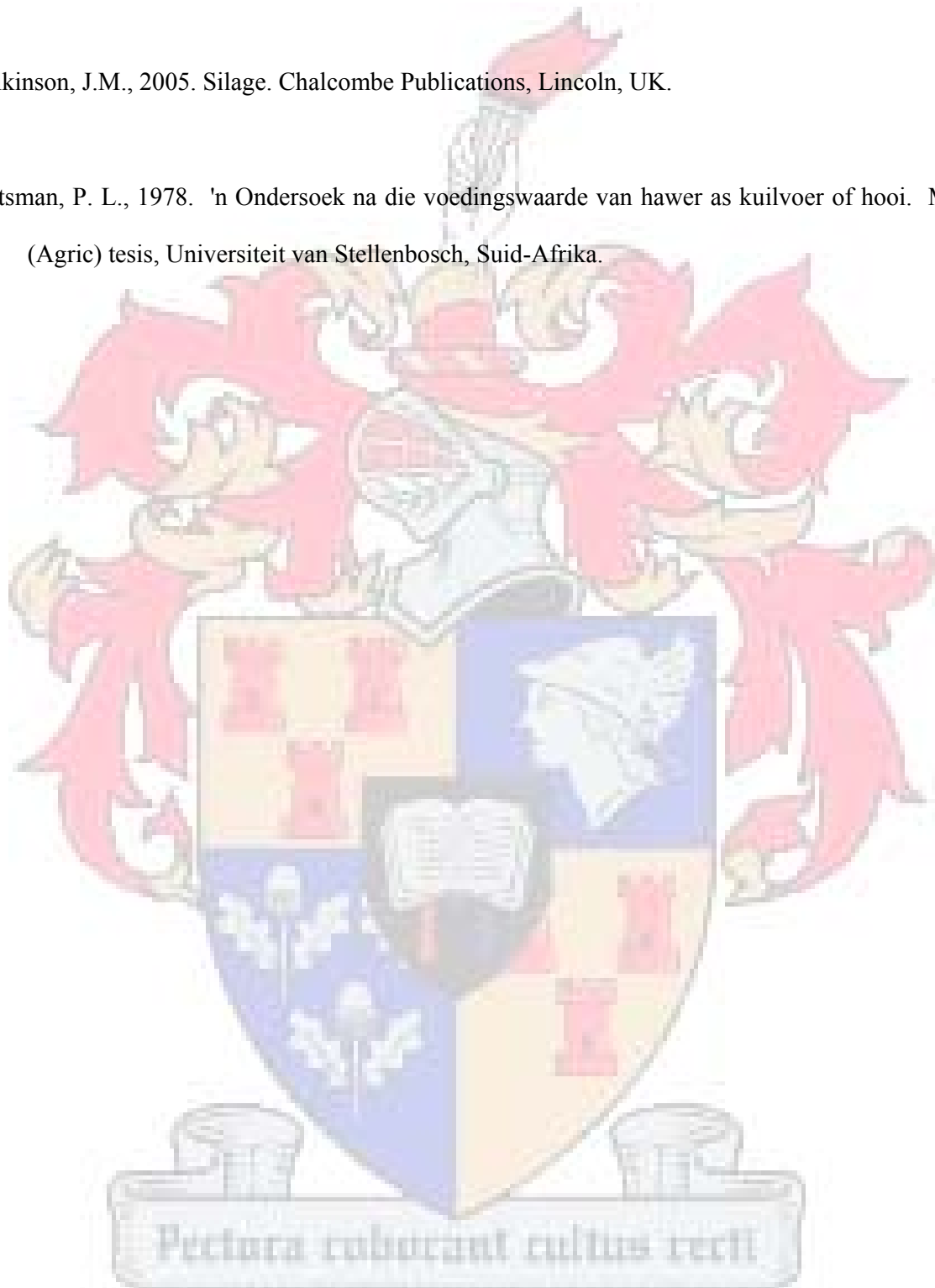
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## CHAPTER 3

### CHEMICAL COMPOSITION, STABILITY AND DEGRADABILITY OF WHOLE-CROP OAT SILAGE INOCULATED WITH A COMMERCIAL INOCULANT AND ENSILED IN MICRO-SILOS

#### ABSTRACT

A study was done to determine the effects of inoculating whole-crop oat silage with Lalsil® Cereal Lactobacilli (*Lactobacillus buchneri* (NCIMB 40788) and *Pediococcus acidilactici* (CNCM MA 18/5M)) LAB on silage fermentation, aerobic stability and nutritional value under micro-silo conditions. Dry matter (DM), organic matter (OM), neutral detergent fibre (NDF), crude protein (CP), lactic acid levels, pH, water soluble carbohydrates (WSC) and *in vitro* organic matter degradability (IVOMD) of silage were determined at 0,60 and 102 days of fermentation. A portion of the silage was exposed to air for 10 days after 60 days of ensiling and a second portion was exposed after 102 days of ensiling to determine aerobic stability of silage.

At harvesting (day 0) the forage had an average CP content of 9.7%, NDF of 58.2%, DM of 42.9%, pH 6, lactic acid 0.27% and IVOMD of about 54.2%. At day 60 there was a sharp decline in pH from 6 (day 0) to 3.7. Water soluble carbohydrates were higher in the inoculated silage 6.95% compared to 2.28% in the control batch whilst lactic acid was higher in the control 7.21% than that of the treated silage 6.14%. After 102 days of fermentation the WSC declined sharply, but still remained higher than the inoculant-treated silage. The levels of ammonia nitrogen (% of total nitrogen) in the oat silage were low ( $\approx 3.4\%$ ).

During the aerobic phase (60 to 70 days) WSC of untreated silage was almost consumed, but remained significant ( $P < 0.01$ ) in the treated silage. During the aerobic phase of 102-112 days lactic acid levels declined sharply; WSC in treated silage decreased by 40% and CO<sub>2</sub> levels were higher compared to the control. It is concluded that the inoculant Lalsil® Cereal had the effect of reducing the rate of consumption of WSC during the anaerobic phase and aerobic exposure. Silage

spoilage due to yeasts and moulds was however more evident with the inoculated silage due the presence of sugars. Silage spoilage due to yeasts and moulds was however more evident with the inoculated silage due to the presence of sugars.

### 3.1 Introduction

There is a high level of oat production in the Western Cape region of South Africa that could be utilised for silage. However, large variations in weather conditions and fluctuating wet and dry conditions are conducive to silage deterioration due to mould contamination and yeasts (Wyss & Jans, 1993). Over the past years several additives have been developed to promote and stabilize the fermentation of ensiled crops. Nevertheless, efficiency of inoculants is affected by levels of microflora present on the crop at the time of ensiling. If the number of LAB present on a crop before ensiling is low, fermentation of WSC may be poor, resulting in poor silage (Meeske *et al.*, 2002).

Inoculants, such as Lalsil® Cereal that contain *L. buchneri* and *P. acidilactici*, are still being evaluated for use on cereal crops and other forages in both bunkers and micro-silos. Micro-silos are a more practical method of assessing silage fermentation kinetics (Cherney *et al.*, 2006) as fermentation occurs under more controlled conditions.

The aim of this study was therefore to determine the effects of Lalsil® Cereal, an inoculant containing *L. buchneri* (NCIMB 40788) and *P. acidilactici* (CNCM MA 18/5M) LAB, on

- (1) fermentation of whole-crop oats (in micro-silos 1.5 L glass jars),
- (2) aerobic stability and
- (3) nutritional value of the silage.

## 3.2 Materials and methods

### *Study Site*

The study to evaluate the effect of fermentation, stability and digestibility of whole-crop oat silage inoculated with Lalsil® Cereal was conducted at Elsenburg (33° 50' 32.51"S, 18° 49' 51.56"E) and Stellenbosch University (33° 55' 53.62"S, 18° 52' 03.39"E, Western Cape, South Africa.

### **3.2.1 Cropping and harvesting**

Oats (*Avena sativa*, cv SSH 405) were planted on 30 May 2006 on 60 ha under dryland conditions at Elsenburg (33°51,485' S, 018° 50,188' E) in the Western Cape province of South Africa. Soil pH was 5.9; calcium (Ca) 2.7 cmol(+)/kg, magnesium (Mg) 0.2 cmol(+)/kg and potassium (K) 37.0 mg/kg. At planting 200 kg of fertilizer (15% nitrogen (N), 10% phosphorus (P) and 5% potassium (K) were applied per hectare. Oats were planted with a 3 m Piket planter at 120 kg/ha. Thirty days after planting a top dressing of fertilizer (18% N and 18% K) was applied at 200 kg/ha and after 60 days potassium ammonia nitrogen (28%) was applied at 100 kg/ha. At 123 days the crop was harvested at soft dough stage and length of the chopped material was 9 mm.

### **3.2.2 Silage preparation - ensiling whole-crop oats**

At day 0 (day of ensiling) 60 kg of fresh chopped material was collected and thoroughly mixed on a sterile plastic sheet (sterilized with ethanol). The material was divided in two portions of 30 kg each, which were randomly allocated to either the control or the inoculant treatment. The inoculant, Lalsil® Cereal containing *L. buchneri* (NCIMB 40788) and *P. acidilactici* (CNCM MA 18/5M), was sprayed onto one batch (30 kg of chop) to provide about  $5.79 \times 10^9$  colony forming



units (CFU) of LAB per gram of fresh material. After the inoculation, the silage was mixed thoroughly for ensiling in micro-silos (1.5 L glass jars) (WECK, GmbH u. Co., Wehr-Oflingen, W. Germany).

Forty-eight micro-silos were randomly divided into two groups (control and inoculant). Approximately 670 g of the chopped material was ensiled in each of the 24 glass jars for each treatment. All the jars were stored at ambient temperature in a dark room provided for light-sensitive microbes.

### **3.2.3 Sample collection**

Three micro-silos were opened for each treatment on days 1, 2, 4, 8, 15, 30, 60 and 102 post-ensiling. At these days gaseous losses were also determined by weighing the jars before and after removing the lid. Samples were also collected for chemical analysis. A portion ( $\pm 200$  g) from each sample was vacuum-packed and frozen at  $-20$  °C pending analysis for lactic acid, WSC, volatile fatty acids (VFA) and ammonia nitrogen. Another portion ( $\pm 180$  g) was dried in a conventional oven at  $60$  °C for 72 hours and milled through a 1 mm screen using a hammer mill (Scientific RSA, Hammer mill, Ser No 372), pending chemical analyses for IVOMD, NDF, CP and OM. A third portion ( $\pm 25$  g) was oven-dried at  $100$  °C for 48 hours to determine DM.

### **3.2.4 Aerobic stability**

Determination of aerobic stability was done according to the method describe by Ashbell *et al.* (1991). After 60 and 102 days, temperature changes and CO<sub>2</sub> production in silage were monitored using a data logger system (MCS 120) over a period of 10 days. Silage ( $\pm 280$  g) was loosely placed in the upper part of a system and a temperature sensor was placed in the material. The system consisted of two parts. An upper part, which was made out of a 2 L polyethylene



terephthalate bottle and a lower part made out of a honey jar. Three holes, 1 cm in diameter were drilled in the bottom of bottle (2 L polyethylene terephthalate bottle) and another hole was drilled through the cap of the bottle. The latter was covered with nets to ensure that silage would not fall out of the bottle. The base of the bottle was cut and served as a lid. The hole through the cap enabled air circulation. The lower part of the unit was filled with 150 ml of 20% potassium hydroxide (KOH) to absorb CO<sub>2</sub>. After 5 days the 20% KOH solution was changed with a new refill. The solution 20% KOH was titrated with 1 N HCl to expel the CO<sub>2</sub>. The amount of CO<sub>2</sub> (g/kg DM) released was calculated according to Ashbell *et al.* (1991) as shown in the equation below:

$$\text{CO}_2 \text{ (g/kg DM)} = \left[ \frac{(T \times V \times 0.044 \times 1 \times 100)}{(A \times Fm \times \%DM / 100)} \right]$$

where:

T = volume (ml) of 1 N HCl used in titration (ml)

V = total volume (ml) of 20% KOH (ml)

A = volume (ml) of KOH used in determination (ml)

Fm = mass (kg) of fresh material (kg)

DM = fraction of dry matter

### 3.2.5 Chemical analysis

Determination of dry matter was done according the method of AOAC International (2002), AOAC Official method number 934.01. About 180 g material was dried in a conventional oven at 60 °C for 72 hours and milled through a 1 mm screen using a hammer mill (Scientific RSA, Hammer Mill, Ser No 372). A second portion ± 25 g was oven-dried at 100 °C for 48 hours to determine DM. Determination of organic matter was done according to AOAC International (2002), AOAC Official Method 942.05. Approximately 2 g of dry sample was placed in a crucible and incinerated for 6 hours with a muffle furnace at 500 °C. Crude protein was analyzed using a Dumas-type nitrogen analyzer (Leco FP-528, Leco Corporation, St. Joseph, MI). This is based on the method of AOAC International (2002), Official Method 968.06. About 0.1 g dried sample was used. Neutral detergent fibre was determined according to the method of Van Soest *et al.* (1991). Determination

of NDF was done with Ankom 220 Fibre Analyzer (Ankom Technologies, Fairport, NY). Heat stable  $\alpha$ -amylase was used in the analysis and 20 g sodium-sulphite was added to each batch of samples.

### **3.2.6 Lactic acid determination**

Lactic acid was determined according to the colorimetric method of Pryce (1969), which is a modification of the Barker & Summerson (1941) method for the determination of lactic acid. Lactic acid was determined in a 20 ml diluted solution. The dilute was prepared as follows: 50 g frozen silage diluted with 250 ml distilled water. This mixture was shaken by hand for about 3 minutes and stored in a fridge (5 °C) for up to 24 hours. During the cooling period, the mixture was shaken twice for about 3 minutes. After cooling down, the diluent was filtrated through Whatman no 4 paper to remove the plant matter. The supernatant was transferred to bottles and kept refrigerated until the samples were sent to the Agricultural Research Council at Irene, Pretoria for lactic analysis.

### **3.2.7 Water soluble carbohydrates**

Water soluble carbohydrates were determined based on the phenol-sulphuric acid method of Dubois *et al.* (1956). The WSC were determined on 40 g of frozen sample diluted with 360 ml of distilled water which was homogenized for 4 minutes with a bamix and filtrated through a Whatman no 1 to remove the plant material. The pH of the supernatant was measured using a pH measurer.

A 1 ml supernatant was diluted with 9 ml distilled water (solution A). Exactly 1 ml of solution A was pipetted and diluted with 9 ml distilled water (solution B), giving a 1:1000 solution. One ml of solution B was placed in a test-tube as well as 1 ml distilled water in another test-tube, which served as the blank. Phenol (80%) 0.15 ml was pipetted to the 1 ml of solution B and

vortexed 10 seconds. A 5 ml sulphuric acid 98% ( $\text{H}_2\text{SO}_4$ ) was placed in the middle of the latter solution and vortexed another 10 seconds. It was read on the spectrophotometer after a waiting period of 30 minutes. The amount of WSC was determined by referring to a standard curve which was constructed for the particular sugar under examination.

### **3.2.8 Volatile fatty acids**

Volatile fatty acids were determined at Nutrilab, University of Pretoria, South Africa, using a Gas Chromatograph (Varian 3300 FID Detector Gas Chromatograph, Varian Associates, Inc. 1985, United States of America, Column: CP Wax 58 (FFAP)CB Cat no 7654 25 m, 0.53 mm, 2.0  $\mu\text{m}$ ) according to the method of Webb *et al.* (1994) and Suzuki & Lund (1980). About 50 g of sample was used and diluted with 200 ml distilled water. This mixture was shaken on a horizontal shaker at 180 rpm for 6 hours and filtrated through four layers of cheesecloth to remove the plant matter. The supernatant was transferred to bottles and centrifuged at 4500 rpm for 20 minutes in a cooled chamber and filtrated through a Cameo 30 (0.45  $\mu\text{m}$ ) filter. About 1  $\mu\text{l}$  sample was injected into the gas chromatograph and the standard was repeatedly injected until consecutive results were comparable. The following conditions were maintained for the gas chromatograph:

Initial column temperature 50 °C; initial column hold time 2.00 °C; final column temp 190 °C; column rate in °/min 15; end time 16.33 min; injector temperature 250 °C; detector temperature 260 °C.

### **3.2.9 Ammonia nitrogen**

Ammonia nitrogen ( $\text{NH}_3\text{-N}$ ) of silage was determined by homogenizing 50 g of silage in 250 ml of 0.1 N  $\text{H}_2\text{SO}_4$  solution for three minutes with a bamix. The homogenate was filtrated through a Whatman no 4 filter paper. The ammonia content in the filtrate was determined by distillation

using a Buchi 342 apparatus and a MettröhM 655 Dosimat with an E526 titrator in accordance with Pearson & Muslemuddin (1968).

### **3.2.10 Yeasts and moulds**

The number of CFU of yeast and moulds were done by the Department of Microbiology, Stellenbosch University. About 40 g silage was used and diluted with 360 ml distilled water per sample. This is a 1:10 dilution. The dilution was spread out on three different agars namely: yeast morphological media, 1/2 potato dextrose agar and potato dextrose agar. After 10 days the yeast and moulds were counted.

### **3.2.11 In vitro degradability**

*In vitro* degradability was done according the method of Van Soest & Robertson (1985). Dried silage samples that were milled through a 1 mm screen (Scientific RSA, Hammer mill, Ser No 372) were weighed into F57 Ankom bags. About 0.5 g of material was weighed into each bag and heat-sealed.

A medium containing distilled water, macro-mineral solution, buffer solution, tryptose, micro-mineral solution and rezasurin was prepared and warmed to about 39 °C in a water bath. Reducing solution containing distilled water, potassium hydroxide pellets, cysteine-HCL and sodium sulphite nonahydrate was prepared prior to rumen fluid collection.

Rumen fluid was collected in the morning (09:30 am) from two cannulated Holstein cows that were fed a diet of oat hay, lucerne, wheat straw and 19% protein concentrate mixture twice a day in the morning at 07:30 am and in the afternoon at 17:00 pm. Rumen fluid was collected and squeezed through two layers of pre-warmed cheese cloth into warmed 39 °C thermo flasks. Rumen fluid pH was measured before and after blending was done at low speed for 15-20 seconds.

Blended rumen fluid was strained twice through two layers of pre-warmed cheese cloth into a thermos flask while gassing with CO<sub>2</sub>.

About 1 076 ml of medium (macro-minerals + buffer + micro-mineral + rezasurin) and 54 ml reducing solution were transferred into each incubation vessel while gassing with CO<sub>2</sub> and placed in an incubator (Daisy<sup>II</sup>, Ankom technology, Fairport, New York) set at 39 °C. When the medium was fully reduced (cleared), about 270 ml of rumen fluid was added to each incubation vessel and CO<sub>2</sub> was used maintaining anaerobic conditions. Samples were added into each vessel. The incubation period was 48 hours. Afterwards incubation samples were rinsed in cold water and air-dried. NDF analysis (Van Soest *et al.*, 1991) was done on air-dried samples; the samples were subsequently ashed in a muffle furnace at 500 °C for 6 hours to determine ash content.

### **3.3 Experimental design**

The experiment was a complete randomized design with one treatment: Inoculant Lalsil® Cereal (LAB) was the only factor. Material for ensiling was divided into two groups and one group was randomly selected for treatment with Lalsil® Cereal. No inoculant was added to the control crop.

### **3.4 Statistical analysis**

Data were analysed using a one-way ANOVA in SAS statistical software, SAS Institute, Inc. (1999), SAS/STAT User's Guide, Version 9, 1<sup>st</sup> printing, Volume 2. (SAS Institute Inc, SAS Campus Drive, Cary, North Carolina 27513.) Difference between means was tested using GLM procedure Least squares means.

Pectora roburant cultus recti

### 3.5 Results and discussion

#### *Aerobic Phase*

#### **3.5.1 Chemical composition of harvested whole-crop oats at day 0**

The chemical composition, pH levels and IVOMD of whole-crop oats harvested for silage are presented in Table 3.1.

**Table 3.1** Chemical profile and degradability of whole-crop oats at the start of fermentation (day 0).

	Day 0			
	Control	Inoculant	SEM	P-Value
<b><u>Descriptive parameters</u></b>				
Dry matter (g/kg)	429.0	429.0	1.90	0.853
Organic matter (g/kg DM)	960.0	959.0	0.90	0.738
aNDFom (g/kg DM)	578.0	586.0	4.60	0.248
Crude Protein (g/kg DM)	99.0	95.0	2.00	0.241
<b><u>Fermentation and degradability</u></b>				
pH	5.98	6.01	0.024	0.481
Lactic acid (% DM)	0.27	0.27	0.014	0.872
WSC (% DM)	9.26	11.36	2.940	0.639
<i>In vitro</i> organic matter degradability (%)	54.40	53.90	0.760	0.641

SEM = Standard Error of Means; WSC = Water Soluble Carbohydrates; NDF<sub>OM</sub> = Neutral detergent fibre in Organic Matter\*

The dry matter content of harvested oats was about 43%. Dry matter of less than 25% and pH lower than 4.5, would result in poor quality silage (Wilkinson, 2005). Dry matter of 40% and an average pH of 5.5 are preferable at ensiling because undesirable bacteria favour more moist conditions in the silo. Herbage with high DM content has low risk of spoiling (Wilkinson, 2005). The initial CP content of the ensiled material (control and inoculant) is above the minimum requirement of 7.5 % (Ørskov, 1992) which indicate there is enough protein for the microbial bacteria.



There was no significant difference between the WSC levels of the control and inoculation-treated material.

*In vitro* degradable organic matter values of oat silage were 54.4% and 53.9% respectively for the control and inoculated material. Digestibility of oat silage was less than 60% comparable to that of mature tropical grasses such as *Penisetum clandestinum*. The average IVOMD values of maize are 67% (Garcia-Rodriguez *et al.*, 2005). Maize silage is more digestible because of the high WSC content (McDonald *et al.*, 1991).

### 3.5.2 Chemical composition, fermentation and *in vitro* degradability at 60 days

The chemical composition of oat silage ensiled in micro-silos for 60 days is given in Table 3.2.

**Table 3.2** Chemical profile, organic matter and degradability of Lalsil-treated silage at day 60 of ensiling.

	Day 60			
	Control	Inoculant	SEM	P-Value
<b><u>Descriptive parameters</u></b>				
Dry matter (g/kg)	429.0	424.0	1.20	0.043
Organic matter (g/kg DM)	957.0	957.0	1.10	0.852
aNDFom (g/kg DM)	589.0	588.0	14.90	0.980
Crude protein (g/kg DM)	101.0	100.0	1.30	0.470
<b><u>Fermentation and degradability</u></b>				
pH	3.69	3.71	0.012	0.305
Lactic acid (% DM)	7.21	6.14	0.089	<0.01
WSC (% DM)	2.28	6.95	0.931	0.024
Volatile fatty acids (% DM)	1.09	1.00	0.029	0.098
Acetic acid (% DM)	1.06	0.97	0.028	0.089
Propionic acid (% DM)	0.02	0.03	0.002	0.116
Ammonia nitrogen (% of total nitrogen)	3.87	3.28	0.761	0.611
<i>In vitro</i> organic matter degradability (%)	52.80	53.10	0.610	0.741

SEM = Standard Error of Means; WSC = Water Soluble Carbohydrates; NDF<sub>OM</sub> = Neutral detergent fibre in Organic Matter \*

Dry matter content was relatively unchanged over the 60 days (averaged 42.5 %). There was a sharp decline in pH from 6.00 (day 0) to about 3.7 after 60 days of ensiling; although there were no

significant differences between treated silage and control. There was an inverse relationship between lactic acid and WSC content. Lactic acid content increased with fermentation as WSC were consumed by anaerobic microbes. The WSC of Lalsil® Cereal inoculated silage were almost three times the amount detected in the control silage. The Lactic acid content of treated material was significantly lower than the control silage. We can conclude that the Lalsil treatment had the effect of reducing WSC consumption during ensiling. There was, however, no improvement *in vitro* degradability, which supports the view that low WSC in grass silage can contribute to the poor efficiency with which silage-N is used by ruminants (Davies *et al.*, 2005). Energy in grass is usually in short supply thereby reducing microbial efficiency (Clark *et al.*, 1992).

### 3.5.3 Chemical composition, fermentation and *in vitro* degradability at 102 days

The chemical composition, fermentation and *in vitro* degradability of oat silage ensiled in micro-silos for 102 days are given in Table 3.3.

**Table 3.3** Chemical composition, volatile fatty acids, ammonia nitrogen and digestibility of whole-crop oats at 102 days of ensiling.

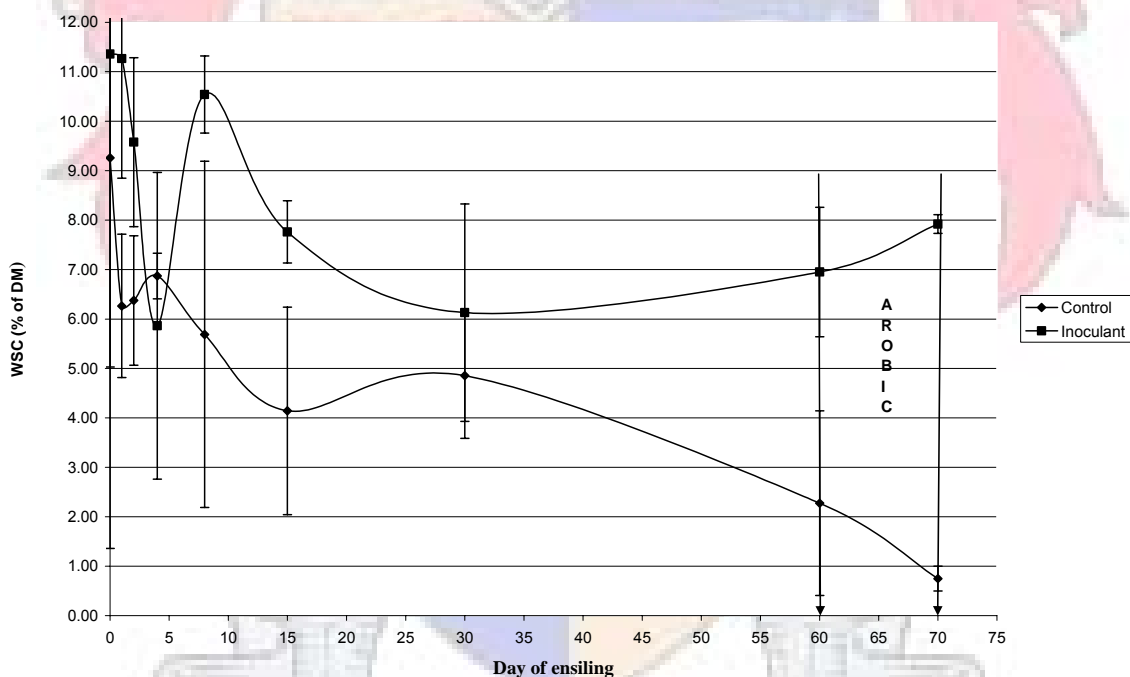
	Day 102			
	Control	Inoculant	SEM	P-Value
<b><u>Descriptive parameters</u></b>				
Dry matter (g/kg)	430.0	424.0	1.40	0.034
Organic matter (g/kg DM)	957.0	955.0	0.30	0.035
aNDFom (g/kg DM)	595.0	586.0	1.50	0.012
Crude protein (g/kg DM)	101.0	103.0	2.00	0.572
<b><u>Fermentation and degradability</u></b>				
pH	3.67	3.74	0.011	0.013
Lactic acid (% DM)	7.42	6.48	0.113	<0.01
WSC (% DM)	1.62	5.44	0.342	<0.01
Volatile fatty acids (% DM)	1.07	1.35	0.035	<0.01
Acetic acid (% DM)	1.06	1.32	0.034	<0.01
Propionic acid (% DM)	0.016	0.023	0.003	0.230
Ammonia nitrogen (% of total nitrogen)	3.21	3.60	0.226	0.289
<i>In vitro</i> organic matter degradability (%)	53.90	53.10	0.670	0.419
Yeast and moulds (g DM)	999	432	302	0.255

SEM = Standard Error of Means; WSC = Water Soluble Carbohydrates; NDF<sub>OM</sub> = Neutral detergent fibre in Organic Matter\*

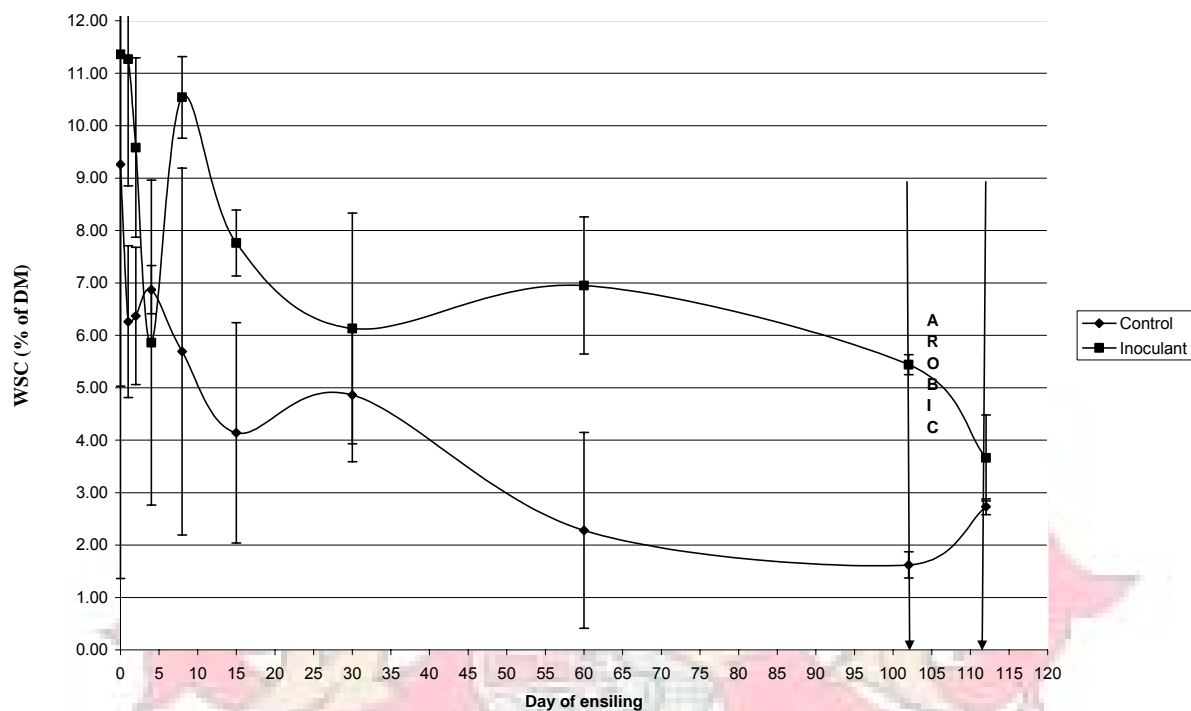


Although content of WSC declined from day 60 to 102, it however remained higher for the inoculant-treated silage. The inoculant reduced the rate of WSC fermentation; also confirmed by the low levels of lactic acid in treated silage. This indicates the high levels of lactic acid. The lactic acid production is a vital factor in inhibiting the growth of undesirable bacteria and in reducing fermentation losses (McDonald *et al.*, 1991). The levels of ammonia nitrogen (% of total nitrogen) in oat silage were low ( $\approx 3.4\%$ ) compared to that normally reported for maize silage (6.31%) (Borreani *et al.*, 2007). This may indicate that proteolysis did not occur that much in oat silage. Borreani *et al.* (2007) reported that maize silage had an acetic acid content of 2.7%; this was double the amount that was observed in our silage.

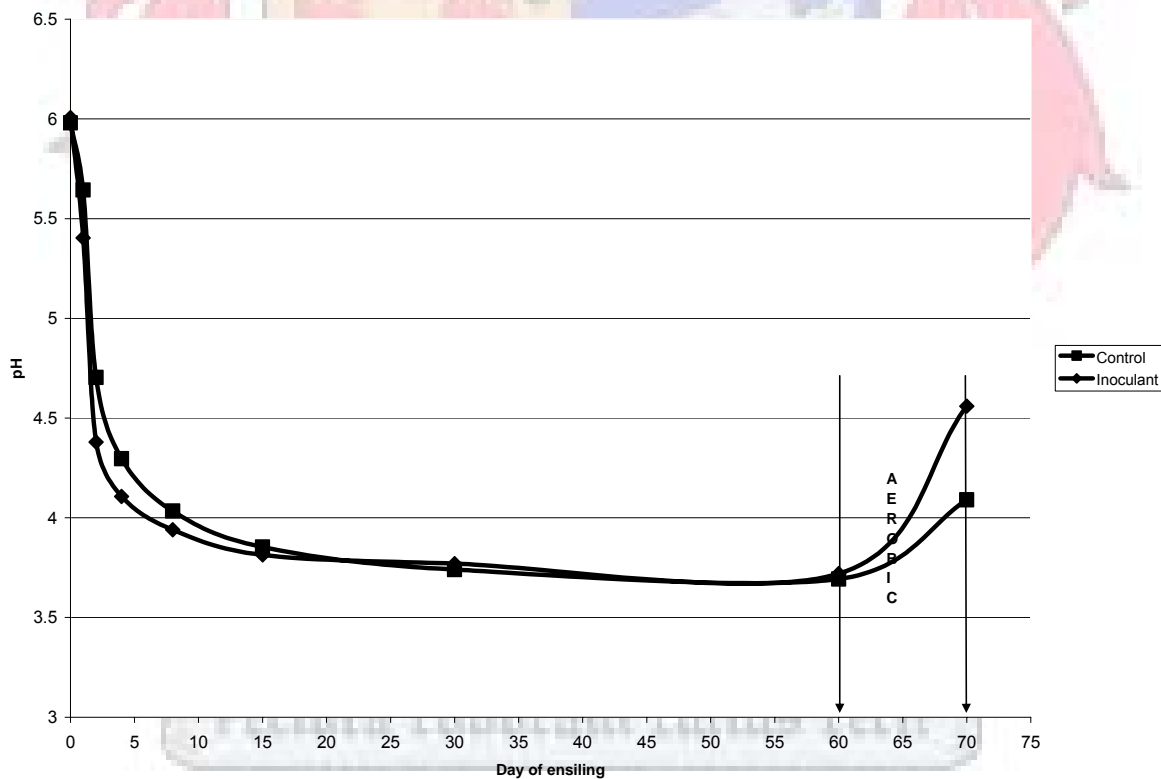
Figures 3.1, 3.2, 3.3, 3.4, 3.5 and 3.6 illustrate changes in WSC, pH and lactic acid contents of inoculated and control silages at 102 days of fermentation in micro-silos.



**Figure 3.1** The change in water soluble carbohydrates of oat silage ensiled with or without a lactic acid bacterial inoculant after 60 days of ensiling

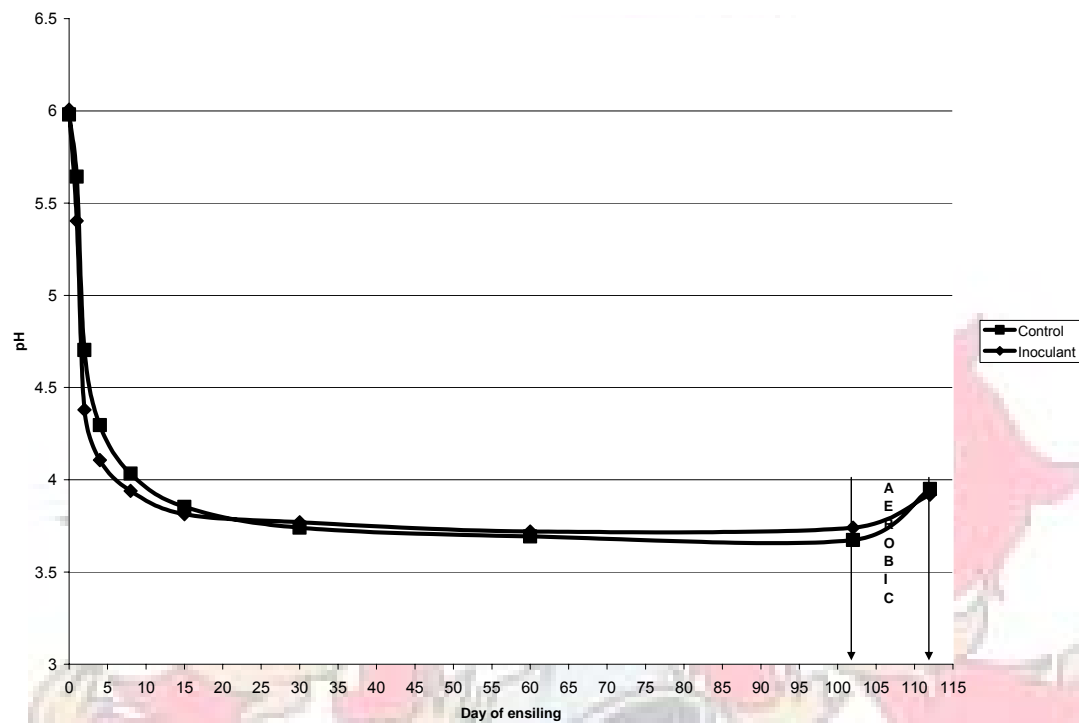


**Figure 3.2** The change in water soluble carbohydrates of oat silage ensiled with or without a lactic acid bacterial inoculant after 102 days of ensiling

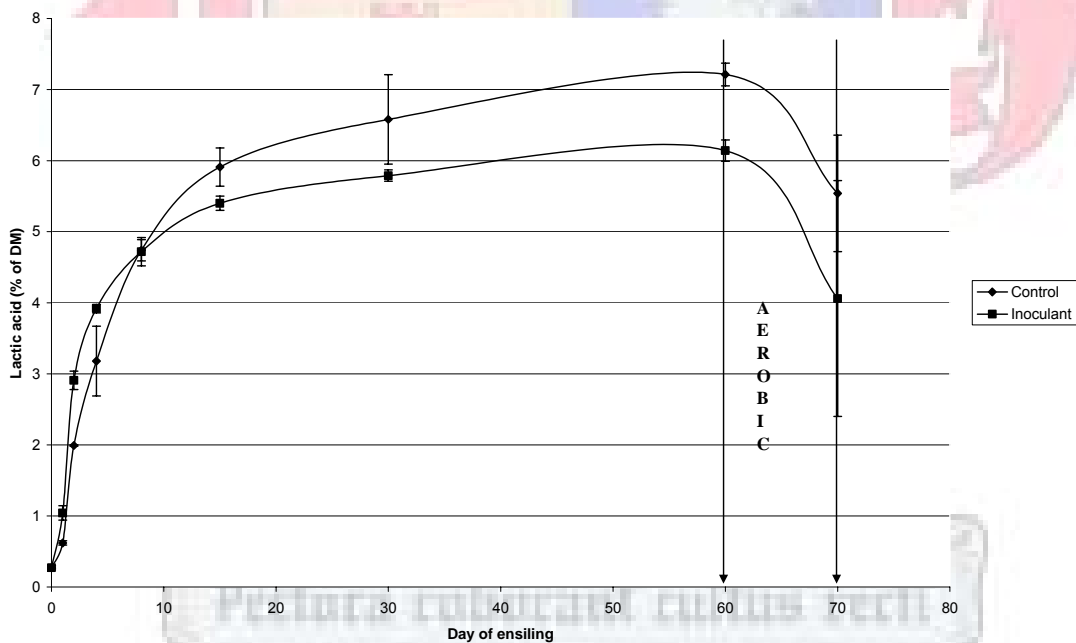


**Figure 3.3** The change in pH of oat silage ensiled with or without a lactic acid bacterial inoculant at 60 days of ensiling

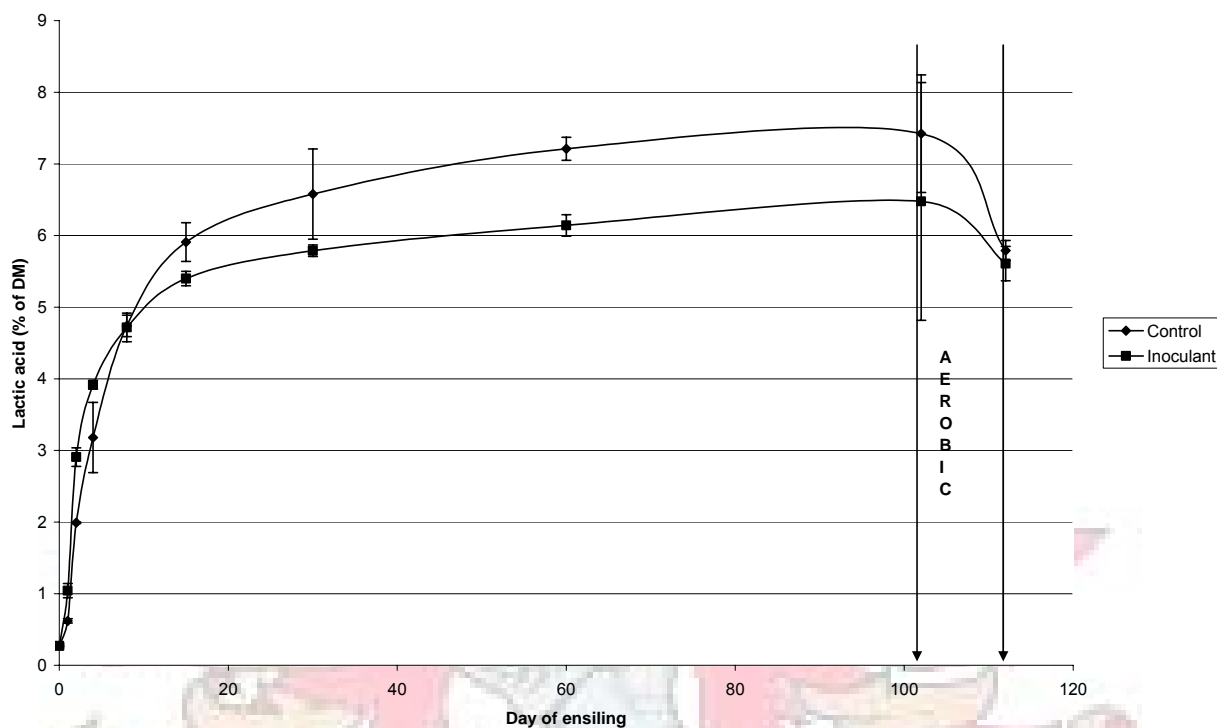
The pH of both silages was less than 4 and were well preserved.



**Figure 3.4** The change in pH of oat silage ensiled with or without a lactic acid bacterial inoculant at 102 days of ensiling



**Figure 3.5** The change in lactic acid of oat silage ensiled with or without a lactic acid bacterial inoculant at 60 days of ensiling



**Figure 3.6** The change in lactic acid of oat silage ensiled with or without a lactic acid bacterial inoculant at 102 days of ensiling

#### *Aerobic Study*

#### **3.5.4 Aerobic stability at 60 days**

The chemical composition of whole-crop oat silage exposed to aerobic conditions for ten days after being ensiled for 60 days is given in Table 3.4.

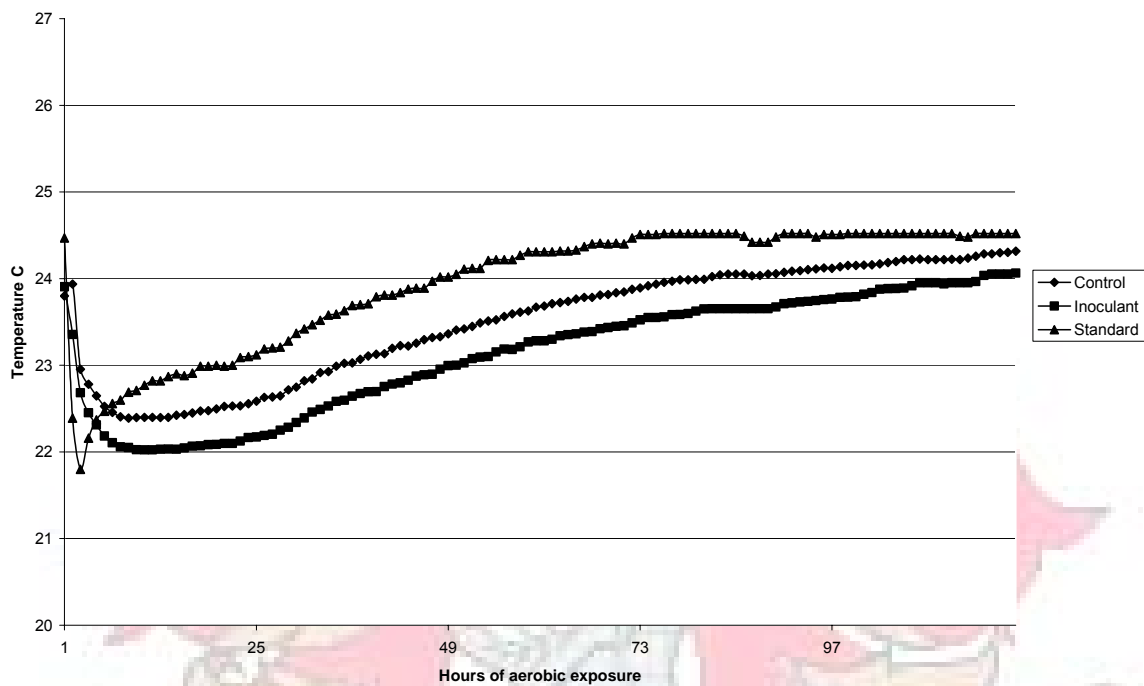


**Table 3.4** Composition of whole-crop oat silage exposed to aerobic conditions during the period 60-70 days and organic matter and neutral detergent fibre disappearance.

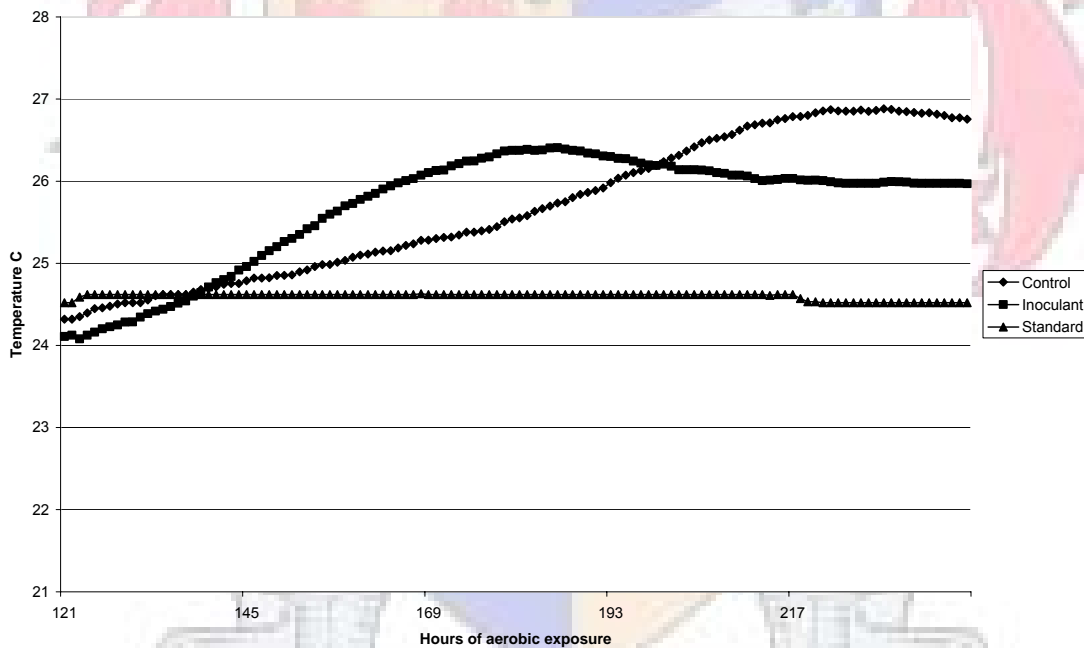
	Day 70			
	Control	Inoculant	SEM	P-Value
<b><u>Descriptive parameters</u></b>				
Dry matter (g/kg)	424.0	418.0	2.60	0.181
Organic matter (g/kg DM)	954.0	956.0	0.80	0.183
aNDFom (g/kg DM)	597.0	619.0	7.10	0.091
<b><u>Fermentation and degradability</u></b>				
pH	4.09	4.56	0.365	0.415
Lactic acid (% DM)	5.54	4.05	0.756	0.237
WSC (% DM)	0.74	7.92	0.248	<0.01
<i>In vitro</i> organic matter degradability (%)	53.30	51.70	0.420	0.056
Carbon dioxide (g/kg DM)	6.45	3.90	2.248	0.468

SEM = Standard Error of Means; WSC = water soluble carbohydrates; NDF<sub>OM</sub> = Neutral detergent fibre in Organic Matter \*

*In vitro* organic matter degradability was anomalously and significantly lower for the treated silage compared to the control. The pH was 4.1 and 4.6 for control and treated silage, respectively. Water soluble carbohydrates of the control silage were almost totally entirely consumed but content in the treated silage was significantly higher; WSC in the treated silage was 10.7 times more than the control. Carbon dioxide was 6.45 g/kg DM in the control and it could be an indicator of sugars being metabolised by aerobic microbes that yield CO<sub>2</sub> as by-product. This silage was unstable under aerobic conditions - there was an increase of pH and CO<sub>2</sub> content and the lactic acid concentration dropped. Consumption of lactate by yeast, fungi and *Bacillus* spp during the aerobic phase decrease the potential stability of silage as lactate is converted to acetate or degraded to butyric resulting in a pH rise (Lindgren *et al.*, 1985, Kung Jr., 2001). Aerobic stability is determined from the time the silage is exposed to aerobic conditions till the temperature of the silage rise by 2 °C above the ambient temperature (Kung, Jr, 2001).



**Figure 3.7** Changes in temperature for control and inoculated oat silage during the first 120 hours of aerobic exposure after 60 days of ensiling.



**Figure 3.8** Changes in temperature for control and inoculated oat silage during the last 120 hours of aerobic exposure after 60 days of ensiling.

### 3.5.5 Aerobic stability at 102 days

The chemical composition of whole-crop oat silage exposed to aerobic conditions for ten days after being ensiled for 102 days is given in Table 3.5.

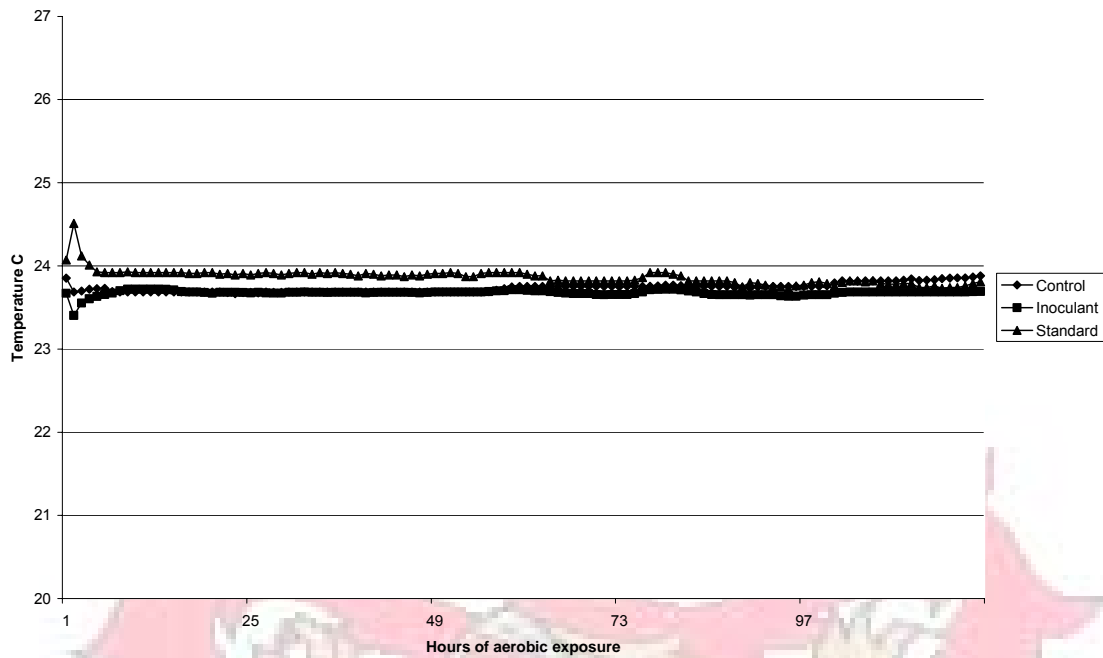
**Table 3.5** Profile of whole-crop oat silage exposed to aerobic conditions for 10 days after an ensiling period of 102 days.

	Day 112				SEM = Standar rd Error of Means; WSC = Water Solubl e Carboh ydrates ; NDF <sub>O</sub>
	Control	Inoculant	SEM	P-Value	
<b><u>Descriptive parameters</u></b>					
Dry matter (g/kg)	443.0	442.0	3.10	0.827	
Organic matter (g/kg DM)	955.0	956.0	0.70	0.456	
aDFom (g/kg DM)	599.0	601.0	2.80	0.739	
<b><u>Fermentation and degradability</u></b>					
pH	3.95	3.92	0.020	0.481	
Lactic acid (% DM)	5.79	5.61	0.230	0.596	
WSC (% DM)	2.73	3.66	0.430	0.206	
<i>In vitro</i> organic matter degradability (%)	52.40	51.70	0.410	0.263	
Carbon dioxide (g/kg DM)	5.73	17.30	7.700	0.348	
Yeast and moulds (g DM)	4.3x10 <sup>8</sup>	2.3x10 <sup>9</sup>	3.5x10 <sup>8</sup>	0.018	

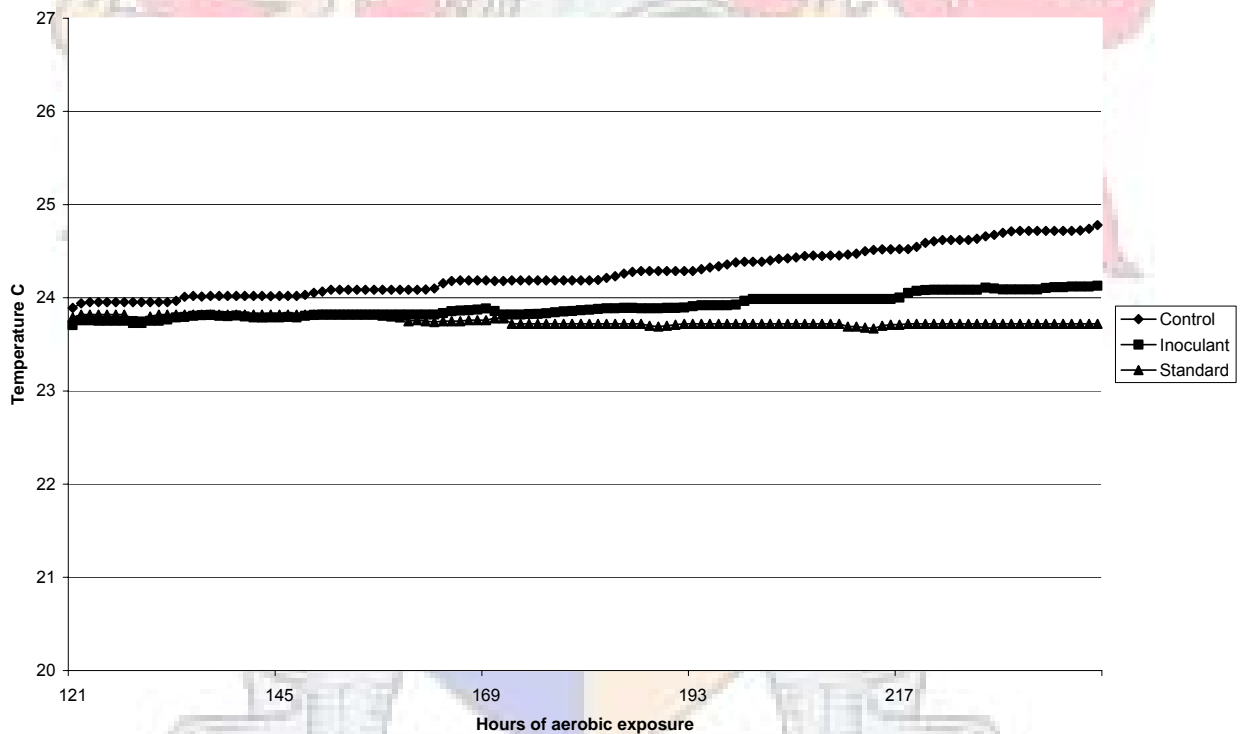
M = Neutral detergent fibre in Organic Matter\*

The lactic acid level dropped as silage was exposed to aerobic conditions indicating a decrease in population of lactic acid microbes. Water soluble carbohydrates decreased by 40% in the inoculated silage as sugars were metabolized for energy and CO<sub>2</sub> production. The inoculated silage had higher CO<sub>2</sub> production due to the high content of WSC in the silage compared to the control group. Inoculated silage had 5.44% WSC which declined to 3.66% after ten days of aerobic exposure. The WSC in the control silage were almost consumed by 102 days of ensiling. Yeast and mould counts could be suspected of the high WSC content.

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**Figure 3.9** Changes in temperature for control and inoculated oat silage during the first 120 hours of aerobic exposure after 102 days of ensiling.



**Figure 3.10** Changes in temperature for control and inoculated oat silage during the last 120 hours of aerobic exposure after 102 days of ensiling.



### 3.6 Conclusion

The inoculant Lalsil®Cereal reduced consumption of WSC during the anaerobic phase and aerobic exposure. Silage spoilage due to yeasts and moulds was however more evident with the inoculated silage due to the presence of sugars. However there was significant differences between lactic acid values. There is potential for improving oat silage preservation with Lalsil® Cereal.



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## CHAPTER 4

### CHEMICAL COMPOSITION, STABILITY AND DEGRADABILITY OF WHOLE-CROP OAT SILAGE INOCULATED WITH A LACTOBACILLI- BASED INOCULANT AND ENSILED IN A BUNKER

#### ABSTRACT

A study was done to determine the effects of inoculating whole-crop oat silage with Lalsil® Cereal Lactobacilli (*Lactobacillus buchneri* (NCIMB 40788) and *Pediococcus acidilactici* (CNCM MA 18/5M)) LAB on silage fermentation, aerobic stability and nutritional value of silage ensiled in a bunker under outdoor conditions of a Mediterranean summer. Dry matter (DM), organic matter (OM), neutral detergent fibre (NDF), crude protein (CP), lactic acid levels, pH, water soluble carbohydrates (WSC) and *in vitro* organic matter degradability (IVOMD) of silage were determined at 0, 186 and 196 days of fermentation. A portion of the silage was exposed to aerobic conditions after opening the bunker from day 186-196 to determine aerobic stability.

At harvesting (day 0) the forage had an average crude protein content of 9.8%, NDF of 55.2%, DM of 45.8%, pH 5.81, lactic acid 0.27% and IVOMD of 56.6%. After 186 days of fermentation the WSC declined with  $\approx 37\%$ , but still remained higher for the inoculant-treated silage. During the aerobic phase (186 to 196 days) WSC of untreated silage was almost consumed, but remained significantly higher ( $P < 0.01$ ) in the treated silage compared to the control batch. Lactic acid content of inoculum-treated silage dropped ( $P > 0.05$ ) from 4.49 to 4.24 and  $\text{CO}_2$  levels were lower compared to the control. It is concluded that the inoculant Lalsil® Cereal had the effect of reducing the rate of consumption of WSC during the anaerobic phase and aerobic exposure.

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## 4.1 Introduction

Several additives have been developed over the past years to promote and stabilize ensiled crops. Efficiency of inoculants is however affected by levels of microflora present on the crop at the time of ensiling. If the numbers of LAB present on a crop before ensiling are low, fermentation of WSC may be poor resulting in poor silage (Meeske *et al.*, 2002).

Inoculants such as Lalsil® Cereal that containing *L. buchneri* and *P. acidilactici* are still being tested for their use on whole-crop oats and other cereals. The aim of this study was to determine the effects of a Lalsil® Cereal inoculant containing *L. buchneri* (NCIMB 40788) and *P. acidilactici* (CNCM MA 18/5M) on fermentation of whole-crop oats ensiled in a bunker silo on

- (1) fermentation characteristics,
- (2) aerobic stability and
- (3) nutritional value of the silage.

## 4.2 Materials and methods

### *Study site*

The study to evaluate the effect of fermentation, stability and digestibility of whole-crop oat silage inoculated with Lalsil® Cereal was conducted at Stellenbosch University, South Africa.

### ***4.2.1 Cropping and harvesting***

Oats (*Avena sativa*, cv SSH 405) were planted on 30 May 2006 on 60 ha under dryland conditions at Elsenburg (33°51,485' S, 018° 50,188' E) in the Western Cape province of South Africa. Soil pH was 5.9; calcium (Ca) 2.7 cmol(+)/kg, magnesium (Mg) 0.2 cmol(+)/kg and potassium (K) 37.0 mg/kg. At planting 200 kg of fertilizer (15% nitrogen (N), 10% phosphorus (P) and 5% potassium



(K)) was applied per hectare. Oats were planted with a 3 m Picket planter at 120 kg/ha. Thirty days after planting a top dressing of fertilizer (18% N and 18% K) was applied at 200 kg/ha and after 60 days potassium ammonia nitrogen (28%) was applied at 100 kg/ha. At 123 days the crop was harvested at soft dough stage and chopped to a length of 9 mm. The silage was compacted in the bunker with a Landini 8500, kW 63 tractor; and the bunker was closed with a plastic sheet, 200  $\mu$ m thick.

#### **4.2.2 Silage preparation - Ensiling whole-crop oats**

Whole-crop oats were packed in net bags and placed in a bunker (4 m x 68 m x 3 m) according to the buried bag technique (Allred *et al.*, 1955). About 80 kg of chopped oats (chop length  $\approx$  9 mm) were sampled from the harvested material, mixed and divided into two portions (on a sterile plastic sheet, cleaned with ethanol). The inoculant (Lalsil® Cereal) containing *L. buchneri* (NCIMB 40788) and *P. acidilactici* (CNCM MA 18/5M) was applied to one portion (40 kg of chop) to provide  $5.79 \times 10^9$  CFU of LAB per gram of fresh material. After inoculation, silage was mixed thoroughly and packed into net bags. Six bags per treatment were weighed and buried at 1 m and 2 m depths in the same bunker. White (control) and yellow (inoculant) nylon lines (3 m lengths) were attached to each bag and stretched out towards the front end of the bunker for easy retrieval of bags after 186 days. On day 186 all the 12 bags were retrieved and weighed before opening to determine changes in bag weight between day 0 and day 186.

### **4.2.3 Sample collection**

Before ensiling, samples (400 g) were collected from each bag and frozen at -20 °C pending further analyses. After 186 days of ensiling the samples were collected from each bag for chemical analyses. A portion (approximately 200 g) of each sample was vacuum-packed and frozen at -20 °C pending chemical analysis for lactic acid, WSC, VFA and ammonia nitrogen.

A second portion ( $\pm$  180 g) of the sampled material was dried in a conventional oven at 60 °C for 72 hours and subsequently milled through a 1 mm (Scientific RSA, Hammer mill, Ser No 372) screen, pending determination of IVOMD and NDF digestibility, CP and OM content. A third portion (about 25 g) was oven-dried at 100 °C for 48 hours to determine dry matter content.

### **4.2.4 Aerobic stability**

Determination of aerobic stability was done according to the method describe by Ashbell *et al.* (1991). After 184 days, temperature changes and CO<sub>2</sub> production in silage were monitored using a data logger system (MCS 120) over a period of 10 days. Aerobic stability was done in a temperature controlled room with the temperature set at 24 °C. Silage ( $\pm$  280 g) was loosely placed in the upper part of a system and a temperature sensor was placed in the material. The system consisted out of two parts. An upper part, which was made out of a 2 L polyethylene terephthalate bottle and a lower part made out of a honey jar. Three holes, 1 cm in diameter were drilled in the bottom of the bottle (2 L polyethylene terephthalate bottle) and another hole was drilled through the cap of the bottle. The latter was covered with nets to ensure that silage would not fall out of the bottle. The base of the bottle was cut and served as a lid. The hole through the cap enabled air circulation. The lower part of the unit was filled with 150 ml of 20% potassium hydroxide (KOH) to absorb CO<sub>2</sub>. After 5 days the 20% KOH solution was changed with a new refill. The solution

20% KOH was titrated with 1 N HCl to expel the CO<sub>2</sub>. The amount of CO<sub>2</sub> (g/kg DM) released was calculated according to Ashbell *et al.* (1991) as shown in the equation below:

$$\text{CO}_2 \text{ (g/kg DM)} = [(T \times V \times 0.044 \times 1 \times 100) / (A \times Fm \times \%DM / 100)]$$

where:

T = volume (ml) of 1 N HCl used in titration (ml)

V = total volume (ml) of 20% KOH (ml)

A = volume (ml) of KOH used in determination (ml)

Fm = mass (kg) of fresh material (kg)

DM = fraction of dry matter

#### 4.2.5 Chemical analysis

Determination of DM was done according to the method of AOAC International (2002), AOAC Official method number 934.01. About 180 g material was dried in a conventional oven at 60 °C for 72 hours and milled through a 1 mm screen using a hammer mill (Scientific RSA, Hammer Mill, Ser No 372). A second portion ± 25 g was oven-dried at 100 °C for 48 hours to determine DM. Determination of OM was done according AOAC International (2002), AOAC Official Method 942.05. Approximately 2 g of dry sample was placed in a crucible and incinerated for 6 hours with a muffle furnace at 500 °C. Crude protein was analyzed using a Dumas-type nitrogen analyzer (Leco FP-528, Leco Corporation, St. Joseph, MI). This is based on the method of AOAC International (1990), Official Method 968.06. About 0.1 g dried sample was used. NDF was determined according the method of Van Soest *et al.* (1991). Determination of NDF was done with Ankom 220 Fibre Analyzer (Ankom Technologies, Fairport, NY). Heat stable α-amylase was used in the analysis and 20 g sodium-sulphite was added to each batch of samples.

#### 4.2.6 Lactic acid determination

Lactic acid was determined according to the colorimetric method of Pryce (1969), which is a modification of the Barker & Summerson (1941) method for the determination of lactic acid.

Lactic acid was determined in a 20 ml diluted solution. The dilute was prepared as follows: 50 g frozen silage diluted with 250 ml distilled water. This mixture was shaken by hand for about 3 minutes and stored in a fridge (5 °C) for up to 24 hours. During the cooling period, the mixture was shaken twice for about 3 minutes. After cooling down, the diluent was filtrated through Whatman no 4 paper to remove the plant matter. The supernatant was transferred to bottles and kept refrigerated until the samples were sent to the Agricultural Research Council at Irene, Pretoria for lactic analysis.

#### **4.2.7 Water soluble carbohydrates**

Water soluble carbohydrates were determined based on the phenol-sulphuric acid method of Dubois *et al.* (1956). The WSC were determined on 40 g of frozen sample diluted with 360 ml of distilled water which was homogenized for 4 minutes with a bamix and filtrated through a Whatman no 1 to remove the plant material. The pH of the supernatant was measured.

A 1 ml supernatant was diluted with 9 ml distilled water (solution A). Exactly 1 ml of solution A was pipetted and diluted with 9 ml distilled water (solution B), giving a 1:1000 solution. One ml of solution B was placed in a test-tube as well as 1 ml distilled water in another test-tube, which served as the blanko. Phenol (80%) 0.15 ml was pipetted to the 1 ml of solution B and vortexed 10 seconds. A 5 ml sulphuric acid 98% (H<sub>2</sub>SO<sub>4</sub>) was placed in the middle of the latter solution and vortexed another 10 seconds. It was read on the spectrophotometer after a waiting period of 30 minutes. The amount of WSC was determined by referring to a standard curve which was constructed for the particular sugar under examination.

#### **4.2.8 Volatile fatty acids**

Volatile fatty acids were determined at Nutrilab, University of Pretoria, South Africa, using a Gas Chromatograph (Varian 3300 FID Detector Gas Chromatograph, Varian Associates, Inc. 1985,

United States of America, Column: CP Wax 58 (FFAP)CB Cat no 7654 25 m, 0.53 mm, 2.0  $\mu\text{m}$ ) according to the method of Webb *et al.* (1994) and Suzuki & Lund (1980). About 50 g of sample was used and diluted with 200 ml distilled water. This mixture was shaken on a horizontal shaker at 180 rpm for 6 hours and filtrated through four layers of cheesecloth to remove the plant matter. The supernatant was transferred to bottles and centrifuged at 4500 rpm for 20 minutes in a cooled chamber and filtrated through a Cameo 30 (0.45  $\mu\text{m}$ ) filters. About 1  $\mu\text{l}$  sample was injected into the gas chromatograph and the standard was repeatedly injected until consecutive results were comparable. The following conditions were maintained for the gas chromatograph:

Initial column temperature 50  $^{\circ}\text{C}$ ; initial column hold time 2.00  $^{\circ}\text{C}$ ; final column temp 190 $^{\circ}\text{C}$ ; column rate in  $^{\circ}/\text{min}$  15; end time 16.33 min; injector temperature 250  $^{\circ}\text{C}$ ; detector temperature 260  $^{\circ}\text{C}$ .

#### **4.2.9 Ammonia nitrogen**

Ammonia nitrogen of silage was determined by homogenizing 50 g of silage in 250 ml of 0.1 N  $\text{H}_2\text{SO}_4$  solution for three minutes with a bamix. The homogenate was filtrated through a Whatman no 4 filter paper. The ammonia content in the filtrate was determined by distillation using a Buchi 342 apparatus and a Metröhlm 655 Dosimat with an E526 titrator according to Pearson & Muslemuddin (1968).



#### **4.2.10 Measurement of yeasts and moulds**

Measurement of yeast and moulds were done by the Department of Microbiology, University of Stellenbosch. About 40 g silage was used and diluted with 360 ml distilled water per sample. This is a 1:10 dilution. The dilution was spread out on three different agars namely: yeast morphological media, 1/2 potato dextrose agar and potato dextrose agar. After 10 days the yeast and moulds were counted.

#### **4.2.11 In vitro degradability**

*In vitro* degradability was done according the method of Van Soest & Robertson (1985). Dried silage samples that were milled through a 1 mm screen (Scientific RSA, Hammer mill, Ser No 372) were weighed into F57 Ankom bags. About 0.5 g of material was weighed into each bag and heat-sealed.

A medium containing distilled water, macro-mineral solution, buffer solution, tryptose, micro-mineral solution and rezasurin was prepared and warmed to about 39 °C in a water bath. Reducing solution containing distilled water, potassium hydroxide pellets, cysteine-HCL and sodium sulphite nonahydrate was prepared prior to rumen collection.

Rumen fluid was collected in the morning (09:30 am) from two cannulated Holstein cows that were fed a diet of oat hay, lucerne, wheat straw and 19% protein concentrate mixture twice a day in the morning at 07:30 am and in the afternoon at 17:00 pm. Rumen fluid was collected and squeezed through two layers of pre-warmed cheese cloth into warmed 39 °C thermo flasks. Rumen fluid pH was measured before and after blending was done at low speed for 15-20 seconds. Blended rumen fluid was strained twice through two layers of pre-warmed cheese cloth into a thermos flask while gassing with CO<sub>2</sub>.



About 1 076 ml of medium (macro-minerals + buffer + micro-mineral + rezasurin) and 54 ml reducing solution were transferred into each incubation vessel while gassing with CO<sub>2</sub> and placed in an incubator (Daisy<sup>II</sup>, Ankom technology, Fairport, New York) set at 39 °C. When the medium was fully reduced (cleared), about 270 ml of rumen fluid was added to each incubation vessel and CO<sub>2</sub> was used maintaining anaerobic conditions. Samples were added into each vessel. The incubation period was 48 hours. Afterwards incubation samples were rinsed in cold water and air-dried. NDF analysis (Van Soest *et al.*, 1991) was done on air-dried samples; the samples were subsequently ashed in a muffle furnace at 500 °C for 6 hours to determine ash content.

### 4.3 Experimental design

The experiment was a complete randomized design with one treatment: Inoculant Lalsil® Cereal (*L. buchneri* (NCIMB 40788) and *P. acidilactici* (CNCM MA 18/5M)) was the only factor. Material for ensiling was divided into two groups and one group was randomly allocated for treatment. No inoculant was added to the control crop.

### 4.4 Statistical analysis

Data were analysed using a one-way ANOVA in SAS statistical software, SAS Institute, Inc. (1999), SAS/STAT User's Guide, Version 9, 1<sup>st</sup> printing, Volume 2. (SAS Institute Inc, SAS Campus Drive, Cary, North Carolina 27513.) Difference between means was tested using GLM procedure Least squares means.



## 4.5 Results and discussion

### *Anaerobic Phase*

#### **4.5.1 Chemical composition of harvested whole-crop oats at day 0 of ensiling**

The chemical composition, pH levels and *in vitro* degradability of whole-crop oats and ensiled in the bunker are presented in Table 4.1.

**Table 4.1** Chemical profile and degradability of whole-crop oats at point of ensiling (day 0).

	Day 0			
	Control	Inoculant	SEM	P-Value
<b><u>Descriptive parameters</u></b>				
Dry matter (g/kg)	461.0	455.0	3.30	0.320
Organic matter (g/kg DM)	964.0	963.0	0.60	0.201
aNDF <sub>om</sub> (g/kg DM)	551.0	553.0	2.60	0.658
Crude protein (g/kg DM)	96.8	99.3	1.40	0.293
<b><u>Fermentation and degradability</u></b>				
pH	5.80	5.82	0.024	0.593
WSC (% DM)	9.26	11.36	2.940	0.639
Lactic acid (% DM)	0.27	0.27	0.014	0.872
<i>In vitro</i> organic matter degradability (%)	56.70	56.40	0.300	0.628

SEM = Standard Error of Means; WSC = Water Soluble Carbohydrates; aNDF<sub>OM</sub> = Neutral detergent fibre in Organic Matter\*

The DM content of harvested oats was about 46% and hence had low risk of spoiling. Initial CP contents of the ensiled material were 96.8 g/kg and 99.3 g/kg for control and inoculant-treated silage, respectively. There was a difference between the WSC levels of the control and inoculated material, though not significant probably due to inconsistency in the sampled material. *In vitro* degradability of oat silage was less than 60% but comparable to that of mature tropical grasses such as *Pennisetum clandestinum*.

#### 4.5.2 Chemical composition, fermentation and *in vitro* degradability at 186 days

The chemical composition, fermentation and *in vitro* degradability of oat silage ensiled in the bunker for 186 days are given in Table 4.2.

**Table 4.2** Chemical composition, volatile fatty acids, ammonia nitrogen and digestibility of whole-crop oats at 186 days of ensiling.

	Day 186			
	Control	Inoculant	SEM	P-Value
<b><u>Descriptive parameters</u></b>				
Dry matter (g/kg)	430.0	442.0	2.60	0.007
Organic matter (g/kg DM)	958.0	961.0	1.60	0.258
aNDFom (g/kg DM)	577.0	540.0	6.60	0.003
Crude protein (g/kg DM)	108.0	109.0	0.90	0.420
<b><u>Fermentation and degradability</u></b>				
pH	4.03	3.91	0.007	<0.01
Lactic acid (% DM)	4.44	4.49	0.180	0.853
WSC (% DM)	6.09	6.77	0.696	0.504
Volatile fatty acids (% DM)	1.76	0.93	0.048	<0.01
Acetic acid (% DM)	1.74	0.91	0.047	<0.01
Propionic acid (% DM)	0.018	0.012	0.048	<0.01
Ammonia nitrogen (% of total nitrogen)	5.65	5.39	0.389	0.651
<i>In vitro</i> organic matter degradability (%)	58.70	59.00	0.900	0.795

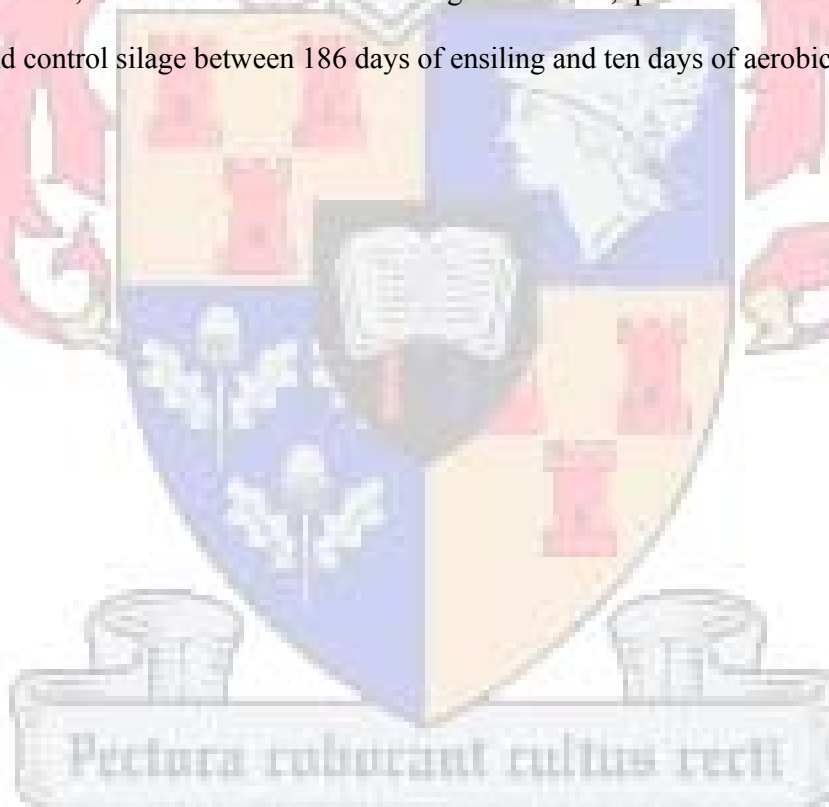
SEM = Standard Error of Means; WSC = Water Soluble Carbohydrates; NDF<sub>OM</sub> = Neutral detergent fibre in Organic Matter\*

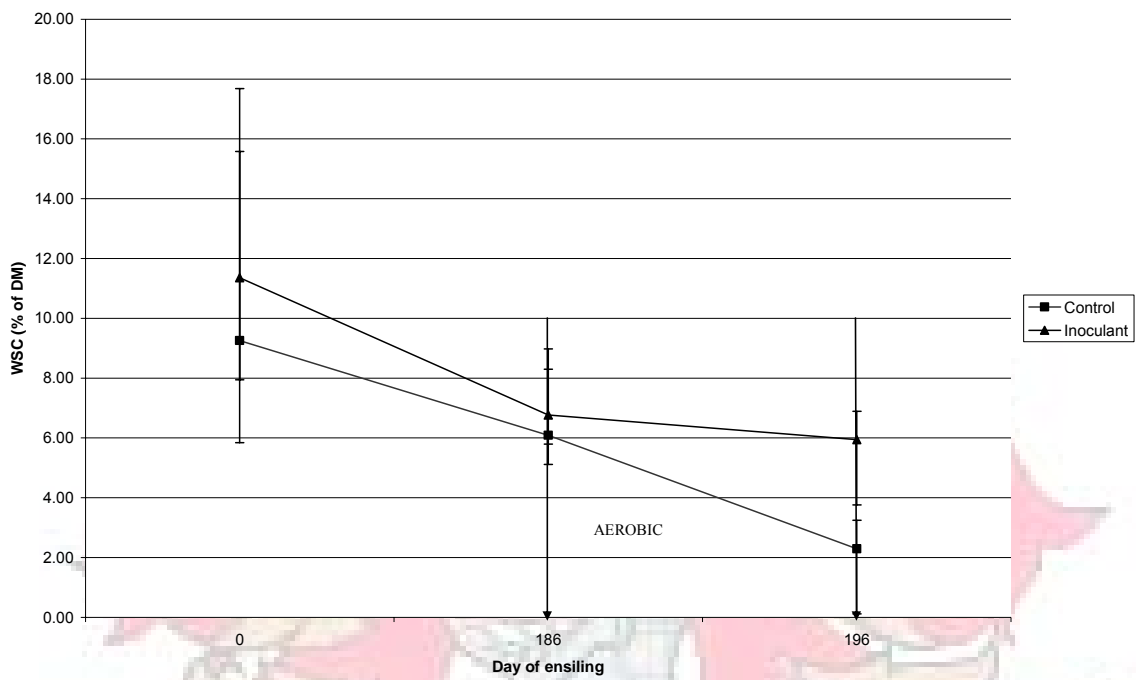
The pH value for both the control and inoculant silage dropped significantly from a pH value of 5.8 to 4.0 from day 0 to day 186 of ensiling. This indicates that LAB fermented carbohydrates to lactic acid. Lactic acid increases hydrogen ion (H<sup>+</sup>) concentration inhibiting growth of undesirable bacteria such as *C. butyricum*, *C. tyrobutyricum*, *Escherichia coli* and *Erwinia herbicola* (McDonald *et al.*, 2002) and is therefore desirable as a silage preservative. Clostridia are present on harvested forage in the form of spores and start to multiply as soon as conditions in the silo become anaerobic. The growth of these organisms is undesirable; they produce butyric acid and degrade amino acids to a variety of products which degrade the quality of silage (McDonald *et al.*,

1991). Optimal pH for preservation however depends on the DM content of the silage; at 44% DM content pH of 5.02 would be optimal for silage preservation (ED d'H d' Yvoy & Meeske, 1999). The pH at 186 days of ensiling averaged 4.0 in treated and untreated silage. The pH level of silage was less than the optimal value of five and could have a negative effect on palatability. Dry matter content was 43.0% and 44.2% for control and treated silage, respectively.

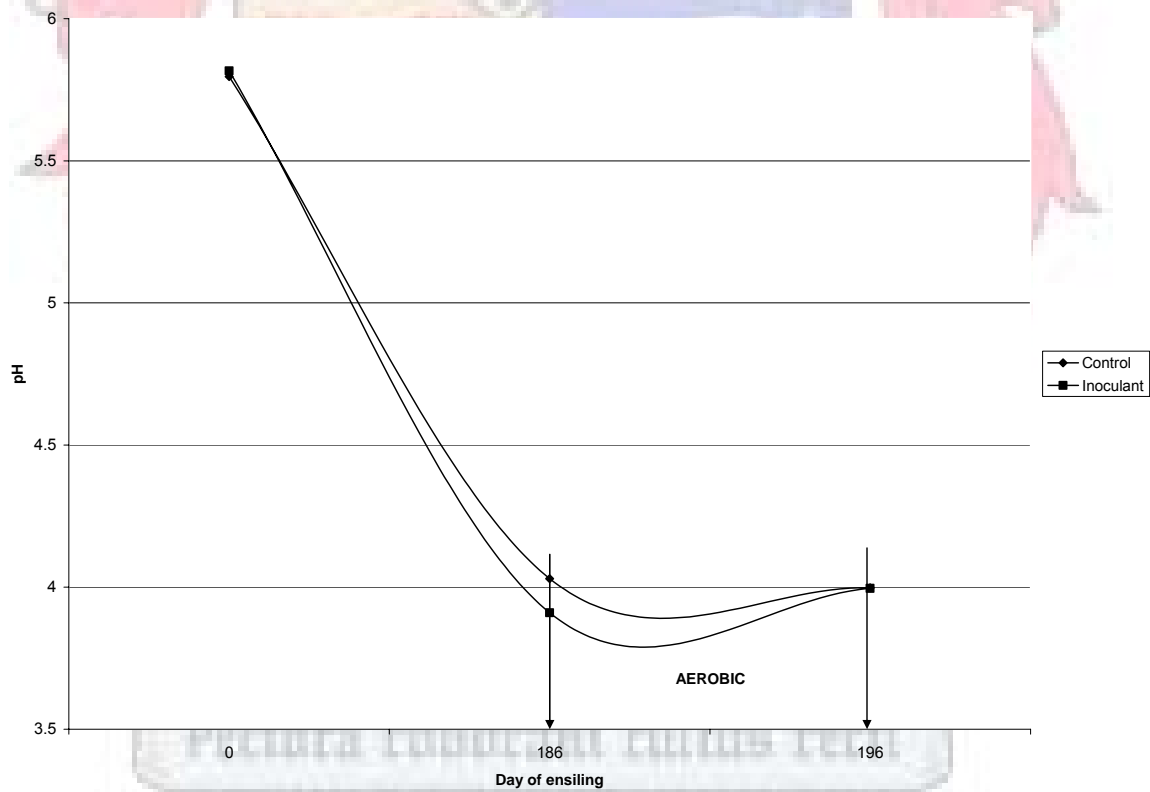
The CP content at day 186 of ensiling averaged 109 g/kg DM, a minimum of 75 g/kg DM CP is required for meeting ruminal microbial requirements (Ørskov, 1992). The drop in WSC of untreated silage was 3.2% units (about 30% WSC consumption) compared to 5% unit drop in inoculum-treated silage (50% WSC consumption). These results are anomalous. Levels of ammonia nitrogen (% of total nitrogen) in the oat silage were almost the same ( $\approx 5.5\%$ ), which is lower compared to that normally reported for maize silage ( $\approx 6.31\%$ ) (Borreani *et al.*, 2007). This indicates less protein degradation, which is desirable.

Figures 4.1, 4.2 and 4.3 illustrate changes in WSC, pH and lactic acid contents of inoculated and control silage between 186 days of ensiling and ten days of aerobic exposure.

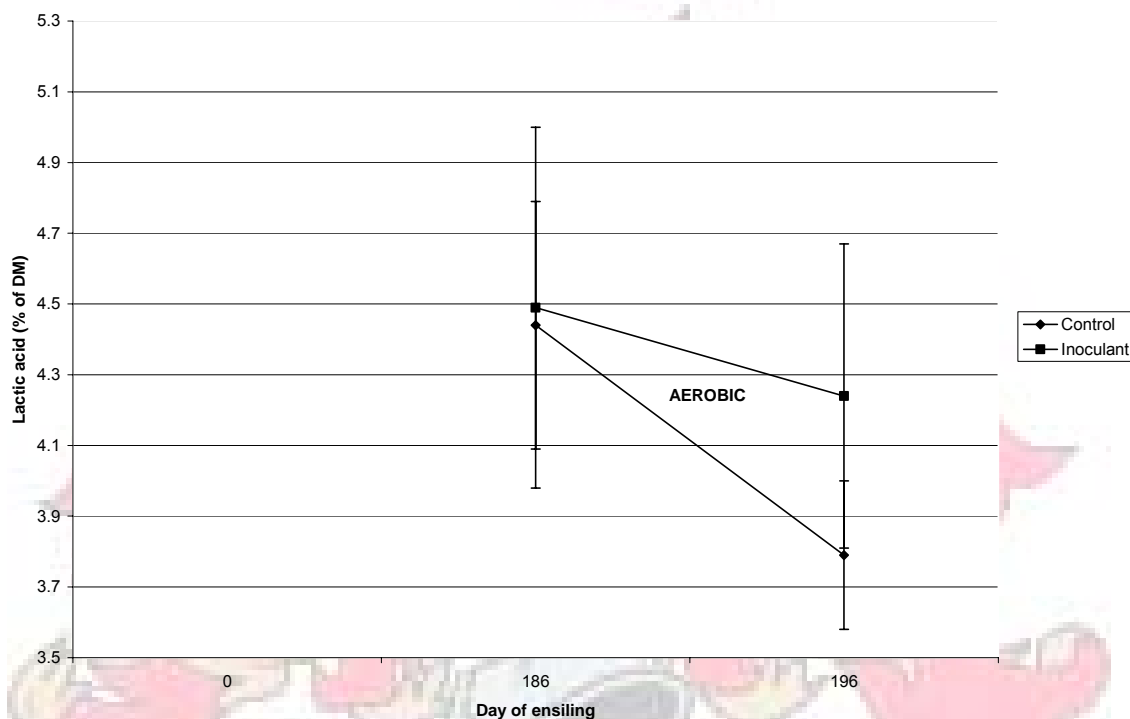




**Figure 4.1** The change in water soluble carbohydrates of oat silage ensiled with or without a lactic acid bacterial inoculant after 186 days of ensiling.



**Figure 4.2** The change in pH of oat silage ensiled with or without a lactic acid bacterial inoculant.



**Figure 4.3** The change in lactic acid of oat silage ensiled with or without a lactic acid bacterial inoculant.

#### *Aerobic Study*

#### **4.5.3 Aerobic stability at 196 days**

The chemical composition of whole-crop oat silage exposed to aerobic conditions for ten days after being ensiled for 186 days is given in Table 4.3.



**Table 4.3** Profile of whole-crop oat silage exposed to aerobic conditions for 10 days after ensiling, period of 186 days.

	Day 196			
	Control	Inoculant	SEM	P-Value
<b><u>Descriptive parameters</u></b>				
Dry matter (g/kg)	487.0	487.0	7.60	0.957
Organic matter (g/kg DM)	959.0	960.0	1.40	0.588
aNDFom (g/kg DM)	562.0	548.0	8.20	0.237
<b><u>Fermentation and degradability</u></b>				
pH	4.00	4.00	0.010	0.932
Lactic acid (% DM)	3.79	4.24	0.110	0.024
WSC (% DM)	2.29	5.94	0.660	0.003
<i>In vitro</i> organic matter degradability (%)	58.10	58.30	1.570	0.935
Carbon dioxide (g/kg DM)	0.47	0.40	0.140	0.719

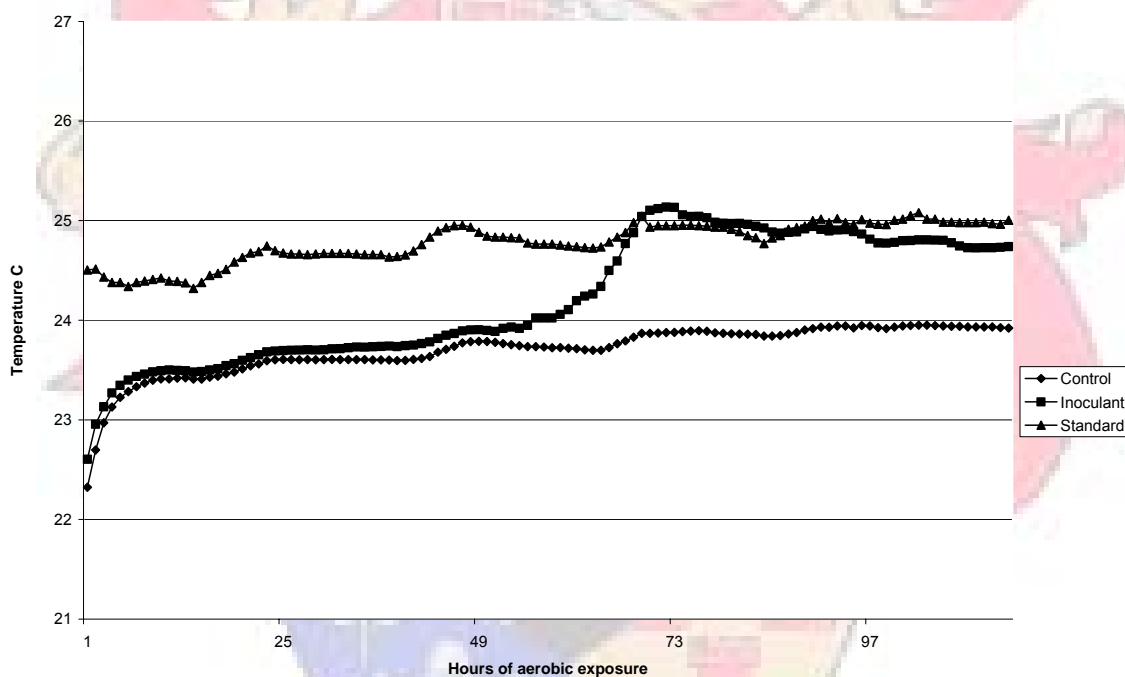
SEM = Standard Error of Means; WSC = Water Soluble Carbohydrates; NDF<sub>OM</sub> = Neutral detergent fibre in Organic Matter\*

After ten days of aerobic exposure the moisture content of silage decreased slightly upon aerobic exposure ( $P > 0.05$ ). The pH of inoculant-treated silage increased slightly after aerobic exposure (Fig 4.1); indicating that the treated silage was not stable with a greater portion of the WSC degrading to lactic acid ( $P > 0.05$ ). Depletion of lactate by fungi, yeast and *Bacillus* during the aerobic phase reduces the potential stability of silage as lactate is converted to acetate or butyric acid with a consequent pH rise (Lindgren *et al.*, 1985). Water soluble carbohydrates of the control silage dropped significantly after 10 days of aerobic exposure but remained the same for inoculant-treated silage, which means the inoculant could probably have an effect. Carbon dioxide was high in the control crop, although not significantly different. This indicates that the sugars are being metabolised by aerobic microbes that yield CO<sub>2</sub> as by-product. Lactic acid concentration for both control and inoculant-treated silage dropped slightly, which points to a reduction in growth and activity of lactic acid microbes, hence keeping the silage stable.

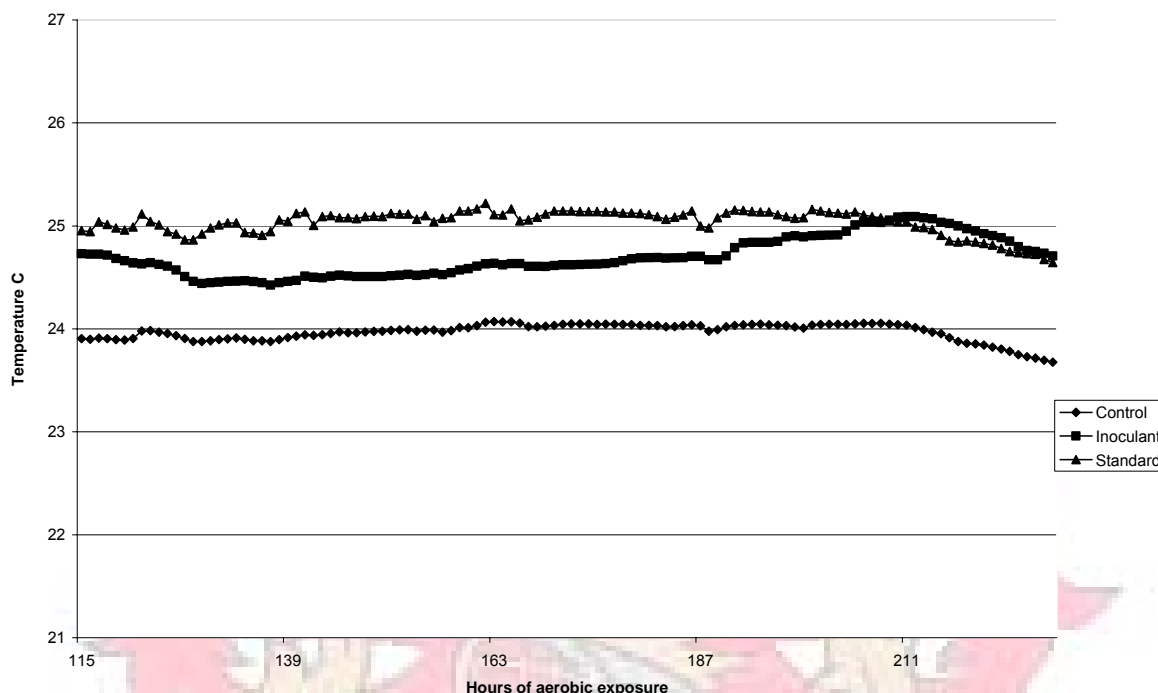
Reviews on silage additives (McDonald *et al.*, 1991; Lindgren, 1999; Weinberg & Muck, 1996; Vilela, 1998) show that effectiveness of inoculums varies with forage type and ensiling

conditions. Coan *et al.* (2001) found that enzymatic-bacterial inoculants did not improve the quality, fermentation and nutritional characteristics of different guinea grass (*Panicum maximum*) varieties (Tanzania or Mombaca) and the effect of the regrowth stage (45 or 60 days) was not significant. Nussio *et al.*, (2001) evaluated the use of enzymatic-bacterial inoculant in Tifton 85 (*Cynodon dactylon*) silage varying in DM content (25 to 65%) and they reported positive results from the use of inoculant in silage of higher DM content (>45%).

It seems that the inoculated (Lalsil® Cereal) silage made in micro silo's was more stable under aerobic conditions compared to inoculated silage made in a bunker due to the fact that the environment in the micro-silos was easier to control.



**Figure 4.4** Changes in temperature for control and inoculated oat silage during the first 114 hours of aerobic exposure after 186 days of ensiling.



**Figure 4.5** Changes in temperature for control and inoculated oat silage during the last 114 hours of aerobic exposure after 186 days of ensiling.

## 4.6 Conclusion

Both inoculated and control silage batches were well preserved, control pH of 4.03 and inoculant pH of 3.91. Lalsil® Cereal however, reduced the consumption of WSC during the aerobic exposure confirming the results reported in Chapter 3. This indicates that the silage was well preserved and that the inoculant could not improve the silage quality. Further studies are recommended to confirm these findings. Research using other inoculants such as Lalsil Dry® is also recommended for ensiling forages that have a lower moisture content.



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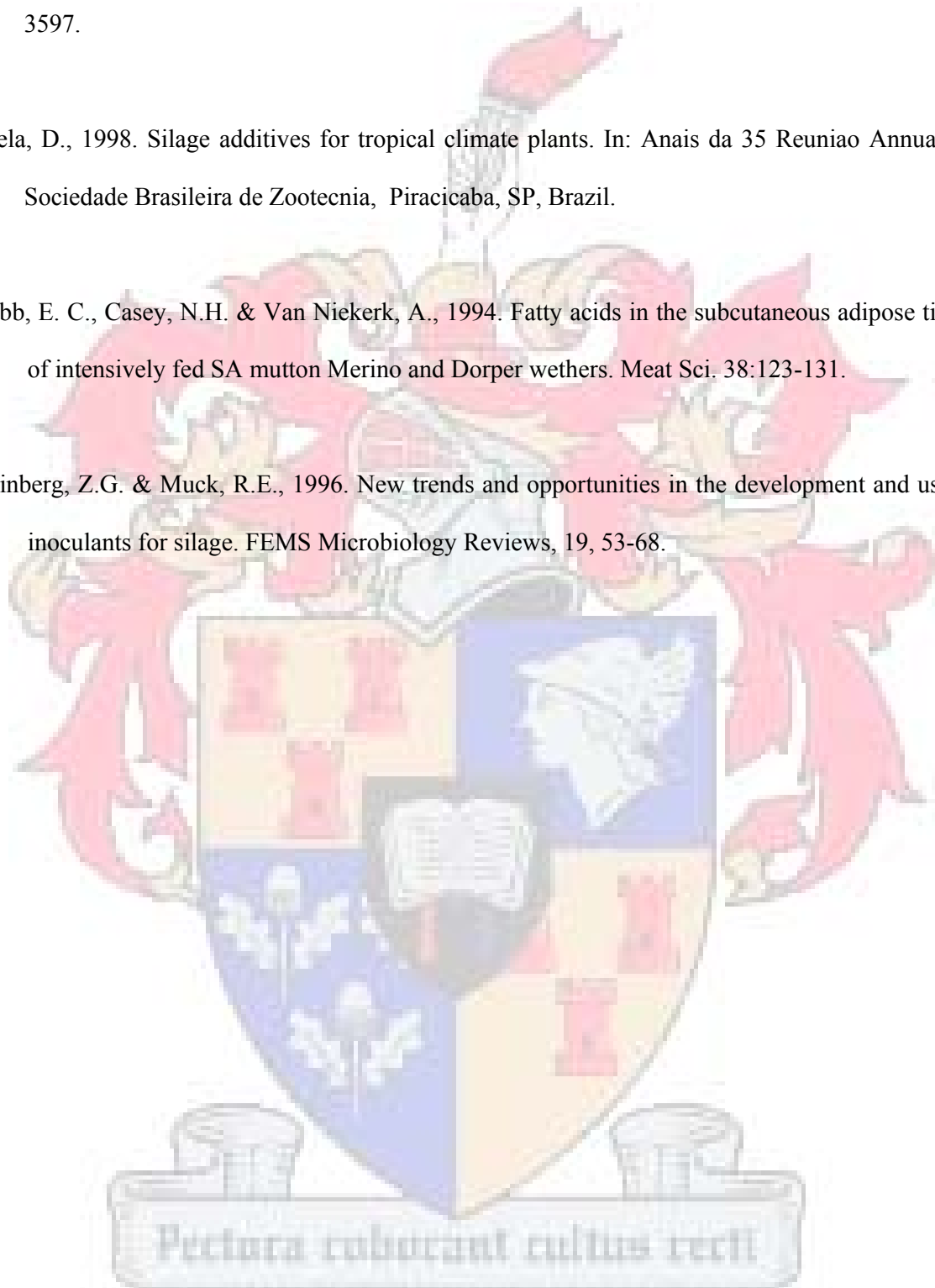
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## CHAPTER 5

### GENERAL CONCLUSION

Oat silage is an important source of fibre and energy for dairy cows especially in the Western Cape, South Africa. Successful ensiling depends mainly on the quantity of fermentable WSC available and DM content. High WSC content of oats (in particular before grain formation) allows the making of good quality silage, particularly in combination with preliminary wilting. However, silage spoilage is a major source of concern resulting in significant forage and energy loss. Preservation is important in achieving consistency in feed supply in those seasons when forage availability is a major source of concern especially in the summer months when outdoor temperatures range between 30 °C and 40 °C.

Both the control and inoculated silages were well preserved as indicated by a low pH. Dry matter content of ensiled material was high (43%). Adding a LAB containing inoculant (Lalsil® Cereal) did not appear to improve the quality of silage. Although on the fermentation side the drop of pH from day 0 of ensiling to 186 days of ensiling in the bunker was significant for the inoculant. The inoculant Lalsil® Cereal reduced consumption of WSC during the anaerobic phase and aerobic exposure for both studies, as sugars were metabolized for energy and CO<sub>2</sub> production. When the silage was exposed to aerobic conditions for 10 days lactic acid levels dropped from day 102 of fermentation to day 112 of fermentation indicating a decrease in population of LAB. We can conclude that the inoculant Lalsil® Cereal was not effective as a silage additive on oat silage with a high DM content in this particular study.

Adding a different inoculant (Lalsil® Dry specific for dry silage) could have a positive effect on silage quality. The concentration of Lalsil® Cereal used on the silage was medium, but using higher concentration levels could have a positive effect.

Further studies are recommended on the same inoculant but with different DM levels.