EFFECTS OF INOCULATION ON ALFALFA SILAGE QUALITY AND ITS FEEDING ON THE PERFORMANCE OF DAIRY CATTLE

By

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Abstract

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EFFECTS OF INOCULATION ON ALFALFA SILAGE QUALITY AND ITS FEEDING ON THE PERFORMANCE OF DAIRY CATTLE

Five studies were conducted to determine the effect of inoculating (Pioneer Sila-Bac 11H50®) on the silage quality and the feeding value of high DM (55.3 %) alfalfa silage. The inoculant contained multi strains of *Lactobacillus plantarum* developed by Pioneer Hi-Bred Inc. In the first study, the effects of inoculation on ensiling characteristics of alfalfa were determined in a completely randomized design using 30 mini-silos. In the 2nd study, 9 containers were used in a completely randomized design to determine the effect of inoculation on the aerobic stability of alfalfa silage. In the 3d study, 2 ruminally fistulated cows were used in a randomized complete block design to determine the effects of inoculation on ruminal degradation of alfalfa silage. In the 4th study, 4 ruminally fistulated cows were used in a switch back experiment to determine the effects of feeding inoculated alfalfa on total tract nutrient utilization. In the last study, 27 Holstein cows in early lactation were used in a randomized complete block design to determine the effects of feeding inoculated alfalfa on intake, milk yield, and milk composition. Results showed that inoculation improved fermentation in the mini silos in terms of rate of acidification, and lactic acid production. Inoculation increased proteolysis as indicated by a reduction in true protein and an increase in non-protein nitrogen (NPN). However after 45 days of ensiling, differences in true protein between the two silage treatments were minimal. When the silages were exposed to air, inoculation significantly reduced the rise in pH ($P<0.05$), and the inoculated silage remained heat stable during the 21 days experiment. Treatment with Sila-Bac 11H50® had no effect on ruminal degradability of the silage. Feeding inoculated alfalfa did not affect total tract digestibility of the total mixed diet. Results from the dairy study showed that feeding inoculated relative to untreated alfalfa silage had no effect on intake, milk yield or milk composition. It was concluded that the inoculant used in this study improved the ensiling characteristics of alfalfa silage with no significant effects on dairy cow performance.

Resumé

Maîtrise en science Science animale (Nutrition)

Charbel Rizk

LES EFFETS DE L'INOCULATION DE LA LUCERNE SUR LA QUALITE DE L'ENSILAGE ET SUR LA PERFORMANCE DES VACHES LAITIÈRES

Nos objectifs étaient de déterminer les effets de l'inoculation de la Lucerne (55.3 % de matière sèche) avec Sila-Bac 11H50® sur la qualité de l'ensilage et sa valeur nutritive. L'inoculant contenait plusieurs souches de *Lactobacillus plantarum* développées par Pioneer Hi-Bred Inc. Dans une première étude, 30 minis silos ont été utilisés dans un plan aléatoire complet pour déterminer les effets de l'inoculation sur la fermentation de la Lucerne. Dans une deuxième étude, 9 contenants ont été utilisés dans un plan aléatoire complet pour déterminer les effets de l'inoculation sur la stabilité aerobique de l'ensilage. Dans une troisième étude, deux vaches avec une fistule ruminale ont été utilisées dans un plan en bloc aléatoire complets pour déterminer les effets de l'inoculation sur la dégradation ruminale de l'ensilage. Dans une quatrième étude, quatre vaches avec une fistule ruminale ont été utilisées dans un chassé-croisé afin de déterminer les effets des rations sur la nutrition des aliments. Dans une dernière, 27 vaches en début de lactation ont été utilisées dans un plan en bloc aléatoire complet pour déterminer les effets des rations sur la consommation des vaches, la production et la composition du lait. Les résultats ont démontré que l'inoculation a favorisé la fermentation dans les minis silos en augmentant le taux d'acidification et la production d'acide lactique. L'inoculation a augmenté la protéolyse indiquée par une réduction en protéine vraie et une augmentation en azote non protéique. Toutefois après 45 jours de fermentation, la différence en protéine véritable entre les deux ensilages était minimale. Quand les ensilages ont été expose à l'air, l'inoculation a ralentit l'élévation du pH ($P<0.05$) et l'ensilage inoculé a gardé une température stable durant les 21 jours de l'expérience. L'application du Sila-Bac 11H50® n'a eu aucun effet sur la dégradation ruminale de l'ensilage et l'incorporation de l'ensilage inoculé n'a pas affecté la nutrition de la ration. Les résultats de la dernière expérience ont montré que la ration contenant l'ensilage inoculé n'a eu aucun effet sur la consommation, la production et la composition du lait. Il a été conclut

que l'inoculation a augmente le taux d'acidification dans les minis silos sans améliorer la performance des vaches laitières.

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Contribution of Authors

In accordance to McGill thesis submission guidelines, this is a manuscript-based thesis and includes a table of contents, a brief abstract in both English and French, an introduction, a comprehensive review of literature, a final conclusion and summary, a thorough bibliography and appendices where appropriate.

Part of this thesis has been sent for publication in the Archives of Animal Nutrition (C. Rizk, A. F. Mustafa and L. E. Phillip. 2004. Effects of inoculation of high DM alfalfa silage on ensiling characteristics, ruminant nutrient degradability and dairy cow performance. This manuscript was coauthored by Dr. A. F Mustafa who contributed to the design of the experiment and proof reading of the manuscript and Dr. L. E. Phillip who contributed to the proof reading of the manuscript.

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1 Introduction

In most ruminant production systems, livestock derive 40 to 90% of their nutritional requirements from forages (Charmley, 2001). Therefore the successful storage of forages during the season of abundance for later animal consumption is a crucial matter. Forages are stored in two possible forms, either as hay or as silage. Hay is formed when forage crops are dried under 20% dry matter (DM; Dwain and Valentine, 1999), whereas silage is formed when herbage or other material with sufficient moisture is stored anaerobically in a confined structure (silo). During ensiling, epiphytic bacteria produce acids (mainly lactic acid), which lower the pH and stabilize the silage.

Lactic acid bacteria inoculant could be added to the epiphytic microorganisms to support the fermentation. The activity of bacteria inoculants used in silage making is largely dependent of moisture content of the ensiled forages (Whiter and Kung, 2001). The term water activity is used to more accurately describe the amount of moisture available for microbial growth during ensiling (Albert et al., 1989). Forages with high DM content (i.e. low water activity) ferment at slower rates than forages with low DM content due to the fact that low water activity reduced microbial growth (Whiter and Kung, 2001; Hristov and McAllister, 2002).

Alfalfa is the main legume forage in eastern Canada. The forage is usually wilted to DM higher than 30% to reduce the risk of clostridial fermentation (Whiter and Kung, 2001). High DM alfalfa (54% DM) treated with a single strain of *Lactobacillus plantarum* had a lower pH and a higher lactic acid concentration than untreated alfalfa after 2 d of ensiling Whiter and Kung, 2001). However, effects of inoculants containing more than one strain of lactic acid bacteria on fermentation of high DM alfalfa have not been determined. Furthermore, information on the effects of such inoculants on animal performance is not available.

The objectives of this study were to determine the effects of a multi strain of *Lactobacillus plantarum* inoculant (Sila-Bac 11H50®) on:

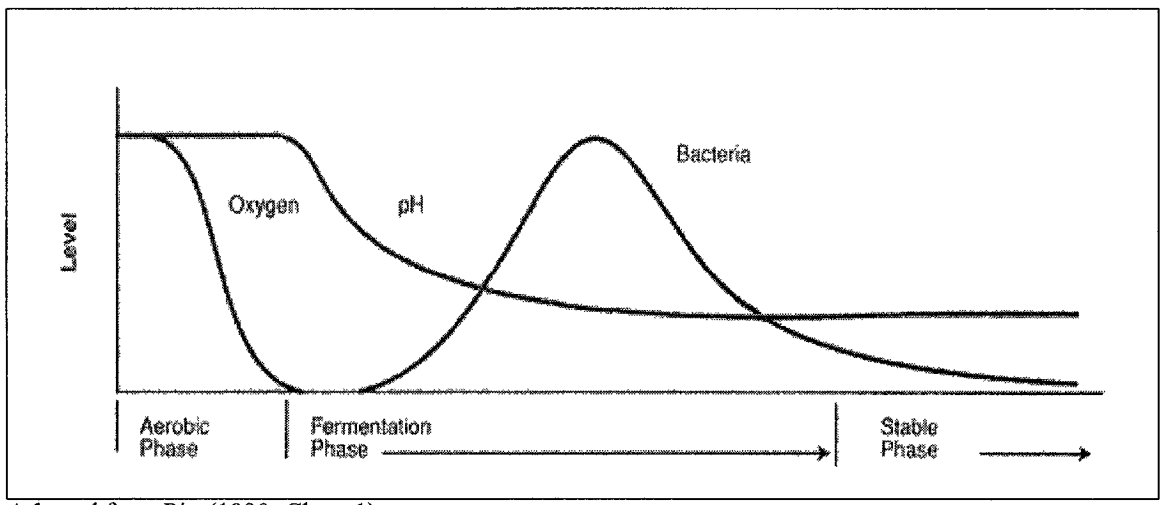
- The ensiling characteristics of high DM alfalfa silage.
- Aerobic stability of high DM alfalfa silage.
- Ruminal degradability of high DM alfalfa silage.
- To determine the effect of feeding inoculated high dry matter alfalfa silage on total tract nutrient digestibility of dairy cows.
- To determine the effect of feeding inoculated high dry matter alfalfa silage on dry matter intake, milk yield and milk composition of dairy cows.

2 Literature Review

2.1 Silage fermentation

The basis of silage preservation is to place plant materials in an aerobic environment so that lactic acid bacteria (LAB) can produce enough acid (preferably lactic acid) to drop the pH to 4.0-4.5 (McDonald, 1991, Chap. 1). This will lower plant respiration and enzymatic reactions, and inhibit undesirable microbial reactions. The fermentation process involves several physical and chemical reactions. The major phases of the ensiling process include aerobic phase, fermentation phase, stable phase and feed out phase (Figure 2.1).

Figure 2.1 – Aerobic, fermentation and stable phase



Adapted from Pitt (1990; Chap. 1)

2.1.1 Aerobic phase

The aerobic phase of ensiling begins at harvest, continues during wilting and for several hours after filling the silos until the oxygen is depleted. At this stage, pH and oxygen are at their highest levels. Plant respiratory enzymes catabolize sugars to yield CO_2, H_2O and energy resulting in dry matter and nutrient losses (McDonald, 1991, Chap. 1). Plant proteases and amino acidases catabolize true protein, which will result in increased levels

of non-protein nitrogen (Ohyama, 1970). Heating may further reduces protein availability via Maillard reaction. Factors affecting the aerobic phase of ensiling include O_2 and CO_2 concentrations, temperature, pH, and dry matter content (Table 2.1, Pitt et al., 1985).

Table 2.1 – Factors affecting aerobic phase of ensiling

Oxygen	Elongates the aerobic phase
Carbon dioxide	Inhibits respiration, proteases, amino acidases
Temperature	Stimulates respiration, proteases, amino acidases May inactivate enzymes if it is too high
PH	Low pH Inhibits respiration, proteases, amino acidases
Dry matter	Controversial

Adapted from Fairbairn (1983)

2.1.2 Fermentation phase

At this stage oxygen is depleted and CO_2 accumulates. Plant enzymes as well as aerobic organisms may be inhibited. Aerobic microorganisms are replaced by anaerobic and facultative microbes. Three main types of bacteria can alternate and dominate the fermentation process resulting in different types of fermentation (Table 2.2).

Table 2.2 – Major types of bacteria during fermentation phase

Bacteria	**Major organic acids**	**pH**	**Proteolysis**	**Silage conservation**
Enterobacteriaceae	Acetic, propionic acid	Intermediate	Intermediate	Intermediate, toxins
Lactic acid bacteria	Lactic, acetic acid	Low	Low	Good
Clostridia	Butyric acid	High	High	Bad, toxins

Adapted from McDonald et al. (1991, Chap. 4)

In a normal desired fermentation, LAB dominates other microorganisms as they produce lactic acid from water-soluble carbohydrates. The accumulation of lactic acid is the main reason behind the rapid decrease of silage pH to 4.0-4.5 (Figure 2.2 and 2.3; Winters et al., 2000). The fermentation phase can last for up to four weeks (Mustafa and Seguin, 2003c) and during this phase temperature, O_2 and pH will be at their lowest.

Figure 2.2 – Changes in pH during ensiling

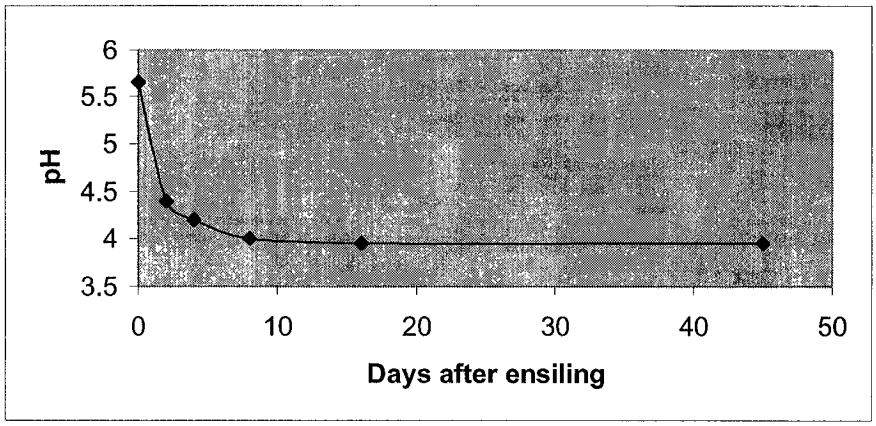
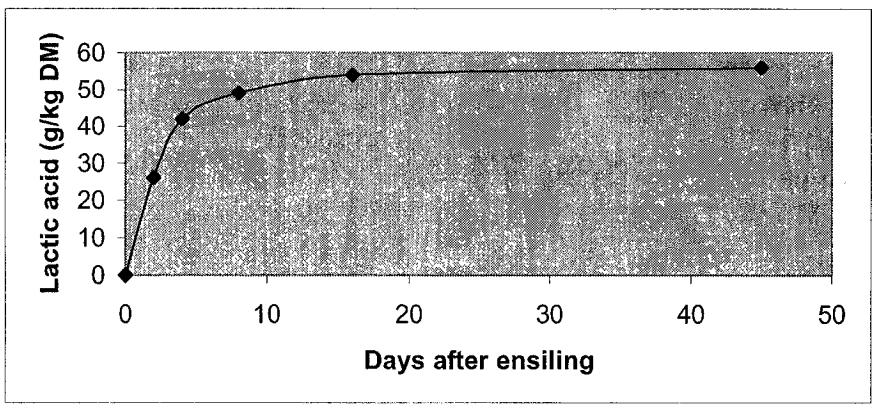


Figure 2.3 – Changes in lactic acid concentration during ensiling



Adapted from Mustafa and Seguin (2003c)

Undesirable fermentation by enterobacteria and clostridia can occur if the pH does not drop rapidly. These conditions are most likely to occur when the forage is relatively low in LAB and water-soluble carbohydrates, high in buffering capacity, or too wet (McDonald et al., 1991, Chap. 4). Improper management practices could also lead to bad fermentation. If the silo is not sealed rapidly, aerobic organisms such as yeasts will break down the lactic acid as it is being produced, pH will not decrease and clostridial fermentation is most likely to occur (McDonald et al., 1991, Chap. 5).

2.1.3 Stable phase

At this stage, pH has dropped to approximately 4.0 to 4.5, all microorganisms found in the silage are inhibited including the LAB and most of metabolic activity has stopped. This phase will last until the silo is opened and silage is exposed to air.

2.1.4 Feed out phase

This stage begins when the silage is exposed to air. In the presence of oxygen, molds and yeasts will eventually metabolize organic sugars and acids (including lactic acid). Temperature and pH will gradually increase allowing other microorganism to grow and spoil the silage (Woolford, 1984, Chap. 3)

2.2 End products of silage fermentation

The end products of fermentation reflect the type of microorganisms that dominate during the ensiling process and can be used to assess the quality of the silage. Lactate is an indicator of fermentation by homofermentative lactic acid bacteria (^{Ho}LAB). A Mixture of lactate and acetate indicate heterofermentative lactic acid bacteria (^{Het}LAB) dominance whereas butyrate and ammonia reflect undesirable clostridial activity (McDonald et al., 1991, Chap. 4). Ammonia N can also arise from the action of plant enzymes and enterobacteria and the reduction of nitrates and nitrites (McDonald et al., 1991, Chap. 4).

Silage microorganisms catabolize water-soluble carbohydrates mostly to organic acids and some alcohol. The main sugars present in the water-soluble carbohydrates fraction of legume forages are fructose, glucose and sucrose (McDonald et al., 1991, Chap. 4). Possible fermentation end products of different sugars by different types of bacteria are shown in Table 2.3.

Table 2.3 – Some theoretical fermentation end products of sugars

Micro-organism	Substrate	End product
Homofermentative Lactic Acid Bacteria (ex. L. Plantarum)	1 glucose	2 lactate
	1 fructose	2 lactate
	1 pentose (xylose, arabinose)	1 lactate + 1 acetate
Heterofermentative Lactic Acid Bacteria (ex. L. Buchneri)	1 glucose	1 lactate + 1 ethanol + 1 CO_2
	1 fructose	1 lactate + 1 ethanol + 1 CO_2
	3 fructose	2 mannitol + 1 lactate + 1 acetate + 1 CO_2
	1 glucose + 2 fructose	2 mannitol + 1 lactate + 1 acetate + 1 CO_2
	1 pentose	1 lactate + 1 acetate
Sacharolytic Clostridia	1 glucose	1 butyrate + 2 CO_2
	2 lactate	1 butyrate + 2 CO_2
Yeasts	1 glucose	2 ethanol + 2 CO_2

Adapted from McDonald (1991, Chap. 4)

Microorganisms are also responsible for amino acid catabolism. The ability of LAB to ferment amino acids appears to be restricted and it is believed that only two amino acids, serine and arginine are attacked by some but not all LAB. Limited amount of NH_4 can be produced from the fermentation of these two amino acids by LAB (Table 2.4). The concentration of NH_4 present in the silage is a reliable indicator of the extent of proteolytic activity of clostridia. It is produced in relatively small amounts by other silage microorganisms, plant enzymes and nitrate reduction (McDonald, 1991, Chap. 4). The effects of microorganisms on amino acid catabolism are shown in Table 2.4.

Table 2.4 – Catabolism of amino acids during fermentation

Micro-organism	**Substrate**	**End product**
Heterofermentative & Homofermentative Lactic Acid Bacteria	1 Arginine 2 Serine	1 Ornithine + 2 NH_3 + 1 CO_2 1 Acetoin + 1 NH_3
Proteolytic Clostridia	Amino acids	Amino acids + amides + fatty acids+ NH_3 + CO_2

Adapted from McDonald (1991, Chap. 4)

Homolactic fermentation is more desirable than other types of fermentation because dry matter and energy recoveries are greatest and proteolysis is minimal (Woolford, 1984, Chap. 5). Table 2.5 shows some typical values for a successful fermentation of different silages at different dry matter content.

Table 2.5 – Fermentation end products in different silages

	Silage type			
	Alfalfa silage (30-35% DM)	Alfalfa silage (45-55% DM)	Grass silage (25-35% DM)	Corn silage (35-40% DM)
pH	4.3 – 4.5	4.7 – 5.0	4.3 – 4.7	4.0 – 4.5
Lactic acid (%)	7 - 8	2 – 4	6 – 10	4 – 7
Acetic acid (%)	2 - 3	0.5 - 2.0	1 – 3	1 - 3
Propionic acid (%)	< 0.5	< 0.1	< 0.1	< 0.1
Butyric acid (%)	< 0.1	< 0.1	< 0.1	< 0.1
Ethanol (%)	0.5 – 1.0	0.5	0.5 – 1.0	1 - 3
NH_3 N (% CP)	10 - 15	< 12	8 – 12	5 – 7

Adapted from Kung (2000)

2.3 Changes in protein and fiber composition during ensiling

Ensiled forages undergo several chemical changes during ensiling. In fresh forages, true protein constitutes 75 to 90% of the total nitrogen and the remainder is non-protein nitrogen (Mustafa et al., 2000; Oshima and McDonald, 1978). After ensiling non-protein nitrogen may account for as much as 80% total nitrogen (Papadopoulos and McKersie, 1983; Albrecht and Muck, 1991; Mustafa et al., 2000). Proteolysis is extensive during the first few days of ensiling (Mustafa et al., 2002; Mustafa and Seguin, 2003a,b). The degradation of forage protein during ensiling is mediated by a group of plant enzymes collectively known as proteases and results in a reduction in true protein (TP) and an increase in non-protein nitrogen (NPN) content (Figure 2.4 and 2.5; Ohshima and McDonald, 1978; Heron and Edward, 1989).

In term of ruminal protein degradation, plant protease drastically decreases the ruminal-undegraded protein of the silage. In a survey of 35 silages, Tamminga et al. (1991) found that on average 61% of protein is instantly solubilized in the rumen, and only 9% was ruminally undegraded. Mustafa et al. (2003) reported ruminal protein degradability of more that 80% of total protein for three different cultivars of pea silage. Ruminal

undegraded protein is particularly low in alfalfa silage compared with other legume silages (Glen, 1995; Mustafa and Seguin, 2003). One reason for the low ruminal degradability of alfalfa silage protein is the fact that it is low in tannin content (Albrecht and Muck, 1991) and lacks polyphenol oxidase (Jones et al., 1995). These metabolites reduce rate of proteolysis during ensiling and therefore reduce ruminal degradability of some legume forages such as clover silages (Broderick, 1995; Mustafa and Seguin, 2003a)

Figure 2.4 – Changes in true protein during ensiling

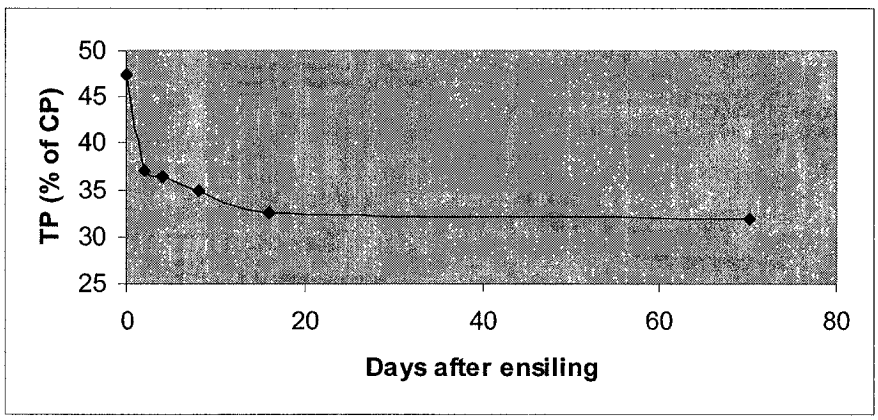
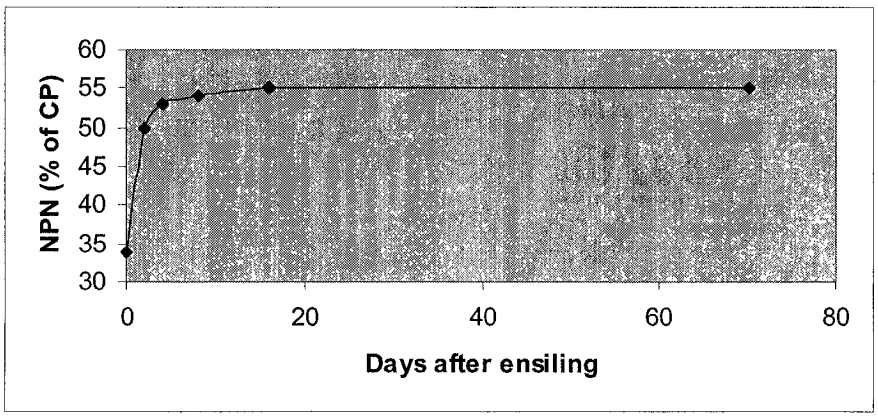


Figure 2.5 – Changes in non-protein nitrogen during ensiling



Adapted from Mustafa et al. (2002)

Mustafa et al. (2002) and Mustafa and Seguin (2003a,b) studied changes in protein fractions during ensiling of several legume forages. The authors reported a rapid increase in soluble protein and non-protein nitrogen and a sharp decline in neutral detergent insoluble protein and true protein fractions as ensiling progresses. Most of these changes took place within 2 days of ensiling with little or no changes thereafter. Results of those studies and those of Papadopoulos and McKersie (1983) and Heron and Edward (1989) confirmed the belief that most the proteolytic activities take place within a very short time following ensiling.

Reasons for the short-term proteolysis during ensiling are not clear. However, McKersie and Buchanan-Smith (1982) indicated that the cessation of proteolysis in alfalfa silage after few days of ensiling was not due to loss of enzyme activity and that pH was not inhibiting. Other factors such as availability of substrate and end product inhibition ought to be responsible (Heron and Edward, 1989). On the other hand, Carpintero et al. (1979) showed that although proteolysis was not inhibited by lowering ryegrass-clover silage pH, overall protein degradation during 50 days of fermentation was reduced. Similar results were obtained by McKersie (1985) suggesting that the rate of acidification could decrease overall proteolysis.

Several researchers reported a reduction in fiber content of forages during ensiling particularity neutral detergent fiber (NDF). Mustafa and Seguin (2003a) found that the NDF content of berseem clover silage (70 days post-ensiling) was lower than that of the fresh forage. However, acid detergent fiber (ADF) content was not affected by ensiling. Selmer-Olsen et al. (1993) reported similar reduction in NDF content during ensiling of alfalfa and Italian rye grass. The reduction in NDF during ensiling is likely due to the breakdown of neutral detergent insoluble protein, which is mediated by plant proteolytic enzymes (Mustafa et al., 2002; Mustafa and Seguin, 2003a,b). This hypothesis may help to explain the small reduction in NDF content of forages as a result of ensiling (McAllister et al., 1995; Mustafa et al., 2002). Keady and Murphy (1996) attributed the decrease in NDF while ensiling perennial ryegrass to a reduction in the hemicellulose fraction during ensilage. This fraction could be broken down during ensilage by

hemicellulases and / or hydrolysis by organic acids produced during fermentation (McDonald et al., 1991, Chap. 8). Some studies have found a rise in ADF content of silage as a result of ensiling (Gordon, 1989; Keady and Steen, 1995). Keady et al. (1995) attributed this increase to the loss of soluble cell components during fermentation.

2.4 Factors affecting ensilability of alfalfa silage

Compared with other forages, legumes such as alfalfa are hard to ensile due to their high buffering capacity and low fermentable sugars (McDonald et al. Chap. 2, 1991; Pitt, 1990, Chap. 1). Table 2.6 compares the chemical composition of alfalfa, corn and barely prior and after ensiling.

Table 2.6 – Chemical composition of different forages before and after ensiling

	Forage			Silage		
	Barley	Corn	Alfalfa	Barley	Corn	Alfalfa
pH	6.64	5.60	6.56	3.70	4.20	4.52
DM (%)	25.7	37.3	30.1	25.5	36.4	35.3
Buffering Capacity[z]	411	91	940	NM	NM	NM
CP (g/kg DM)	103	97	167	109	106	179
NDF (g/kg DM)	454	463	496	432	496	509
ADF (g/kg DM)	281	224	374	278	240	400
Starch (g/kg DM)	223	262	NM	172	283	NM
WSC (g/kg DM)	109	78.8	36	36	10.9	27.7
Ammonia (% N)	6.3	0.02	23	7.1	3.3	6.7

[z] Expressed as meq/kg DM, calculated from titration from pH 6.6 to 4.0 with 0.1 N HCL

DM, dry matter; CP, crude protein; NDF, neutral detergent fiber; ADF, acid detergent fiber; WSC, water soluble carbohydrates;

Adapted from McAllister et al. (1995, 1998) and McAllister and Hristov (Unpublished data)

Several factors could affect silage fermentation. These include cultivar, maturity, and cut, cutting time and wilting.

2.4.1 Effect of cultivar on ensilability of alfalfa

Tremblay et al. (2001) ensiled 27 different alfalfa cultivars at the same dry matter level from spring and summer re-growth. The authors found significant cultivar variations for

non-protein nitrogen (612 to 717 g kg^{-1} total N) in summer regrowth silage, indicating that proteolysis might be lower in some cultivars. Based on this study, the authors recommended the "Rangelander" cultivar for its low non-protein nitrogen (measured in silage) and high ruminal undegraded protein content (measured in forage). Bowley and McKersie (1987) evaluated three populations of alfalfa and found that proteolysis is affected by cultivar type.

2.4.2 Effect of maturity and cut on ensilability of alfalfa

The effects of stage of maturity on water-soluble carbohydrates (WSC) and buffering capacity of forages are well known. Raguse and Smith (1966) measured WSC of alfalfa at the vegetative, pre-bud, mid-bud, early bloom, full-bloom and green seed pod stage. The authors showed that WSC decreases as maturity advances with the highest level reported at the pre-bud stage (109 g kg^{-1}). On the other hand, Couchman (1959) found no relationship between WSC content and stage of maturity.

High buffering capacity of forages could result in less successful ensiling (Muck and Walgenbach, 1985). Buffering capacity of legume forages decreases as maturity progresses (Melvin, 1965; Muck and Walgenbach, 1985). The reason for this is a combination of two factors: a general drop in the buffering capacity of plant tissue and a lower leaf to stem ratio as maturity advances. (Stems have lower buffering capacity than leaves). According to Muck and Walgenbach (1985), the 1st cut tends to have a higher buffering capacity than the 2nd and 3rd cut. Similar observations were reported by Melvin (1965).

Tremblay et al. (2001) reported higher proteolysis in summer regrowth when compared with spring cut alfalfa (at 10% bloom). This agrees with the work of McKersie (1985) and Papadoupoulos and McKersie (1983) but is opposite to that of Muck (1987).

2.4.3 Effect of diurnal variation on ensilability of alfalfa

Owens et al. (1999) studied protein degradation and ensiling characteristics of alfalfa and red clover when forages harvested at different times of the day. The authors found significant effects of cutting time on total non-structural carbohydrate content. These effects were more pronounced in alfalfa than red clover. Differences in total non-structural carbohydrate were mainly due to changes in starch content. Starch content (% of total non-structural carbohydrates) ranged from 2.5 to 18.8% in the 6:00 h cutting and from 25 to 42% in the 18:00 h cutting. However, year-to-year variations in total non-structural carbohydrate concentration were greater than cutting time differences. Ensiled, forages from the later cutting times had significantly lower pH, higher lactate and lower acetate content. However, the improved fermentation did not result in lower proteolysis for alfalfa and red clover.

2.5 Effect of wilting on fermentation of alfalfa

Wilting is performed to optimize forage dry matter content before ensiling. Silages with very low dry matter content are often associated with increased seepage losses and clostridial fermentation while very dry ensiled forages do not compact properly resulting in a lower aerobic stability. Optimal dry matter level for ensiling alfalfa depends on environmental conditions and type of silo used. Ishler et al. (1992) recommended that alfalfa should be ensiled at 30 to 35, 35 to 40 and 45 to 60% dry matter when using bunker, tower, or O_2 limiting storage system, respectively.

2.5.1 Chemical and microbial changes during wilting

During wilting, forages undergo several chemical and microbial changes. Hristov and McAllister (2002) found that wilting reduces sugars concentration of whole crop barley forage by 32 %. Owens et al. (1999) reported 16 to 43% reduction in total non-structural carbohydrates when alfalfa was wilted to 35% dry matter. Reduction in plant sugar content during air exposure has been attributed to the hydrolysis of non-structural carbohydrates and subsequent respiration of released hexoses (Marsh, 1979).

Papadoupoulos and McKersie (1983) reported higher non-protein nitrogen levels in wilted silages than for directly ensiled crops, suggesting increased proteolytic activities as the forage being dried (Owens et al., 1999). However, other studies showed no effect of wilting on the proportion of different nitrogen fractions in alfalfa silage (Muck, 1987; Hristov and Sandev, 1998). Furthermore, wilting forages prior to ensiling reduces epiphytic LAB populations and thus likely results in lower LAB counts on silage as compared with directly ensiled crops (Muck, 1990a).

2.5.2 Effects of wilting on fermentation and fermentation parameters

The effects of wilting on fermentation have been investigated by several researchers. Silage fermentation proceeds at a slower rate and usually to a lesser extent with increasing dry matter content (Jones et al., 1992; Muck, 1990b). This is because low

water activity (a_w) restricts the growth of bacteria (Toller and Christian, 1978). Relative to fresh, wilted alfalfa is characterized by silage with lower lactic and acetic acid, lower ammonia and acidification rate (Whiter and Kung, 2001; Colombari et al., 2001), and higher pH (Muck, 1990b). These observations support the belief that wilting reduces fermentation rate of ensiled forages. Whiter and Kung (2001) compared lactic with acetic acid ratio of alfalfa at different dry matter content. The ratio was 2.6-3.0 for 30% dry matter alfalfa and increased to more than 8.5 for 54% dry matter alfalfa indicating a shift to a more homolactic fermentation as dry matter increases.

Wilted forages have also been associated with lower proteolysis in the silo than unwilted forages (Papadopoulos and McKersie, 1983). Muck (1987) studied the effect of dry matter content on proteolysis of ensiled alfalfa. The author reported a significant decrease in proteolysis as dry matter increases. Similar results have also been reported by Colombari et al. (2001). Luchini et al. (1997) found that wilted forages contained less non-protein nitrogen and NH_3 than unwilted forages. However, neutral and acid detergent insoluble proteins and fiber fractions were not affected by dry matter content of ensiled forages (Luchini et al., 1997).

2.5.3 Effect of wilting on aerobic stability

High DM silages are generally more susceptible to aerobic deterioration than low DM silages (Crawshaw and Woolford, 1979). Colombari et al. (2001) found higher microbial activity with higher yeast and mold counts in wilted silages and they attributed their findings to lower bulk density in drier silages.

2.6 Silage additives

The main objective of silage additives is to ensure well-preserved silage, and minimize spoilage prior and during feeding. Silage additives can be grouped into four main categories (Table 2.7). Additives in the first category stimulate fermentation by providing the proper inoculant and / or enough substrate to assure the dominance of ^{Ho}LAB. Additives in the second category enable proper silage preservation by preventing microbial activity (fermentation inhibitors). Aerobic deterioration inhibitors are important when the silage is exposed to air and nutrients are added to crops before ensiling in order to enhance the nutritional value of the silage.

Table 2.7 – Classification of silage additives

Fermentation stimulants		**Fermentation inhibitors**		**Aerobic deterioration inhibitors**		**Nutrients**
^{Ho}LAB	Carbohydrate sources	Acids	Others	Acids	Bacteria	
Lactobacillus plantarum	Mollasses	Propionic acids	Formaldehyde	Acetic acid	Propionic acid bacteria	Urea
Lactobacillus acidophilus	Sugars	Formic acid	CO_2	Propionic acid	^{Het}LAB	Ammonia
Pediococcus cerevisiae	High-sugar crops	Acetic acid				
Pediococcus acidilactici		Mineral acids				
Streptococcus faecium	Cellulases					
Enterococcus faecium	Hemicellulases					

Adapted from McDonald (1991, Chap. 7)

2.6.1 Fermentation stimulants

Additives in this category stimulate fermentation by providing proper bacterial population and enough substrate to assure the dominance of ^{Ho}LAB during ensiling. These include ^{Ho}LAB inoculants and different carbohydrate sources.

2.6.1.1 Homofermentative lactic acid bacteria as fermentation stimulants

Many species and strains of ^{Ho}LAB have been tested for their suitability as an inoculant in silage making. These include *Lactobacillus plantarum*, *Lactobacillus acidophilus*, *Enterococcus faecium*, *Pediococcus acidactili*, *Pediococcus pentosaceus* and others. Within the same species, different strains may have different fermentation response. The action of ^{Ho}LAB is discussed later in the literature review

2.6.1.2 Carbohydrates sources as fermentation stimulants

Carbohydrate additives are most useful in crops that are low in WSC. They include sugar rich sources that can be added to the forage before ensiling, and enzymes that degrade non-structural carbohydrates into sugar substrates for microbial fermentation.

The major enzymes used include cellulases, hemicellulases and amylases. The action of these additives works through the conversion of non-structural carbohydrates into soluble carbohydrate that is readily available for lactic acid bacteria. Enzymes are most needed in low WSC crops such as legumes (Nadeau et al., 2000a; Zhu et al., 1999; Muck and Kung, 1997). Several enzymes have been successfully tested on alfalfa. (Nadeau et al., 2000a,b; Sheperd et al., 1995). Nadeau et al. (2000a,b) showed that the addition of cellulases to alfalfa at ensiling increased WSC content, which led to improved ensilability.

Immature plants and grasses seem to be more responsive to enzyme addition. Nadeau (2000b) and Van Vuuren (1989) showed that immature plants are more responsive to cellulase than mature ones. This was attributed to a greater extend of lignification in mature plants (Buxton et al., 1988) which protect cellulose from the action of enzymes.

Nadeau (2000a,b) showed that enzymatic hydrolysis was greater in grasses when than in legumes.

However, controversy still exists about the effect of enzymes on nutrient digestion. Some researchers found that enzymes improve digestibility suggesting that the breakdown of cell components could facilitate microbial digestion (Chamberlain and Robertson, 1992), while others (Van Vuuren et al., 1989; Zhu et al., 1999) claim that enzymes would mainly hydrolyze easily degradable fibers and leave less digestible organic matter in the silage.

Mollasses are byproducts of the sugar cane and sugar beet industries and contains 79% soluble carbohydrates. In a review of the literature on molasses as silage additives, Keady (1996) indicated that molasses are effective in enhancing silage preservation, but did not significantly increased silage digestibility or animal performance. Sucrose is another source of carbohydrate, which can be used as a silage additive. Nishimo and Uchida (1999) added sucrose to already high WSC alfalfa forage. Sucrose was found to significantly increase lactic acid content of the alfalfa silage and decreased the pH.

2.6.2 Fermentation inhibitors

The additives in this category inhibit fermentation by reducing or stopping plant and microbial metabolisms. These include several acids as well as some preservatives such as formaldehyde

2.6.2.1 Acids as fermentation inhibitors

Acidification inhibits plant protease enzymes and microbial growth through rapid reduction in the pH (Charmley, 1994). Acid treatment is usually used with high moisture crops, where rapid acidification is a must for a proper preservation (McDonald et al., 1991, Chap. 7; Muck and Kung, 1997).

Charmley et al. (1994) found that proteolysis in alfalfa silage was reduced as the level of acid (Maxgrass mixture of carboxylic salts) increased. The authors found that feeding acid treated alfalfa silage improved performance of steers. Fellner et al. (2001) treated high moisture ear corn with propionic acid and found a significantly lower lactic acid content in the treated silage indicating that fermentation was reduced. Nadeau et al. (2000 a,b) tested the effect of formic acid on orchard grass and alfalfa. Formic acid restricted silage fermentation and preserved silage sugars. Nagel et al. (1992) and Waldo et al. (1971) positively tested the efficacy of formic acid on alfalfa. O'Kiely (1996) found a small effect of sulphuric acid on grass fermentation.

2.6.2.2 Other fermentation inhibitors

Formaldehyde has been extensively used as a silage additive, particularly in European countries. It is a well-known sterilant and is available commercially as formalin, which contains 40% of the gas in aqueous solution (McDonald, 1991, Chap. 7). Formalin has been shown to inhibit plant and microbial proteases (Brown and Valentine, 1972). It restricts fermentation, decreases lactic acid production and ammonia N levels (Thomas et al., 1973; Valentine and Brown, 1973). Dry matter recovery was not enhanced upon formaldehyde application but ruminants fed treated silage showed a potential improvement in dry matter intake (Davidson and Stevenson, 1973; Waldo, 1977). However, formaldehyde alone was found to be less effective than formic acid in controlling proteolysis (Davidson and Stevenson, 1973).

2.6.3 Aerobic deterioration inhibitors

Aerobic stability inhibitors are added to the forage prior to ensiling to improve the shelf life of silage when it is exposed to air. These include some short chain acids, and bacterial inoculants.

2.6.3.1 Acids as aerobic deterioration inhibitors

Many acids that are used to inhibit fermentation such as propionic and formic acids are also used to increase the bunk life of silage. Propionic acid is known to be a strong inhibitor of yeasts and molds (McDonald, 1991, Chap. 7), and many studies have obtained an improvement in aerobic stability of silage treated with propionic acid (Mann and McDonald 1976; Crawshaw et al., 1980). Propionic acid can be added in buffered mixes as ammonium or sodium salts (pH of 5.5 to 6); however, it is not used widely as a silage preservative because of the high application cost.

Several researchers (Mayne, 1993; Keady and Murphy, 1996) showed the virtues of formic acid on enhancing aerobic stability of grass silage. However, Nadeau et al. (2000b) hypothesized that since formic acid inhibits fermentation and saves WSC it might lead to aerobic deterioration. O'Kiely (1996) found decreased aerobic stability of grass ensiled at 22% DM when treated with formic acid.

2.6.3.2 Bacterial inoculants as aerobic deterioration inhibitors

Due to handling hazards associated with direct acid application, microbial inoculants containing ^{Het}LAB and propionic acid bacteria are more commonly used to improve aerobic stability. Propionic acid bacteria (PAB) can ferment sugars and lactic acid to acetate and propionate, which will inhibit the growth of yeast and molds in silage (Flores-Galarza et al., 1985; Higginbotham et al., 1998). The PAB ferment lactic acid in preference to glucose when both substrates are present (Lee et al., 1974; Parker and Moon, 1982). Therefore, mixed inoculation of LAB and PAB would further benefit aerobic stability (Pitt ,1997).

Corn silage treated with PAB was more aerobically stable than corn silage treated with LAB (Weinberg et al., 1995). However, inoculation with *Propionibacterium shermani* improved aerobic stability of millet silage but not that of corn or sorghum silages (Weinberg et al., 1995). Higginbotham et al. (1996) found that the addition of two

concentrations of PAB inoculant had no effect on aerobic stability of corn silage. Similar observations have been reported for low DM orange pulp silage (Alio et al., 1994).

The ^{Het}LAB have gained popularity in the last few years due to their ability to inhibit yeast growth and prevent aerobic spoilage. However, heterolactic fermentation may result in DM losses (2 to 24%) depending on the substrate used (Oude Elferink et al., 1999). Kung et al. (2003) treated alfalfa (40% DM) with *Lactobacillus buchneri 40788* in laboratory and farm scale silos. The treated silage had higher acetic, propionic acid and ammonia nitrogen and lower lactic acid than control silage. Inoculation significantly improved aerobic stability. Previous studies with the same strain (Weinberg et al., 1999; Kung and Ranjit, 2001; Taylor et al., 2002) and other strains of *Lactobacillus buchneri* (Driehuis et al., 1999; 2001; Kung et al., 1999) on different types of silage support these findings.

2.6.4 Other additives (Nutrients)

Anhydrous ammonia, water-ammonia or molasses ammonia mixes have been used as silage additives. The benefits of ammonia application prior to ensiling include:

1) Increased protein content (Huber et al. 1979).
2) Increase aerobic stability (Britt and Huber 1975).
3) Reduction in proteolysis due to inhibition of plant enzymes (Johnson et al. 1982)
4) Inhibition of mold growth during fermentation.

However, since ammonia increases the buffering capacity of the silage, it results in greater acid production and therefore can have adverse effects on DM recovery (Bolsen et al., 1992). For that same reason, ammonia treatment is not advisable for alfalfa. Corn, high moisture corn and small grain cereal are the main target crops for ammonia use.
In addition of being a cheap source of nitrogen, urea is another ingredient that can be used as a silage additive. Some studies have shown the beneficial effects of urea on silage aerobic stability (Adogla-Bessa et al., 1999) and nutritive value (Hill and Leaver, 1999).

2.7 Homofermentative lactic acid bacteria

The ^{Ho}LAB has been used successfully as a silage additive since the 1950's (Baker and Voelker, 1958). The objective of adding ^{Ho}LAB as inoculants is to help the epiphytic population of ^{Ho}LAB to quickly dominate the fermentation process.

2.7.1 Mode of action of homofermentative lactic acid bacteria

The ^{Ho}LAB produce only lactic acid from glucose (Jones et al., 1992). The strong acidity of lactic acid is expected to rapidly decrease silage pH (Whiter and Kung, 2001). A rapid fall in silage pH is expected to result in less proteolysis, and deamination. In addition, an early dominance of ^{Ho}LAB restricts clostridial and heterolactic fermentations. The result is lower ammonia-nitrogen in the silage (Kung et al., 1991; Nadeau et al., 2000b; Whiter and Kung, 2001), lower volatile fatty acid production, and saving of plant WSC (Whiter and Kung, 2001; Hristov and McAllister, 2002; Gordon, 1989).

2.7.2 Epiphytic homofermentative lactic acid bacteria

At the time of fermentation, the epiphytic micro flora of alfalfa is diversified and variable (Cai, 1999). These include enterobacteria, clostridia, yeasts, molds and the lactic acid bacteria (*Streptococcus*, *Lactobacilli*, *Lactococci*, *Lactococcus*, *Pediococci*, *Enterococci* and *Leuconostoc*; Bolsen et al. 1996). It is believed that the sources of epiphytic LAB include inoculation by mowing, growth in the swath, and inoculation by harvesting equipment (McDonald et al. 1991, Chap. 4).

Some studies tend to point out that the quantity and the quality of epiphytic LAB might be improper to induce homolactic fermentation. According to Pathlow and Weissbach (1996), the epiphytic LAB has a weak osmotolerance, and therefore might not compete well in wilted or high dry matter silage (Whiter and Kung, 2001). Other studies indicated that if the silage is left untreated, ^{Het}LAB tend to dominate the ^{Ho}LAB during the

fermentation. (Winters et al., 2000). Other studies point that adequate LAB might be deficient when the crop is ensiled (Cai, 1999; Stirling and Whittenbury, 1964)

It is clear from the above discussion that the addition of good strains of ^{Ho}LAB is expected to increase the likelihood of desired homolactic fermentation.

2.8 Effects of homofermentative lactic acid bacteria inoculants on fermentation

The concept of applying microbial inoculants to silage is to add fast growing ^{Ho}LAB in order to dominate the fermentation resulting in higher quality silage (Driehuis et al., 1997). Seale (1986) summarized the benefits of adding ^{Ho}LAB: (1) to accelerate the decline of pH during the initial stage of silage fermentation, (2) to preserve plant carbohydrates through homolactic fermentation and (3) to preserve plant protein by decreasing proteolysis and deamination.

2.8.1 Effects of inoculants on fermentation parameters

Several homofermentative lactic acid bacteria species and strains have been tested on alfalfa silage with positive effects on fermentation parameters. Cai et al. (1999) applied *Lactobacillus plantarum FG 10* and *Lactobacillus casei FG 1* at 10^5 CFU g^{-1} fresh matter on 45% DM alfalfa and on Italian ryegrass at flowering stage and sorghum at milk stage. Results showed that inoculation significantly improved fermentation parameters as lactic acid production was increased and WSC were spared. These changes resulted in lower silage pH, reduced concentrations of butyric acid, propionic acid and ammonia nitrogen as well as lesser DM losses.

Inoculation with *Lactobacillus plantarum MTD1* reduced the concentration of ammonia nitrogen in high DM alfalfa silage (Whiter and Kung, 2001; Kung et al., 1991). Williams et al. (1995) found positive results by inoculating alfalfa with *Streptococcus faecium* and *Lactobacillus plantarum* strains. Encouraging results were also obtained with *Lactobacillus paracasei* (Winters, 2000). Muck (1989) inoculated alfalfa forage with a mixture of *Streptococcus faecium*, *Lactobacillus plantarum* and *Pediococcus* species. The author reported improvement in acidification rate and lactic: acetic acid ratio when inoculant was applied at 10% or more of the natural level of lactic acid bacteria.

Data on the effects of inoculation on ensiling characteristics of forages other than alfalfa are numerous. Hristov and McAllister (2002) inoculated whole plant barley with a mixture of *Enterococcus faecium* and *Lactobacillus plantarum*. The treatment increased

lactic acid bacteria count, lactic acid production and lowered the final pH. However, inoculation was more effective for un-wilted (30.7% DM) than wilted (37.8% DM) forage. Other studies have found only limited or even negative effects of inoculation. Ranjit and Kung (2000) treated corn silage at half milk line with two strains of *Lactobacillus plantarum*. Inoculation had no effect on residual WSC, ammonia nitrogen or acetic: lactic acid ratio. The authors concluded that the strains used might not have been robust enough to dominate the epiphytic micro flora. Keady and Murphy (1996) used a combination of *Lactobacillus plantarum*, *Pediococcus acidilactici, Lactococcus lactis*, enzymes and a rumen enhancer on ryegrass silage. Results showed that fermentation parameters and aerobic stability were not affected. When fed to dairy cows, DM intake, milk fat and protein yields were not affected. However, a significant increase in milk protein content was observed.

2.8.2 Effects of inoculants on proteolysis

Effects of LAB on proteolytic activity of ensiled forages are inconsistent. Muck (1989) treated alfalfa with LAB at different moisture content (18.1 to 49.4% DM). The author found a non-significant but consistently higher non-protein nitrogen of LAB treated alfalfa when compared with the control silage. A similar trend was obtained for 50% DM alfalfa by Philip et al. (1990). In contrast, Jones et al. (1992) inoculated alfalfa forage at different DM and found that LAB reduce proteolysis in all but at the highest DM content (54% DM). More recently, Witers et al. (2000) inoculated gamma-irradiated ryegrass with *Lactobacillus plantarum* or *Lactobacillus paracasei paracasei* and found that in the in 2nd day of fermentation, the soluble protein was lower in the inoculated than in the non-inoculated gamma-irradiated silages. However after 90 days of fermentation, *Lactobacillus plantarum* and *Lactobacillus paracasei* resulted in an average increase in soluble protein of 6.6 and 4.3%, respectively. The authors attributed these changes to a proteolytic activity of the *Lactobacillus* bacteria.

Hristov and McAllister (2002) found no effect of inoculation on non-protein nitrogen content of whole crop barley silage. Other studies have demonstrated the benefits of

Lactobacillus plantarum in restricting proteolysis in grass silage (Cussen et al., 1995; Davis et al., 1998). Shirley et al. (1986) inoculated gamma-irradiated ryegrass with a mixture of *Lactobacillus plantarum* and *Streptococcus faecalis*, and found higher true protein as a result of inoculation. The authors concluded that inoculation resulted in almost 30% increase in true protein after 153 days of ensiling.

The effectiveness of ^{Ho}LAB as inoculants depends on several factors such as the rate of application, form of application, strain of ^{Ho}LAB and plant factors. Muck (1989) showed that the rate of application of the inoculant should be equal or greater than epiphytic LAB in order to reduce ammonia nitrogen production. In order to increase performance however, Satter at al (1987) reported a response in animal productivity only when ^{Ho}LAB was 10 times as numerous as epiphytic LAB. Effects of form of LAB inoculation were studies by Whiter and Kung (2001). The authors found that in alfalfa with 30% DM, both liquid and dry inoculation resulted is silages with more lactic acid and lower pH than untreated silages. However, microbial inoculant was more effective when applied in a liquid rather than a dry form to high DM alfalfa silage (i.e. 54% DM).

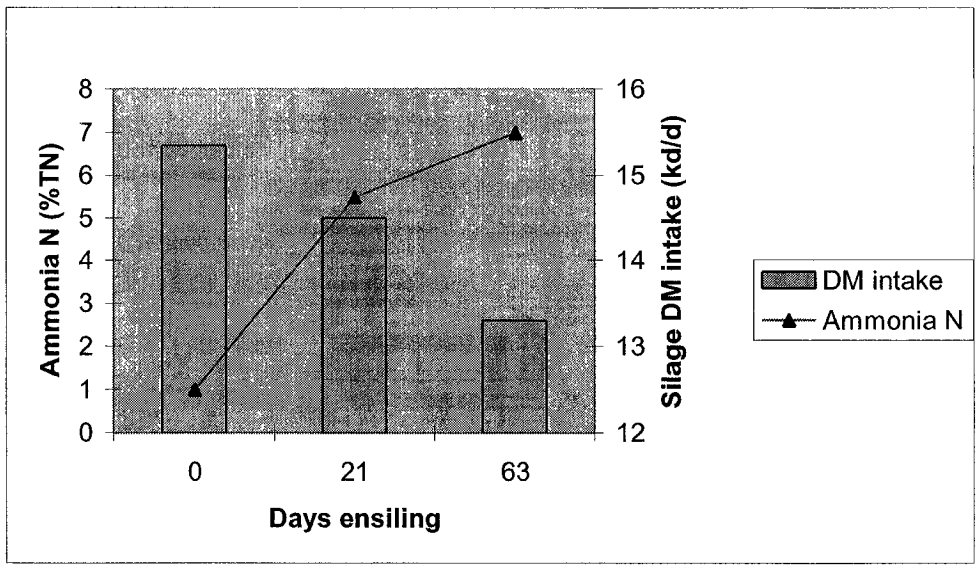
2.9 Effects of homofermentative lactic acid bacteria inoculants on performance

Inoculation of silages is believed to affect the performance of dairy and beef cattle through different means. Researchers have pointed out the effects of LAB inoculants on silage intake, ruminal fermentation, nutrient digestibility and metabolism.

2.9.1 Factors affecting silage intake

Several factors have been shown to affect silage intake. These include ammonia level, volatile fatty acid concentrations, protein solubility, fiber digestibility and lactic acid content. Studies on the effects of fermentation products on DM intake revealed inconsistent results. Crushnahan et al. (1995) reported a negative relationship between intake and silage ammonia and butyric acid levels.

Figure 2.6 – Relationship between silage ammonia content and voluntary intake



Adapted from Crushnahan et al. (1995)

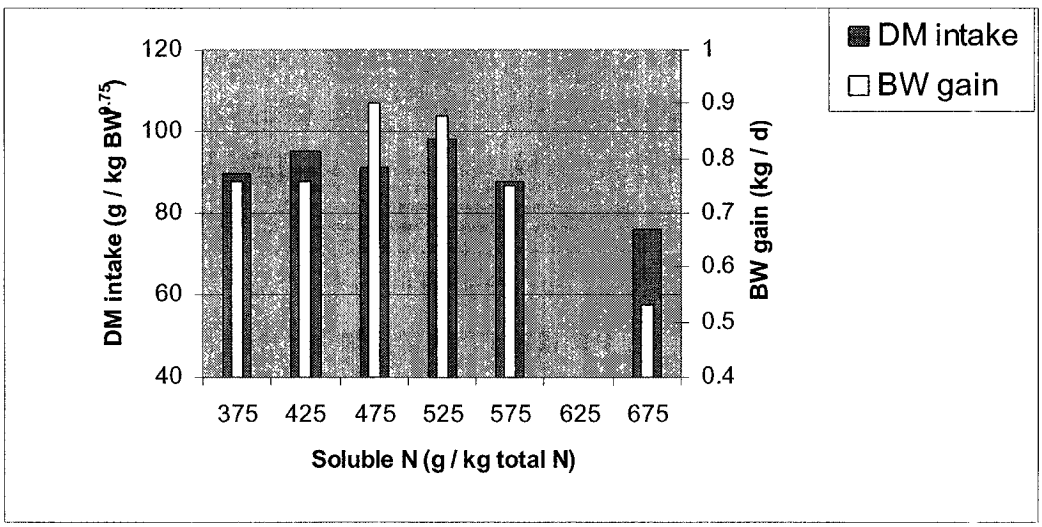
In contrast, Rooke and Gill (1990) found that total DM intake is related to volatile fatty acid concentration, but not to ammonia level. More recently, Steen et al. (1998) concluded that ammonia is an important predictor of silage intake (Figure 2.6) but a weak relation exists between intake and total volatile fatty acid concentrations. The concentrations of individual volatile fatty acids have been studied as possible signals that cause the cessation of feed consumption. The inhibitory effects of acetate (Baile and McLaughlin, 1987; Forbes et al., 1992) and propionate (Charmley, 1996; Quigley and Heitmann, 1991) are well documented. Sheperd and Combs (1998) found a more pronounced inhibitory effect of propionate than acetate.

Other studies have shown the potential negative effect of lactic acid on DM intake (Choung and Chamberlain, 1993; Rooke, 1995). Choung and Chamberlain (1993) found an average decrease of 0.8 kg DM intake when lactic acid content of perennial ryegrass silage was increased from 53 to 96 g kg^{-1}. Rooke (1995) also reported a decrease in DM intake of sheep when lactic acid was added to a perennial ryegrass diet. Schaffer et al. (1989) found lower DM intake and weight gain of finishing steers fed inoculated whole corn silage although the inoculant significantly improved fermentation parameters of the

silage. Choung and Chamberlain (1993) postulated that the reduction in DM intake when lactic acid is added could be due to a disturbance in the animal's acid-base balance. The effect of lactic acid on silage palatability is another explanation for the reduction in DM intake. Lactic acid treated feeds are more acidic and tend to have a more pronounced sour taste and are less palatable. Rooke (1995) showed that DM intake is depressed by the free acids in the silage and not by the acid salts. Grovum and Chapman (1998) found that salty taste (e.g. acid salts) increases palatability of silage whereas sour taste (e.g. free acids) reduces it.

Other studies have reported relationship between soluble protein content and silage DM intake. Charmley (2001) found a quadratic relationship between silage DM intake and soluble protein content. Silages with high CP and high solubility, such as alfalfa can result in high ruminal ammonia concentration, which is associated with depressed DM intake (Figure 2.7; Charmley and Veira, 1990; Choung et al., 1990).

Figure 2.7 – Relationship between protein solubility and voluntary intake or rate of gain by silage fed steers



Adapted from Charmley (2001)

Another factor that affect silage DM intake, is digestibility. Mertens (1994) stated that when high quality forage is fed, the energy demand is usually the most limiting factor of

DM intake, whereas rumen fill normally limits intake of poor quality forages. When intake is limited by rumen fill, increasing the digestible fraction of NDF and / or its rate of digestion may increase ruminal passage rate and therefore, increase DM intake (Dado and Allen, 1995). In order to observe the effect of NDF digestibility of high quality forage on DM intake it is important to use animals with potential for high-energy intake, such as young, growing lambs and cattle, or dairy cows in early lactation to ensure that fill and not energy demand limits DM intake (Nadeau et al., 2000a). In a review of the effects of digestibility on intake, Castle and Watson (1973) concluded that one percentage unit increase in organic matter digestibility would result in an increase in silage DM intake of 0.25 kg d^{-1}.

Several studies have shown that inoculation increased silage DM intake. Petit and Flipot (1990) found that although inoculation of alfalfa-timothy silage by a mixture of LAB (*Lactobacillus plantarum*, *Lactobacillus casei*, *Lactobacillus amylovorus*, *Streptococcus faecium*, and *Cellulomonas flavigena*) had no effect on silage composition, it increased DM intake. Nadeau et al. (2000a) added LAB inoculant to cellulose-treated mixture of alfalfa and barley silage. The authors found that inoculation improved DM intake by lambs. The augmentation in DM intake was associated with lower NDF content of the silage, enhanced fermentation characteristics and higher DM digestibility. Similar observations were reported by Meeske et al. (1999). The authors reported higher DM intake by lambs when inoculated grass silage was fed compared with untreated silage. The increase in DM intake was attributed to improved fermentation parameters and increased organic matter digestibility.

2.9.2 Effects of ^{Ho}LAB inoculation on ruminal fermentation

It has been suggested that inoculants could enhance animal performance by altering ruminal fermentation (Fellner et al., 2001). Although the findings are equivocal, there are indications, that bacterial inoculants do have the potential to alter ruminal fermentation (Keady et al., 1994).

Sharp et al. (1994) attributed improved weight gain in steers fed inoculated high moisture ear corn to improved efficiency of energy utilization due to higher levels of propionic acids in the rumen. Sharp et al. (1994) inoculated grass silage (perennial ryegrass and white clover) with a mixture of *Lactobacillus plantarum* and *Streptococcus faecalis*. The authors found significant changes in ruminal volatile fatty acid pattern with a significantly greater molar proportion of propionate and a corresponding reduction in both acetate and butyrate as a result of inoculation. Charmley et al. (1996) found that inoculation of grass silage improved intake of steers, while inoculation of wheat silage improved feed efficiency. Fellner et al. (2001) reported higher body weight gain for steers fed inoculated high moisture air corn than for those fed untreated feed. However, inoculation had no effect on DM intake or digestion of organic matter. The improvement in animal performance was attributed to higher ruminal pH and iso-acid concentrations observed for steers fed the inoculated high moisture air corn. Indeed, iso-acids have been shown to enhance microbial protein synthesis in the rumen (Russel and Sniffen, 1984) while a pH lower than 6.0 can reduce microbial protein synthesis (Russel et al., 1992).

2.9.3 Effects of ^{Ho}LAB inoculants on silage digestibility

Several studies have shown the potential of LAB inoculants in increasing silage digestibility. Inoculation of alfalfa and barley silages with lactic acid inoculant and cellulase resulted in improvement of *in vivo* NDF digestibility in lambs (Nadeau et al., 2000a). The enhancement reported for the inoculant and cellulase treatment was better than that reported for the cellulase treatment alone. Similarly, Meeske (1999) found that *in vivo* organic matter digestibility of *Digitaria eriantha* grass in lambs increased with the application of a mixture of LAB (*Lactobacillus plantarum*, *Streptococcus faecium* and *Pediococcus acidactili*) and enzymes (cellulase, hemicellulase and amylase). Keady et al. (1994) applied *Lactobacillus plantarum* on grass forages low in WSC and DM. After ensiling, the silage was fed to dairy cows. Treatments had little effect on silage fermentation and DM intake. However, feeding inoculated silage alone or as a part of a complete mixed diet increased organic matter and nitrogen digestibilities.

In situ ruminal degradability of silages does not seem to be affected by LAB inoculation. Hristov and McAllister (2002) found that *in situ* soluble and potentially degradable DM fractions were similar for untreated and inoculated (*Lactobacillus plantarum* and *Enterococcus faecium*) whole crop barley silage. Salawu et al. (2001) found that inoculation of pea and wheat bi-crop with *Lactobacillus plantarum* had no effect on *in situ* dry matter degradability. However, inoculation significantly increased *in situ* crude protein degradability.

2.9.4 Effect of ^{Ho}LAB inoculants on nitrogen utilization

Most of silage protein is in the form of non-protein nitrogen and therefore most silages have low ruminal undegraded protein content (Mustafa et al., 2002; Mustafa et al., 2003). Excessive ruminal protein degradation may be the most limiting nutritional factor in high-quality legume silages (Broderick, 1995).

Two of the factors that can affect efficiency of ruminal microbial protein synthesis include protein solubilization during ensiling and the fermentation of soluble sugars to volatile fatty acids and lactic acid (Charmley, 2001). High proportions of volatile fatty acids are absorbed across the rumen wall and therefore are not available for rumen microbes. The efficiency of silage for microbial protein synthesis is only 60 to 70% compared with fresh forage (Agricultural Research Council, 1984). The effects of WSC level on microbial protein synthesis are important when silage is given as the sole source of feed, but not when offered as a part of a total mixed diet. Homolactic fermentation has been shown to reduce proteolysis (Jones et al., 1992; Cussen et al., 1995; Davis et al., 1998). Therefore it is believed to increase the proportion of true protein available in the rumen and its subsequent incorporation into microbial nitrogen.

Ruminal pH could be another potential mode of action of ^{Ho}LAB on microbial protein synthesis. Fellner et al (2001) reported a higher ruminal pH for animals fed inoculated silage relative to those fed untreated silage. It has been supposed that microbial protein

synthesis per unit of energy is enhanced with increasing ruminal pH (Strobel and Russel, 1986; Fellner et al., 2001).

Keady et al. (1994) reported higher nitrogen retention by animals fed *Lactobacillus plantarum* treated permanent pasture than by those fed untreated pasture. The authors attributed these findings to increased ruminal nitrogen degradability (possibly through the action of the inoculants on the cell wall of the plant cells). McAllister et al. (1995) found that feeding inoculated barley silage to lambs had no effect on total tract nutrient digestibility. However, lambs fed inoculated barley silage gained more weight than those fed untreated silage possibly due to greater nitrogen intake and retention. However, Fraser et al. (2001) reported a reduction in nitrogen retention by lambs as a result of feeding forage peas and field beans treated with *Lactobacillus plantarum.*

2.9.5 Effects of feeding ^{Ho}LAB inoculated silages on animal performance

Several studies have shown improved performance of steers fed inoculated silage. Improvement was mediated through higher DM intake and improved nutrient digestibility and utilization. O' Kiely (1996) treated unwilted grass forage with *Lactobacillus plantarum* prior to ensiling. Results showed that inoculation had no effect on fermentation parameters of the silage. However, steers fed inoculated silage gained more weight than those fed untreated silage. The author attributed the improved animal performance to enhanced *in vivo* DM digestibility. Keady and Steen (1995) treated low DM, low digestibility perennial ryegrass with *Lactobacillus plantarum* and found that inoculation had no major effects on silage quality; however, inoculation significantly improved total tract digestibility of crude protein and fiber and tented to increase nitrogen retention. Increased fiber digestibility was associated with improved DM intake. Higher DM intake and improved total tract nutrient utilization are likely the reasons for the better performance of animals fed inoculated forages.

Several studies reported inconsistent effects of inoculation on animal performance. Wardunski et al. (1993) found that feeding inoculated relative to untreated high moisture

corn had no effect on weight gain, feed efficiency or DM intake. Fermentation parameters were also not affected by inoculation. These results suggest the failure of the inoculant to dominate the fermentation process.

Other researchers found positive effects of LAB inoculant on silage quality with no improvement in animal performance. Schaefer et al. (1989) treated corn silage and high moisture corn with lactic acid bacteria (*Pediococcus acidactili* and *Lactobacillus xylosus*). Results showed that inoculation improved fermentation of high moisture corn but not corn silage. However, in both feeds, beef performance was not affected by inoculation. Rooke and Kafilzadeh (1994) treated perennial ryegrass sward with different inoculant strains (*Lactobacillus plantarum MTD1*, *Pediococcus sp. 6A2*, *Lactobacillus plantarum 6A6*) using 10 kg silos. Treated silage showed improvement in fermentation parameters. However, when the same treatments were applied to 2-ton silos and fed to sheep, only one inoculant (i.e. *Lactobacillus plantarum MTD1*) improved animal performance. Sharp et al. (1994) inoculated grass silage (perennial ryegrass and white clover) with a mixture of *Lactobacillus plantarum* and *Streptococcus faecalis*. The treated silage resulted in improvement in silage fermentation patterns. When fed to heifers, significant increase in DM intake was observed which was attributed to better fermentation patterns. However, DM intake did not result in higher weight gain for animals fed the inoculated silage.

2.9.6 Effects of feeding ^{Ho}LAB inoculated silages on milk yield and milk composition

Data on the effect of inoculation of alfalfa silage on milk yield and composition are limited. Kung et al. (1993) inoculated corn silage with *Lactobacillus plantarum* for 120 days in bag silos. Inoculation had no effect on silage composition; however, DM intake and 3.5% fat-corrected milk production were significantly increased when the inoculated silage was fed to lactating dairy cows as a part of total mixed diet. The author concluded that factors other than commonly measured end products of fermentation might improve DM intake and the nutritive value of the inoculated silage and be responsible for

improved milk yield. Mayne (1990) inoculated perennial ryegrass low in DM and WSC with *Lactobacillus plantarum*. Inoculation slightly enhanced fermentation parameters (rate of pH decline and final ammonia content). However, when fed to lactating dairy cows, inoculated silage increased DM intake, milk yield, and milk protein and fat content. Improvement in DM intake was attributed to a higher *in vivo* digestibility of the treated silage (tested on sheep). Similarly, Gordon (1989) reported a significant increase in silage DM intake and a subsequent increased milk production for cows fed inoculated relative to untreated silage. The authors also reported improvement in *in vivo* digestibility but no effects on fermentation parameters were noted as a result of inoculation. Martinsson (1992) applied LAB inoculant (*Streptococcus faecium*, *Lactobacillus plantarum* and *Pediococcus sp.*) on a mixture of wilted and unwilted grasses (timothy and meadow fescue) prior to ensilage. When fed to lactating dairy cows, inoculated wilted silage increased nutrient digestibility and DM intake as well as milk yield. However, quality of unwilted silage was not improved by inoculation. It was concluded that the response of milk yield to the use of a specific inoculant appears to be mediated through increased intake of metabolisable energy.

Other studies found no effects of LAB inoculants on milk yield. Keady and Murphy (1997) applied LAB inoculants (*Lactobacillus plantarum* and cellulase, gluconase, arabinase and xylanase) on perennial ryegrass, which were fed to lactating dairy cows. Inoculation showed no beneficial effects on silage fermentation, total tract nutrient digestibility, DM intake or milk yield. Mayne (1993) treated first and second cut perennial ryegrass with *Lactobacillus plantarum* and fed to lactating dairy cows. Small increase in DM intake in the first cut was observed but no response was noted for the second cut. Inoculation had no significant effect on milk yield; however, small increases in milk fat and protein concentrations were observed.

2.10 Aerobic stability

Aerobic stability refers to the duration of time before the silage starts deteriorating upon exposure to air (McDonald et al., 1991, Chap. 5). Several measurements of aerobic stability have been proposed. Henderson et al. (1979) suggested three temperature parameters as indicators of aerobic stability: 1) maximum temperature during period of exposure to air, 2) average sum temperature to exposure time to indicate the rate of temperature increase, 3) Mean sum to maximum temperature to indicate total heat produced during the period of air exposure. Other researchers used the time needed for the silage or the total mixed diet to rise 2 °C (Moran et al., 1996; Pitt, 1997) or 1 °C above ambient temperature (Driehuis et al., 2001; Oude Eferink et al., 1999). Other measurements include the rate of pH rise (Cai et al., 1999; Mayne, 1993), DM loss (Mayne, 1993; Henderson et al., 1979), and yeast and molds growth (Cai et al., 1999; Pitt and Muck, 1991).

2.10.1 Microbial, chemical and physical changes during aerobic exposure

Aerobic spoilage of silages is characterized by an increase in pH, loss of fermentation acids and WSC, silage heating and increase in yeast and mould population (Woolford, 1990, Chap. 2; Pitt and Muck, 1991). However, some studies have observed a decrease in pH upon spoilage in corn (Fellner et al., 2001) and in grass silages (Ohyama et al., 1975).

Upon exposure to air during the feed out phase, oxygen promotes the growth of yeasts, molds and aerobic bacteria in the silage. Yeasts (*Pichia*, *Candida* and *Cryptococcus*) initiate aerobic spoilage by metabolizing lactic acid (Barry et al., 1980). The degradation of lactic acid will eventually lead to an increase in silage pH, which in turn promotes the proliferation of other aerobic microorganisms such as proteolytic bacteria, streptomyces and moulds (McDonald et al., 1991; Chap.8). Spoilage can be initiated to a lesser extend by molds, bacilli, acetic acid bacteria and possibly lactic acid bacteria (Woolford, 1990, Chap. 3).

2.10.2 Effect of aerobic spoilage on silage quality

Spoiled silage is associated with lower intake and reduced animal performance (Hoffman and Ocker, 1997; Whitlock et al., 2000). In addition, feeding spoiled silage may lead to increase disease incident due to toxins accumulation (McDonald, 1991, Chap. 5; Driehuis and Oude Elferink, 2000). Aerobic spoilage in silos is associated with high DM loss. Average losses have been estimated at 5% of the total DM with higher losses (10 to 15%) at the surface layers (McGehnan, 1990).

2.10.3 Effect of ^{Ho}LAB on aerobic stability

It is believed that homolactic fermentation, while enhancing silage quality may reduce aerobic stability (Kung et al., 1991; Weinberg et al., 1993). First, inoculation with ^{Ho}LAB is associated with higher residual WSC and lactic acid (Cai et al., 1999), both of which can be used as substrates by yeasts. Second, a dominant heterolactic fermentation is associated with higher concentrations of acetic acid and 1,2 propanediol. Undissociated acetic acid inhibits yeast and mould growth (Courtin and Spoelstra, 1990), and 1,2 propanediol could be converted to propionic acid and 1-propanol (Driehuis et al., 2001) which both have antimycotic properties (Oude Elferink et al., 1999).

Inoculation with ^{Ho}LAB such as *Lactobacillus plantarum* could lead to deterioration in aerobic stability. Cai (1999) inoculated alfalfa, Italian ryegrass and sorghum with an epiphytic strain of *Lactobacillus plantarum*, and *Lactobacillus casei*. Inoculation with both species resulted in higher yeast counts and faster spoilage compared with untreated silage. Most yeast were able to grow at low pH and showed high tolerance to lactic acid but low tolerance to butyric acid. Similar findings were reported for corn and wheat silages (Weinberg et al., 1993). The decline in aerobic stability was attributed to high concentration of residual WSC and lactic acid and low concentrations of volatile fatty acids. Other studies on the other hand found that inoculation improved aerobic stability (Sebastian et al., 1996). Muck and Kung (1997) reviewed the literature of inoculation and found that inoculation improved aerobic stability in 30% of the studies. This may be due

to some bacteriocin and unknown anti-fungal compounds produced by lactobacilli (Laitila et al., 2002). Muck and Bolsen (1991) stated that inoculants could increase or decrease aerobic stability depending on the degree to which pH, lactic acid and acetic acid values are changed. They proposed that lower pH increases toxicity of lactic and acetic acids to yeasts and moulds therefore improving aerobic stability. However, at the same time inoculants tend to reduce acetic acid concentration, which is more toxic to yeasts and moulds than lactic acid at a given pH.

3 Materials and Methods

3.1 Silage preparation

Alfalfa (*Medicago sativa*) was grown in Sainte-Anne de Bellevue and soil nutrient composition was maintained adequate for proper forage growth. A second cut alfalfa was harvested at early bloom stage in July 6th 2002 and was wilted for 24 hours. The wilted forage was chopped to a theoretical cut length of 0.95 cm using a flail forage harvester. Lactic acid bacteria inoculant (11H50 Sila-Bac®; Pioneer Hi-Bred Inc) was applied to portion of the chopped forages following the recommendations of the manufacturers to supply 10^5 CFU per gram of ensiled materials. Alternate loads of the forage was either inoculated or left untreated (control) at the silo blower as the herbage was being uploaded. The inoculated and untreated forages were ensiled in two separate, upright concrete tower-silos (100 tons capacity each) for 2 months.

3.2 Ensiling characteristics of inoculated alfalfa silage

3.2.1 Ensiling procedure

Effect of inoculation on ensiling characteristics of alfalfa silage was determined using mini-silos. Representative herbage samples (1000 g) of inoculated and untreated alfalfa silage were packed manually using a pestle (Sebastian et al., 1996), in triplicates, into mini silo made of PVC tubing (7.6 cm diameter and 25 cm height; capacity one kg). The filled silos were sealed with plastic lids equipped with gas valves, stored at ambient temperature and allowed to ensile for 2, 4, 8, 16 and 45 d. Triplicate samples of fresh forage (0 day after ensiling) from each alfalfa silage treatment were also stored at -20°C for later analysis. Dry matter recoveries for the 45-day silages were estimated by weighing the mini silo before and after the 45-d ensiling period.

3.2.2 Chemical analysis

After the designated ensiling time, silos were opened and the ensiled forage was mixed thoroughly. Twenty-five grams of the fresh and the ensiled forages were homogenized

for 1 hour in 250 ml of distilled water. The pH of the water extract was immediately determined using an Accumet pH meter (Denver Instrument Company, Mansfield, TX). A portion of the extract (20 ml) was filtered through a Whatman 54 filter paper, acidified with 50 µl of 50% H_2SO_4 and frozen before further analysis. Lactic acid and water-soluble carbohydrates were determined following the procedures of Barker and Summerson (1941) and Dubois et al. (1956), respectively.

Sub-samples (500 g) of the fresh (0 d) and the ensiled forages (2, 4, 8, 16 and 45 d) were also dried in a forced-air oven at 55°C for 48 h and then ground through a 1-mm screen using a Wiley Mill (Model 4, A. H. Thomas, Philadelphia, PA, USA). Ground forage samples were analyzed for moisture (method no. 834.01) according to the procedure of the Association of Official Analytical Chemists (AOAC, 1990). Neutral (NDF) and acid (ADF) detergent fiber were determined using the ANKOM System (ANKOM 2000 Fiber Analyzer and F57 filter bags, ANKOM Technology, Fairport, NY, USA). Crude protein (CP; N x 6.25) was determined using a LECO Nitrogen System FP-428 (LECO Corp. St-Joseph, MI, USA). Neutral (NDICP) and acid (ADICP) detergent insoluble protein were determined by measuring the CP content of the NDF and ADF residues, respectively. Non-protein nitrogen (NPN) and soluble protein (SCP) were determined according to the methods of Licitra et al. (1996). True protein was calculated by subtracting NPN and ADICP form total CP.

3.2.3 Statistical analysis

Data of the ensiling characteristics were analyzed using the GLM procedure of SAS. Data of chemical changes during ensiling were analyzed using a completely randomized model with split plot restriction and three replications (Gomez and Gomez, 1984). Main plots were silage treatment (inoculated vs. untreated) and subplots were the ensiling periods. When interactions were significant, data were also analyzed using a completely randomized model for each ensiling period or treatment.

Table 3.1 – Composition of the alfalfa forage before ensiling

	Wilted alfalfa forage
Dry matter (%)	55.3
pH	6
Neutral detergent fiber (% DM)	39.9
Acid detergent fiber (% DM)	29.8
Water soluble carbohydrates (% DM)	8.8
Crude protein (% DM)	19.0
Acid detergent insoluble crude protein (% CP)	6.3
Neutral detergent insoluble crude protein (% CP)	14.9
Non protein nitrogen (% CP)	32.3
Soluble crude protein (% CP)	35.8
True protein (% CP)	61.4

3.3 Dairy production trial

3.3.1 Dietary treatments and animals

The study was conducted at the McDonald Dairy Farm at Sainte-Anne-de-Bellevue, Quebec from September 10th 2002 till March 16th 2003. Two iso-nitrogenous and iso-caloric diets (40% forage and 60% concentrate) were formulated to meet the requirements of dairy cows in early lactation (Table 3.2; NRC, 2001). In both diets, untreated or inoculated alfalfa silage was the only source of forage. Alfalfa silage dry matter (DM) was determined weekly and the TMR were adjusted accordingly to account for changes in DM levels. Diets were offered twice daily (08:00 and 16:00) as total mixed diet and feed quantity were adjusted every two days to allow weigh back of 5 to 10% of intake.

Twenty-seven lactating Holstein cows of mixed parities (16 multiparous cows, 614 kg ± 47.7 and 11 primparous cows, 544 kg ± 29.4) were used. Cows were blocked by parity at calving and randomly assigned to one of the two dietary treatments for 10-week period.

Cows were housed in tie stalls with continuous access to water. Cows were milked three times daily at 04:00, 12:00 and 18:00 and milk yield was recorded at each milking.

Table 3.2 – Ingredients and chemical composition of diets used in the production and the digestibility studies

	Alfalfa haylage treatment	
	Untreated	Inoculated
Diet ingredients (%)		
Untreated alfalfa silage	40	
Inoculated alfalfa silage		40
High moisture corn	45.6	45.6
Soybean meal	6.1	6.1
Beet pulp	2.0	2.0
Commercial dairy supplement[1]	3.0	3.0
Commercial fat supplement	2.0	2.0
Sodium bicarbonate	0.8	0.8
Mineral-vitamin premix[2]	0.6	0.6
Chemical composition (%)		
Dry matter	53.5	51.5
Ash	8.0	8.5
Crude Protein	21.2	20.5
Neutral detergent fiber	36.3	38.0
Acid detergent fiber	27.5	26.7

[1]Contained (% DM basis): 50% CP, 4% crude fat, 5% crude fiber, 2.5% Ca, 1.5% P, 0.8% Mg, 0.5% S, 0.5% K. Supplied (per kg) 160 mg vitamin E, 39,500 IU vitamin A, 11,850 IU vitamin D_3.
[2] Contained (%) 10 Ca, 10 P, 7.8 Na, 8.0 Mg, 3.0 S. Supplied 45 mg I, 3,600 mg Fe, 740 Cu, 2,300 mg Mn, 2,300 mg Zn, 10 mg Co, 500 mg F, 300,000 IU vitamin A, 90,000 IU vitamin D_3, 800 IU vitamin E per kg.

3.3.2 Sampling procedures and measurements

Samples of total mixed diets and silages were collected weekly, dried for 48 h at 55 °C in a forced-air oven (Despatch Industry Inc. Minneapolis USA) and pooled monthly. Samples were later ground through a 1-mm screen for chemical analysis. Additional samples of untreated and inoculated silages were obtained once a week and frozen at -20°C for later analysis. The quantity of feed offered and weigh back were measured daily for each cow to determine daily feed intake. Milk yield was recorded at each milking and milk samples were collected once weekly from the three milkings in plastic bottles containing bronopol preservative for milk component analysis.

3.3.3 Chemical analysis and calculations

Ground samples of total mixed diets and alfalfa silages were analyzed for moisture, ash (method no. 924.05, AOAC, 1990), NDF, ADF and CP. Samples of untreated and inoculated alfalfa silages were also analyzed for NPN, SCP, NDICP, ADICP. Water-soluble carbohydrates and lactic acid were determined on fresh extract as previously described. Ammonia-N was determined by colorimetry using a multichannel Lachat autoanalyzer (Lachat Instruments, Milwaukee WI, USA). Volatile fatty acids (acetic, propionic, butyric and valeric acids) were analyzed by gas chromatography (Varian model 3400; Varian Canada Inc., Ville St-Laurent, QC, Canada) equipped with a 30-m capillary column (Stabilwax-DA, 0.53 mm ID; Restek Corporation, Bellefonte, PA). Initial column temperature was set at 80 °C for 30 s, then temperature was increased at the rate of 15 °C per minute until it reached 180 °C; this temperature was maintained for 1 min. Therefore, run time was 8.16 min. Injector and detector temperatures were 250 and 300 °C, respectively. Gas flows were 30, 300 and 30 ml/min for He, air and H_2, respectively. Volume of sample injected was 0.4 µl.

One 24-h composite milk samples were collected weekly from each cow and analyzed for fat, protein, lactose, milk urea nitrogen and somatic cell count at the Dairy Herd Analysis Service (Programme d'analyse des troupeaux laitiers du Quebec) with an infrared system using an electric Milk-O-Scan 4000 (Foss-Food technology, Hillerød, Denmark)

calibrated with reference standards determined by Mojonnier and Kjeldahl methods (AOAC 1990). Fat, protein and lactose yield were also calculated by multiplying milk yield and the concentration of the corresponding parameters. Daily milk yield and daily DM intake were averaged every week and weekly values were obtained.

3.3.4 Statistical analysis

Data of the dairy study were analyzed using the Mixed model procedures of SAS® (version 8.2, SAS Institute Inc., Cary, NC, USA). Dry matter intake, milk composition, total milk, milk fat and milk protein yield were analyzed using a randomized complete block design with week as repeated measures. The following model was used:

$$Y_{ijklm} = \mu + week_j + diet_i + parity_k + block_l + diet\times week_{ij} + diet\times parity_{ik} + parity\times week_{jk} + parity\times diet\times week_{ijk} + e_{ijklm}$$

Y : variable studied during the week "j" (1,2...), diet "i" ( ) for the "m"th cow of parity "k" and block "l"

μ : overall mean

week : effect of the j^{th} week

diet : effect of the i^{th} diet

parity : effect of the k^{th} parity

block : effect of the l^{th} block

e : random error of the $ijklm^{th}$ measure

3.4 Digestibility study

3.4.1 Dietary treatments and animals

The objective of this study was to determine the total tract nutrient utilization of the diets used in the dairy study. Four lactating cows (164.7 ± 15.4 days post partum) fitted with flexible ruminal cannulas were used in a switchback design with three 17-day periods. The first 10 days of each period were for diet adaptation while the last 7 days were for data collection. Cows were kept in tie stalls with free access to water. Two cows within each period were assigned to one of the total mixed rations. Cows were fed ad libidum during the first 12 days to allow 5 to 10% refusals. Feed intake was then restricted to 90% of voluntary intake from day 13 to 17 to ensure complete consumption. Chromic oxide (Cr_2O_3) was used as an external marker to determine total tract nutrient digestibility. Gelatin capsules containing 10g of Cr_2O_3 were inserted into the rumen of each cow twice daily starting on day 12 until day 17 of each period.

3.4.2 Sampling procedures and measurements

Grabbed fecal samples (800 g) were collected on day 15, 16 and 17, four times per day. On day one, fecal samples were collected at 08:00 and every 3 h till 15:00. First sampling was delayed to 09:00 and to 10:00 on day 16 and 17, respectively, in order to minimize daytime variation on fecal output. Samples were then dried at 55°C in a forced-air oven for 48 hours, ground through a 1mm screen using a Wiley Mill and then pooled by cow within each period. Samples of total mixed diets were also collected during the fecal collection period and samples were then dried at 55°C in a forced-air oven for 48 h and then pooled by treatment within each period.

3.4.3 Chemical analysis and calculations

Ground feed and fecal samples were analyzed for moisture, ash, CP, NDF and ADF as previously described. Gross energy (GE) of feed and fecal samples was determined with an adiabatic calorimeter (Parr Instrument Company, Molilne, Illinois, USA). Fecal samples were also analyzed for chromic oxide as described by Fenton and Fenton (1979).

Chromic oxide concentrations were then used to estimate fecal output. Total tract nutrient digestibilities were calculated as the difference between nutrient uptake in feed and nutrient excretion in feces. Digestibilites were expressed as a proportion of total nutrient intake, using the following equation:

$$Dig\% = ([nut]_{feed} \times int_{feed} - [nut]_{feces} \times exc_{feces}) / ([nut]_{feed} \times int_{feed})$$

where

$[nut]_{feed}$: nutrient concentration in feed (DM basis)

$[nut]_{feces}$: nutrient concentration in feces (DM basis)

int_{feed}: feed intake (DM basis)

exc_{feces} : fecal excretion (DM basis)

3.4.4 Statistical analysis

Data of the digestibility study were analyzed using the Mixed model procedures of SAS® (version 8.2, SAS Institute Inc., Cary, NC, USA). CP, GE, NDF and ADF digestibilities were analyzed using a switch back design. The following model was used:

The Mixed model procedure of SAS® was used to analyze the data using the following design:

$$Y_{ij} = \mu + \beta_i + k_j + Ti + D_k + e_{ijk}$$

Y: variable studied for the cow "i" on sequence "j" at period "i"

μ: overall mean

β: effect of the i^{th} cow within sequence

k: effect of the j^{th} sequence

T: effect of the i^{th} period

D: effect of the k^{th} diet

3.5 *In Situ* nylon bag study

3.5.1 Treatments and Animals

The effects of inoculation on ruminal kinetic parameters and effective ruminal degradability of alfalfa silage were determined using the nylon bag technique. Two lactating Holstein fitted with flexible ruminal cannulas were used. The cows were fed ad libidum 50:50 forage: concentrate total mixed diet (DM basis). The diet contained (DM basis) 17.6% CP, 32.6% NDF and 2.9% ether extract. Animals were fed in equal portions at 08:00 and 16:00 h and had free access to water at all times.

Untreated and inoculated alfalfa silages obtained from the 45-day mini-silos were used. Sub-samples (n = 3) of inoculated and untreated alfalfa silages were ground through a 2-mm screen with a Wiley Mill and composited to obtain a single sample of each treatment. Quadruplicate samples weighing approximately 5 g (air dry basis) of each silage treatment were weighed into nylon bags (25 x 33 cm, 50 μm pore size). Duplicate bags from each silage treatment were incubated in the ventral sac of the rumen of the two cows for 3, 6, 12, 24, 48, 72 and 96 h. Following removal from the rumen, bags were washed in 20 °C tap water and handled as described by McKinnon et al. (1991). The 0 h washout was measured by washing duplicate bags containing samples of the two silage treatments.

3.5.2 Sample analysis and calculation

Residues from the nylon bags at each incubation time were analyzed for DM, CP and NDF as previously described. Ruminal disappearance at each incubation time was calculated from nutrient concentration in the original samples and the ruminal residues. Ruminal disappearance data were used to estimate DM, CP and NDF kinetic parameters using the equation of Dhanoa. (1988):

$$P = a + b\ x\ (1\text{-}e^{-c(t-L)})$$

Where: p is ruminal disappearance at time t (%), a is the soluble fraction (%), b is the slowly degradable fraction (%), c is the rate at which the b fraction is degraded (% h^{-1}),

and ***L*** is a discrete lag phase. Effective ruminal degradability (ED) of DM, CP and NDF were estimated using the equation of Ørskov and McDonald (1979):

$$ED = a + b \times c/(c + k)$$

where ***k*** is the estimated ruminal flow rate of 5.0% h^{-1}.

3.5.3 Statistical analysis

Data of the *in situ* ruminal kinetic parameters and effective digestibility were analyzed as a randomized complete block design with cows as blocks, using the General Linear Model of the SAS Institute, Inc. (1999). Treatment differences were declared when $P < 0.05$.

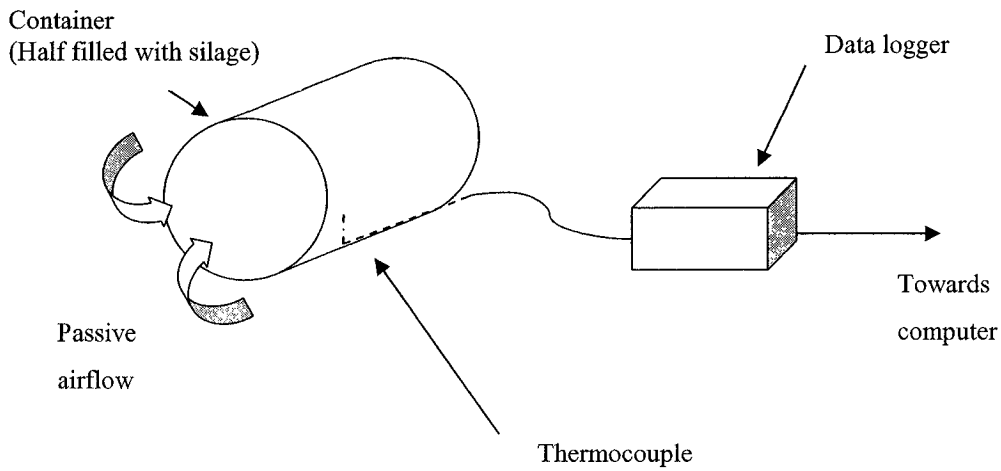
3.6 Aerobic stability

3.6.1 Silage and design preparation

Samples of inoculated and untreated alfalfa silage were collected directly from the tower silos during the dairy production study. Triplicate sub samples were used to measure the effect of inoculation on aerobic stability. Samples were collected one-meter below the surface of the silage to ensure that the silage has not been previously exposed to air. One kg each of inoculated and untreated silage was placed in hermetically sealed plastic containers (20 L capacity; 27 cm diameter and 37 cm height). In total, 6 containers were used (3 for untreated silage and 3 for inoculated silage). Holes were drilled on the top and the bottom sides of the containers to allow passive air infiltration. The containers were placed in a horizontal position before the silages were placed inside. The silages were loosely packed in such a way that they occupied approximately half the container's volume (Figure 3.1). A temperature probe (T-type custom designed thermocouples) was inserted horizontally in each container through one of the sides and was positioned half way the height of the container and 7 cm away from the nearest side. Probes were connected to an Agilent Data Acquisition/Switch Unit System data logger (Agilent

technology Inc., Loveland Colorado, USA) set to measure temperature at one-h intervals. Two T-type thermocouples were immersed in water to measure ambient temperature. The peripheries of the containers were covered by an insulating material to minimize heat loss.

Figure 3.1 – Schematic diagram of the design used to measure aerobic stability



3.6.2 Measurements of aerobic stability and sample analysis

The silages were incubated in the containers for 21 days. At the end of the incubation time, the containers were opened and the silages were removed. Water extracts were obtained from silages before (day 0) and after (day 21) aerobic exposure as previously described. Water extracts were then used to measure pH, water-soluble carbohydrate and lactic acid as previously described. Aerobic stability was defined as the time needed for the silage temperature to rise 1 °C (Driehuis et al., 2001, Oude et al., 1999) and 2 °C above ambient temperature (Moran et al., 1996; MacDonald et al., 1991; Pitt, 1997).

3.6.3 Statistical analysis

Data of the aerobic stability study were analyzed as a completely randomized design (two treatments and three replicates), using the General Linear Model of the SAS® Institute, Inc. (1999).

4 Results and Discussion

4.1 Study I – Effects of inoculation on ensiling characteristics of alfalfa silage

4.1.1 Effect of inoculation on pH, lactic acid and WSC

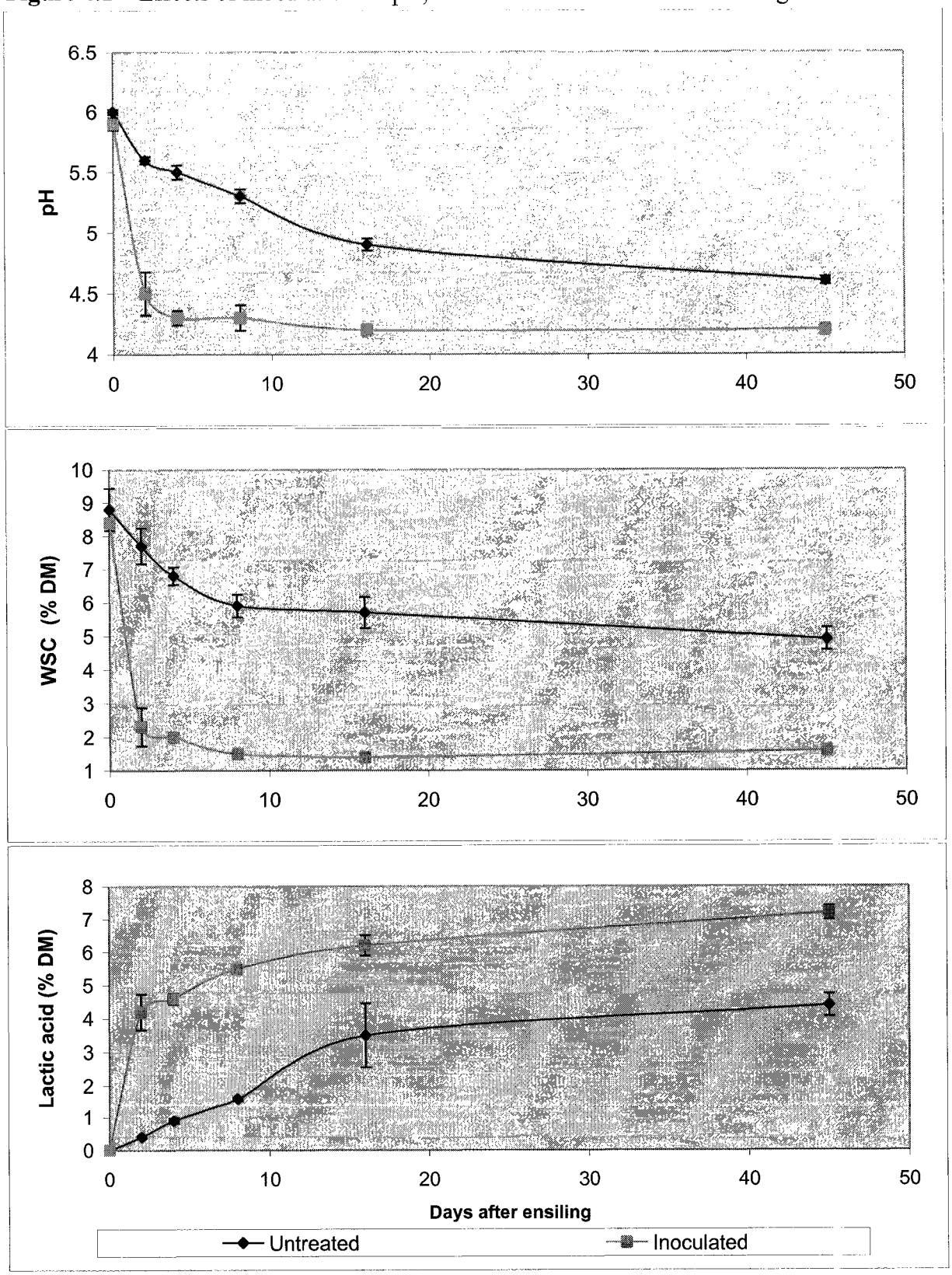
The pH of the silage treated with Sila-Bac® dropped faster ($P < 0.05$) than the pH of the untreated silage (Figure 4.1). The inoculated alfalfa silage reached a pH of 4.5 in the 2nd day post-ensiling whereas it took 45 days for the untreated alfalfa to reach a similar value. The pH of the inoculated alfalfa silage showed little change between day 4 and 45, but remained significantly lower ($P < 0.05$) than the pH of untreated alfalfa silage at all ensiling time. The pH of the untreated silage continued to decline up to day 45 post-ensiling.

Lactic acid concentration was higher ($P < 0.05$) for inoculated than for untreated in alfalfa silage at all ensiling time (Figure 4.1). Lactic acid content of inoculated alfalfa silage in the 2nd day of ensiling is again similar to the value reached in 45 day for the untreated silage. For both silages, the lactic acid content continued to rise up to day 45 post-ensiling. Inoculation seems to have boosted lactic acid bacteria concentration. This is shown by the noticeable increase in lactic acid from day 0 to day 2 after application of Sila-Bac®. In the control silage, lactic acid production was more gradual and slower and reflects the slower build up of lactic acid bacteria from the epiphytic LAB.

Our results are in good agreements with other studies, which showed higher lactic acid concentrations and lower pH for inoculated than untreated silages (Rice, 1989; Sheperd et al., 1995; Kung et al., 2003). Whiter and Kung (2001) studied the effects of inoculation with *Lactobacillus plantarum MTD1* (either in a dry or liquid form) on fermentation of alfalfa wilted at 30 and 54% DM. The authors found that with 30% DM, both forms of inoculation resulted in silages with more lactic acid and a lower pH than untreated silages after 2 days of ensiling. In silages containing 54% DM, dry and liquid inoculation produced more rapid decrease in pH between day 4 to 14 when compared with untreated silage, but the effect was greater when inoculant was applied in a liquid form.

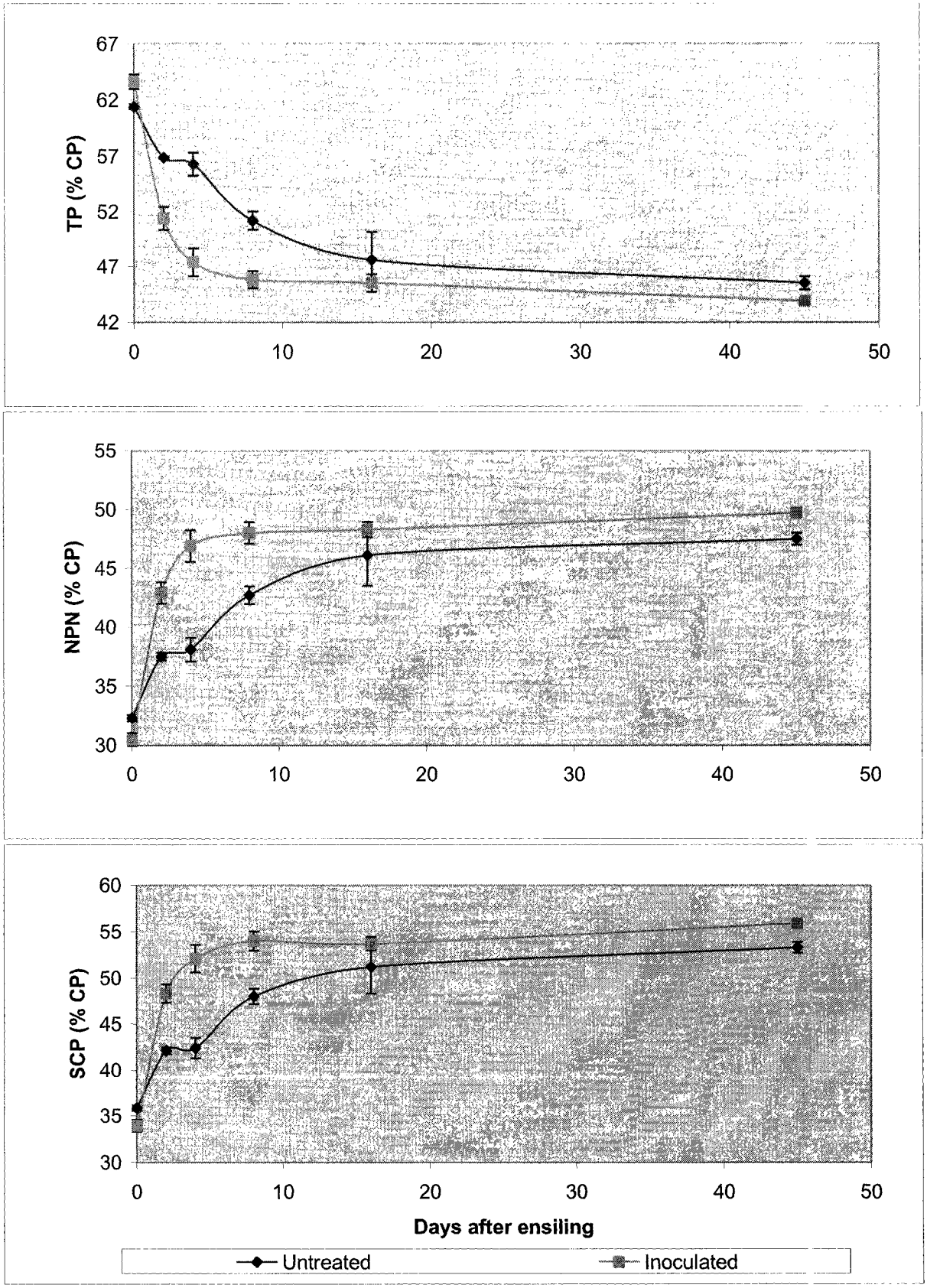
Compared with the work of Whiter and Kung (2001) our inoculated silage reached higher lactic acid content in day 2 (4.2% for Sila-Bac® vs. 1.04% for *Lactobacillus plantarum MTD1* treated alfalfa) and had higher lactic acid concentration at all ensiling times. However, lactic acid concentrations for untreated silage at all ensiling time were lower in our study than in the study of Whiter and Kung (2001). The very slow fermentation of untreated alfalfa silage in our study is likely due to its high DM content. It has been suggested that as the DM content of forages increases, the numbers of lactic acid producing bacteria decrease because low water activity restricts bacterial growth (Jones et al., 1992; Muck, 1990; Whiter and Kung, 2001).

Figure 4.1 – Effects of inoculation on pH, WSC and lactic acid of alfalfa silage



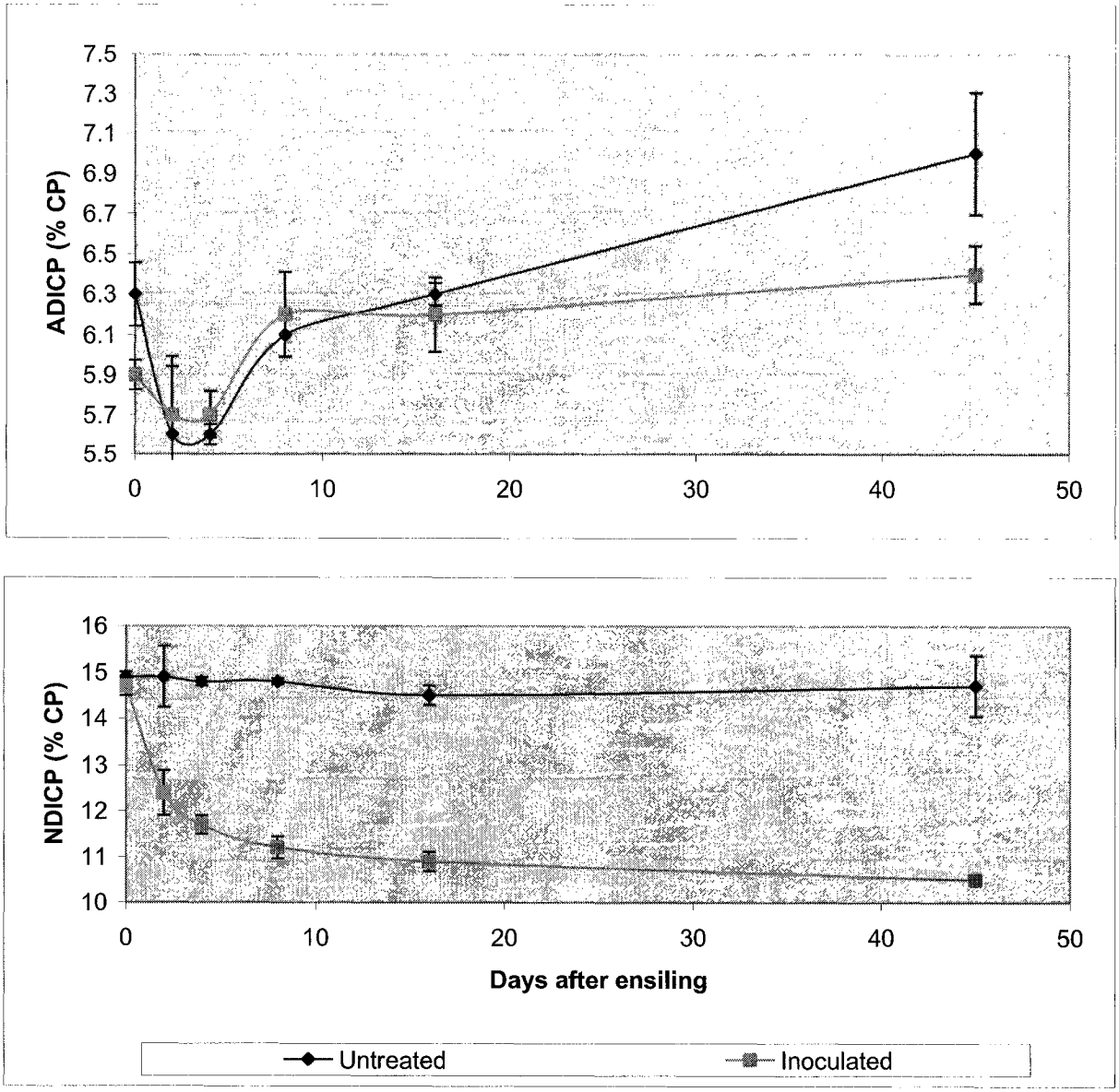
DM: Dry matter; WSC: Water Soluble Carbohydrates

Figure 4.2 – Effects of inoculation on TP, NPN and SCP of alfalfa silage



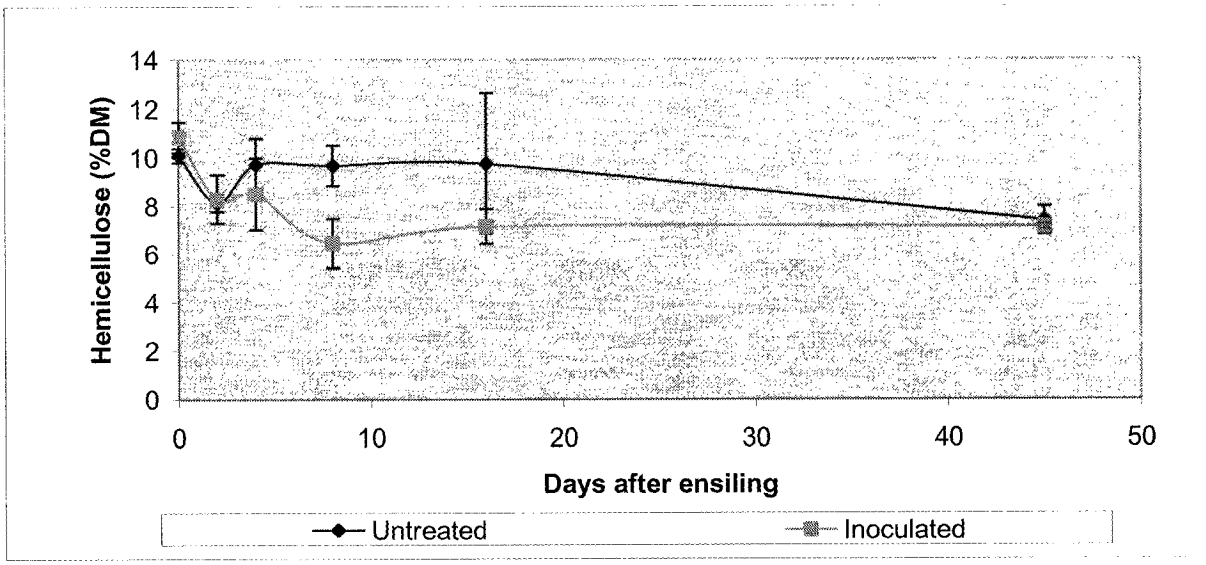
CP: Crude protein; TP: True Proteins; NPN: Non Protein Nitrogen; SCP: Soluble Crude Protein

Figure 4.3 – Effects of inoculation on ADICP and NDICP of alfalfa silage



CP: Crude protein; ADICP: Acid detergent insoluble crude protein; NDICP: Neutral detergent insoluble crude protein

Figure 4.4 – Effects of inoculation on hemicellulose of alfalfa silage



DM: Dry matter

WSC was lower ($P < 0.05$) for inoculated than for untreated alfalfa silage at all ensiling times and decreased more rapidly during the first 2 days of ensiling for inoculated than for untreated silage (Figure 4.1). For the same changes in pH and lactic acid concentration (from day 0 to day 2 for inoculated vs. day 0 to 45 for control), inoculated silage used more of its WSC content. To raise the lactic acid from 0 to 4.2%, 6.1% of WSC was degraded in the Sila-Bac® silage while only 3.9% WSC was degraded in the untreated silage to raise lactic acid from 0 to 4.4%. These results could be attributed to more hemicellulose degraded to WSC after 45 days of fermentation in the untreated silage compared with the hemicellulose degraded after just two days of ensiling in the inoculated silage (Figure 4.4). Dewar et al. (1963) and Morrisson et al. (1979) suggested that during prolonged storage periods, direct acid hydrolysis of cell wall polysaccharides might contribute to the increase in WSC.

DM recovery (after 45 days of ensiling) was higher ($P<0.05$) for the inoculated (97.5%) than the untreated (92.3%) alfalfa haylage. Similar to our results, Cai et al. (1999) found that inoculating silage with lactic acid bacteria reduced dry matter losses.

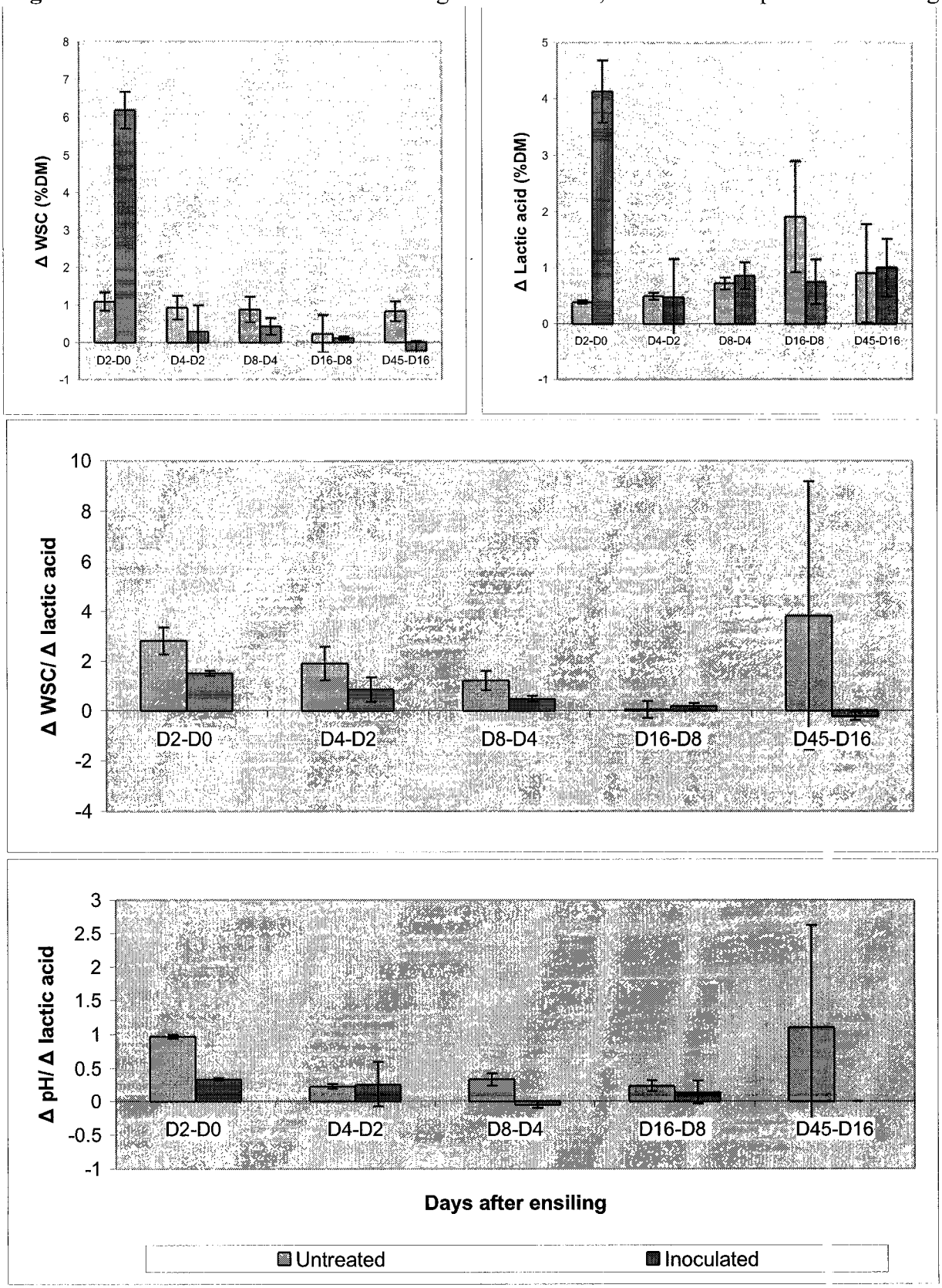
Higher DM recovery, faster pH decline and more rapid lactic acid production has been associated with better homolactic fermentation (Cai et al. 1999; Rooke et al. 1988; McDonald et al. 1991, Chap. 4). It is however interesting to note that similar pHs (4.5 and 4.6) have been obtained at similar lactic acid content (4.4% and 4.2%) for the control and treated silage respectively.

Based on the above results it can be stated that inoculation with Sila-Bac® improved alfalfa fermentation. Our results strongly support the work of White and Kung (2001) who found that microbial inoculation (*Lactobacillus plantarum*) could improve fermentation of high DM alfalfa silage. Other researchers have also reported positive effects of inoculants containing lactic acid bacteria on silage quality (Sheperd et al., 1995; Cai et al. 1999).

The efficiency of lactic acid production (change in lactic acid over change in WSC) seems to become more important as the fermentation progressed for both silages (Figure 4.5). Furthermore, in the inoculated silage while lactic acid rate of production was increasing (from day 2 to 45), WSC disappearance rate was decreasing. Finally from day 16 to 45 both WSC and lactic acid were increasing in the treated silage. Theses observations indicate that more lactic acid is being produced from less WSC. This is probably due to the degradation of hemicellose to WSC therefore compensating for the WSC used by for lactic acid production (Dewar et al., 1963; Morrisson et al., 1979; Figure 4.4). The efficiency of lactic acid production seems to be greater for the treated than for the control silage at all times (except on day 16 where they were equal). This could be explained by a more homolactic fermentation in the inoculated silage. Indeed ^{Ho}LAB are more efficient in converting glucose to lactic acid; they produce 2 moles of lactic acid while heterofermentative bacteria produce only 1 mole of lactic acid per mole of glucose (McDonald et al., 1991, Chap. 4).

The comparisons of the rate of pH change per g of lactic acid (Figure 4.5), showed that 1 g of lactic acid decreased the pH more in the control than in the treated silage. Again this might indicate that other acids may be responsible for the additional decrease in pH, i.e. a more heterolactic fermentation for the untreated alfalfa. The rate of decline in pH per g of lactic acid tends to decrease with time. This mostly reflects higher buffering capacity of silages as the fermentation progresses (McDonald et al., 1991, Chap.9).

Figure 4.5 – Effects of inoculation on change rates of WSC, lactic acid and pH of alfalfa silage



DM: Dry matter; WSC: Water soluble carbohydrates

4.1.2 Effect of inoculation on protein fractions

Soluble crude protein (SCP) and NPN increased rapidly from day 0 to day 4 (more than 53% increase in NPN and SCP; Figure 4.2) in the inoculated alfalfa. There were little changes in SCP and NPN between day 8 and day 45. The untreated silage had a more gradual rise in NPN and SCP from day 0 to 16 (42 % increase in NPN and SCP) followed by a slow increase from day 16 to day 45. At all ensiling times (except for day zero), SCP and NPN content of inoculated alfalfa silage were higher ($P < 0.05$) than untreated alfalfa silage. Changes in NPN and SCP followed a common pattern. At any ensiling time, NPN constituted most of SCP for both inoculated and untreated silage (88.8 to 90.3%).

Neutral detergent insoluble protein declined at a decreasing rate from day 2 to day 45 for the inoculated silage (Figure 4.3). However NDICP of the control silage was not affected by ensiling. Overall the NDICP of the untreated silage was only reduced by 0.2% after 45 days compared with a 4.2% decrease for the inoculated alfalfa silage. Reduction in NDICP reported in this study for inoculated alfalfa silage agrees with similar drop reported for other legume forages (Mustafa et al., 2002; Mustafa and Seguin, 2003a,b).

For both silages, ADICP decreased after 2 days of ensiling, reached a minimum between day 2 and day 4 and rose again between day 4 and day 8 (Figure 4.3). The ADCIP stabilized after 8 days of ensiling for the inoculated alfalfa; however it continued to rise until day 45 days post-ensiling in the untreated silage. Although the overall changes at day 45 were relatively small (0.5% rise for the inoculated alfalfa silage and 0.7% for the untreated alfalfa silage), the behavior of the ADICP curves was different from that observed by Mustafa et all (2002) who noticed an initial rise in ADICP content at day 2 followed by a reduction and stabilization. The low ADICP values suggest that the silages did not over heat during ensiling.

One of the main objectives of silage inoculation is to increase the acidification rate, which in turn is expected to limit protein degradation during early stages of fermentation. It is believed that plant enzymes collectively known as proteases are responsible for the breakdown of TP to NPN in the early stages of fermentation. The higher NPN values of

inoculated alfalfa are rather unexpected since it is thought that plant proteases activity is reduced when pH drops below 5 (McDonald et al., 1991, Chap. 3). Studies on the effects of inoculation on proteolysis are rather limited. In a similar study with high DM alfalfa (50% DM), Phillip et al. (1990) reported a trend for higher NPN in inoculated (Pioneer 1174®) inoculated than untreated alfalfa silages at several ensiling times. Furthermore, the authors found significantly higher NPN in inoculated alfalfa after 21 days of ensiling. Muck (1989) found that of the soluble protein fractions, only ammonia was reduced by inoculation. In contrast to our results, Petit and Flipot (1990) reported lower but non-significant SCP content for inoculated than for untreated alfalfa silage.

Reasons behind the increased proteolysis upon inoculation are unclear. Optimal pH for plant proteolytic activity is reported to range between 5 to 7 (McDonald et al., 1991, Chap. 3), and accordingly proteolytic activity was expected to be higher in the untreated than the inoculated silage. Factors other than plant proteases can also affect proteolytic activities. Heron and Edward (1989) suggested that acid hydrolysis could occur in ryegrass when silage pH is below 4.0. However, acid hydrolysis remains negligible as long as pH is higher than 3.5. Winters et al. (2000) suggested that proteolysis could be mediated by both plant and microbial activities. They suggested that some ^{Het}LAB could have proteolytic activity and Khalid and Marth (1990) isolated proteolytic strains of *Lactobacillus plantarum* from milk. From our results we are tempted to hypothesize that the inoculant added had a small proteolytic effect on high dry matter alfalfa silage.

In both silages, proteolysis was low. This is mainly due to the high DM content of the silages. Muck (1987) found a significant decrease in NPN as DM content of alfalfa increases. For alfalfa wilted to 50% DM (an initial NPN 25.6%), NPN increased to 64.8% after 60 days of ensiling. When alfalfa was wilted to 40% DM, final NPN was 82.6%.

True protein (TP) decreased ($P < 0.05$) more rapidly for the inoculated than for the untreated alfalfa silage (Figure 4.2). Inoculated alfalfa silage lost more than 25% of its TP between day 0 and day 4 (from 63.6 to 51.4% CP) compared with less than 8.5% for the untreated silage (61.4 to 56.9% CP). The drop in TP content stabilized from day 8 to day

45 for the inoculated alfalfa and from day 16 to day 45 for the untreated alfalfa silage. At all ensiling time, inoculated alfalfa had lower TP values. Differences in TP between inoculated and untreated alfalfa silage can be attributed to differences in other protein fractions (i.e. SCP, NPN and NDICP). Although inoculation increased rate of proteolysis, differences in SCP, NPN and TP between inoculated and untreated alfalfa at day 45 post-ensiling do not seem to be of great biological significance. When compared with untreated, inoculated alfalfa silage had only 1.6% lower TP, 2.2% higher NPN and 2.6% higher SCP.

4.2 Study II – Effects of inoculation on aerobic stability of alfalfa silage

Several measurements can be used to assess aerobic stability. Aerobic stability has been measured as the rise in temperature (Pitt, 1997; Oude Elferink et al., 1999), rise in pH (Cai et al., 1999; Mayne, 1993), loss in DM (Mayne, 1993; Henderson et al., 1979), and as change in silage microbial population (Cai et al., 1999; Pitt and Muck, 1991).

4.2.1 Effect of inoculation on temperature measurement

The effects of inoculation on aerobic stability of alfalfa silage are shown in Table 4.1 and 4.2. Our results showed an enhancement in aerobic stability of inoculated relative to untreated alfalfa silage. However, it is interesting to point that it took 14.3, and 16.9 days for the control silage to heat 1, and 2 °C above ambient temperature, respectively. These values are considerably higher than the 7 to 10 days period used to test aerobic stability in other studies (Keady and Murphy, 1997; Kung et al., 1991). These results suggest that on the short run, both inoculated and untreated alfalfa silages tended to be heat stable. However, differences were obvious after 2 weeks of air exposure. A 2-week long aerobic stability should not be an issue in commercial farms where silage-unloading rate is high.

4.2.2 Effect of inoculation on pH

After the silages were exposed to air for 21 days, pH was significantly higher ($P < 0.05$) for untreated than for inoculated alfalfa silage (Table 4.2) indicating that the untreated silage underwent spoilage while the inoculated alfalfa did not. The pH of the Inoculated silage was not altered after 21 days of aerobic exposure. These results support the temperature measurement data that showed no heating of the inoculated silage after 21 days of aerobic exposure. Our pH results are opposite to those of Cai et al. (1999) who found significantly higher pH of alfalfa treated with *Lactobacillus plantarum FG 1* and *FG 10* compared with untreated silage after 5 days of aerobic exposure. Our results are also opposite to the findings of Mayne (1993) who found that perennial ryegrass treated with *Lactobacillus plantarum* showed faster increase in pH when compared with the control since day 4 of aerobic exposure.

Table 4.1 – Temperature measurements of untreated and Sila-Bac® inoculated alfalfa silage after exposure to air

	Alfalfa silage		SEM	P > F
	Untreated	Inoculated		
Days to temperature rise (1°C)	14.3	>21	—	—
Days to temperature rise (2°C)	16.9	>21	—	—
Days to Max temperature (°C)	17.3*	>21	—	—
Max temperature (°C)	7.6*	N.A**	—	—
Temperature at day 10 (°C)	0.33	0.023	0.146	0.216
Accumulated hourly temperature from day 10 till 13 (°C)	35.03	11.25	11.249	0.250
Accumulated hourly temperature from day 10 till 21 (°C)	705	63	191.4	0.077
Accumulated hourly temperature from day 10 to max (°C)	340.87*	N.A**	—	—
Mean accumulated hourly temperature from day 10 to max (°C)	1.757*	N.A**	—	—

*Only two out of the three control samples were used to estimate these values
** Values are not available because the temperature did not reach a maximum

Table 4.2 – Chemical composition of untreated and Sila-Bac® inoculated alfalfa silage before and after exposure to air

	Alfalfa silage					
	Day 0		Day 21			
	Untreated	Inoculated	Untreated	Inoculated	SEM	P > F
pH	4.9	4.5	7.6	4.5	0.50	0.01
WSC (g kg^{-1})	2.93	2.09	1.88	2.40	0.25	0.22
Lactic acid (g kg^{-1})	1.98	3.82	0.78	4.14	0.44	0.01

WSC: Water Soluble Carbohydrates

4.2.3 Effect of inoculation on lactic acid and water soluble carbohydrates

Changes in lactic acid content after exposure to air are shown in Table 4.2. Lactic acid was higher ($P < 0.05$) for inoculated than for untreated alfalfa silage following 21 days of aerobic exposure. About 60% of the initial lactic acid was degraded in the untreated alfalfa silage. Water-soluble carbohydrates were not affected by inoculation. These findings support the changes in pH and temperature previously discussed and gives an insight on the mode of action of aerobic spoilage. Indeed, lactic acid degrading yeast has been associated with initiation of aerobic spoilage by degrading lactic acid and thus allowing the pH to rise (Barry et al., 1980). The aerobic metabolism of lactic acid, WSC and possibly other substrate generates heat therefore lead to rise in silage temperature (Woolford, 1984).

Several researchers studied the effect of ^{Ho}LAB on aerobic stability and found controversial results. While some authors found positive effects of inoculation on aerobic stability of grasses (Meeske et al., 1999; O'Kiely, 1996), corn (Fellner et al., 2001), and barley (McAllister et al., 1995; Moshtaghi et al., 1999) silages, others found no effects on grasses (Keady and Murphy, 1996) or alfalfa (Kung et al., 1987) or even negative effects on aerobic stability of grasses (Keady and Murphy 1997; O'Kiely 1996), corn (Wardynski, 1993; Mayne, 1993), alfalfa (Cai et al. 1999; Kung et al. 1991) or pea/wheat bi-crops (Salawu et al., 2001) silages.

The inconsistent response of aerobic stability to inoculation has been attributed to the initial chemical and microbial composition of ensiled forage. Meeske et al. (1993) postulated that aerobic stability of grass is most likely to be decreased upon inoculation when the fresh forage is high in WSC (more than 60 g kg^{-1} DM), lactic acid (more than 70 g kg^{-1} DM) and lactate assimilating yeasts. Muck and Bolsen (1991) claimed that inoculation could increase or decrease aerobic stability depending on pH, lactic acid, and acetic contents. Low pH increases toxicity of lactic and acetic acid to yeasts, however, homolactic fermentation can decrease aerobic stability since acetic acid is a stronger inhibitor of yeasts than lactic acid. Some studies showed a positive effect of inoculation on aerobic stability without any changes in pH, lactic acid and acetic acid (McAllister et

al., 1995). The authors concluded that there might be some unknown inhibitors of lactate metabolizing microorganisms produced in inoculated silage.

The initial composition of the untreated and inoculated silages that were used for the aerobic stability study is shown in Table 4.2. In our study, inoculated silage had initially 0.4 units lower pH than untreated silage (4.5 vs. 4.9 for the inoculated and untreated silage respectively), 1.84% higher lactic acid (3.82% vs. 1.98%), and 0.84% less WSC (2.09% vs. 2.93%). The undesirable effects of high residual WSC, and low acidity on aerobic stability have been reported by several researchers (Weinberg et al., 1993; Cai et al., 1999). These differences in the initial pH and WSC and lactic acid content of the silages could explain the improved aerobic stability of the inoculated silage.

4.3 Study III - Effects of inoculation on *in situ* nutrient degradability of alfalfa silage

The results of the *in situ* study are presented in Table 4.3. Inoculation had no effects on ruminal DM kinetic parameters. The average values of soluble DM, slowly degradable DM, degradation rate of the slowly degradable DM and effective DM digestibility were 39.4%, 38.7%, 11.6% h^{-1} and 66.3%, respectively. Soluble CP fraction was higher ($P < 0.05$) for inoculated than untreated alfalfa silage. However, inoculation had no effect on the *in situ* slowly degradable CP fraction or on its rate of degradation. Effective ruminal CP degradability was also similar for both silages (average 84.3% of CP) suggesting that the difference in soluble CP fraction was not large enough to affect ruminal CP degradability. *In situ* soluble NDF fraction was small and similar for both silage treatments (Table 4.3). The *in situ* slowly degradable NDF and its rate of degradation were similar for both silages (average 40.4% NDF and 6.4% h^{-1} respectively). Effective ruminal NDF degradability of alfalfa silage was not affected by inoculation and averaged 24.3%.

Few data on the effects of inoculation of alfalfa on ruminal degradabilities are available. In agreement with our findings, Hristov and McAllister (2002) inoculated barley silage with three different PIONEER brand inoculants and found no significant effects on DM ruminal degradability as a result of inoculation. However, other studies reported improved ruminal DM degradability of whole-crop barley silage (McAllister et al., 1995) or grass (Patterson et al. 1997; Mandebvu et al., 1999) as a result of inoculation.

Salawu et al. (2001) found that inoculating wheat/pea bicrop with *Lactobacillus plantarum* decreased soluble N without affecting effective degradability. Our results are more similar to those of Keady et al (1994) who inoculated perennial ryegrass with *Lactobacillus plantarum* and found an increase in soluble N in the inoculated silage. Unlike our results, the increase in soluble N was large enough to result in a higher effective degradability.

Our NDF ruminal degradability results contradicts the work of Salawu et al. (2001) who treated pea/wheat bi crop with 2 LAB inoculants and found a decrease in soluble NDF, an increase in potentially degradable NDF and general decrease in ruminal NDF degradability. However, several studies reported an increase NDF digestibility as a result of inoculation (Martinsson, 1992; Keady and Steen, 1995; Meeske et al., 1999).

Table 4.3 – Ruminal nutrient kinetic parameters and effective degradabilities of inoculated and untreated alfalfa silage

	Alfalfa silage		SEM
	Untreated	Inoculated	
Dry matter (DM)			
Soluble (%DM)	39.3	39.4	0.37
Slowly degradable (%DM)	39.0	38.3	0.53
Degradation rate (% h^{-1})	11.3	11.9	0.79
Lag time (h)	0.2	0.1	0.06
Effective degradability (%)	66.3	66.2	0.71
Crude protein (CP)			
Soluble (% of CP)	57.8b	59.4a	0.47
Slowly degradable (% of CP)	35.5	33.0	0.97
Degradation rate (% h^{-1})	14.3	16.2	0.73
Lag time (h)	0.2	0.4	0.16
Effective degradability (%)	84.0	84.6	0.25
Neutral detergent fiber (NDF)			
Soluble (% NDF)	2.0	1.8	0.10
Slowly degradable (%NDF)	40.5	40.3	0.40
Degradation rate (% h^{-1})	6.7	6.0	0.27
Lag time (h)	0.2	0.3	0.19
Effective degradability (%)	23.4	25.2	0.56

a,b Means within row followed by different letters are different ($P<0.05$)

4.4 Study IV - Effect of inoculation on total tract digestibility

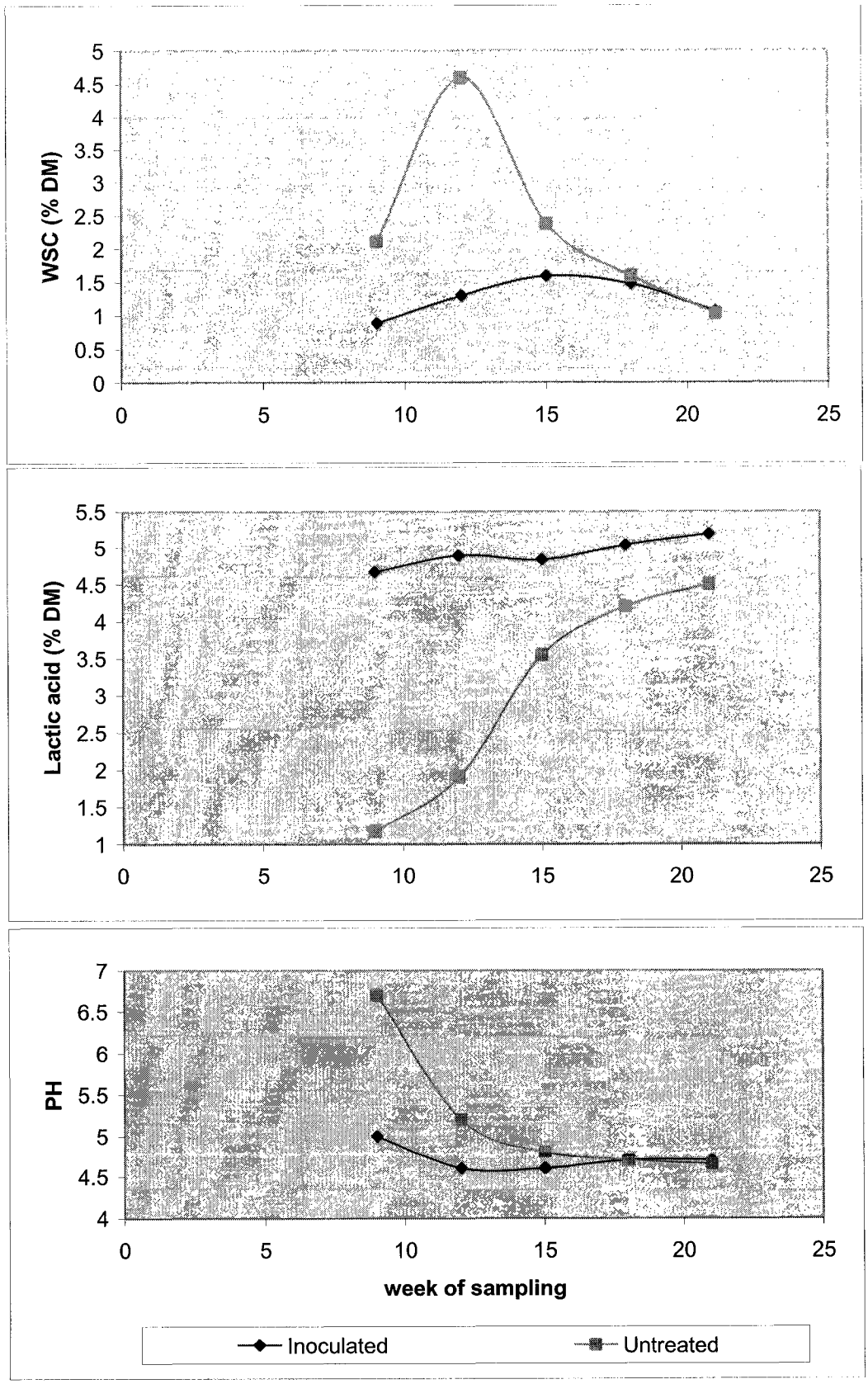
4.4.1 Silage chemical composition

The fermentation parameters of untreated and inoculated alfalfa silage used in the dairy and digestibility studies are shown in Figure 4.6 and 4.7. The pH was higher for the untreated than inoculated silage (Figure 4.6). Difference in pH between silages was more pronounced in week 9 and 12 (pH 6.7 for control in week 9 vs. pH 5 for the inoculated silage) and gradually decreased until week 18 where both silages had a pH of 4.7. A similar trend was observed for lactic acid. Lactic acid was particularly low in the control silage in week 9 and 12 (1.17 and 1.9% DM), and increased to 4.2 and 4.5% in week 18 and 21, respectively. WSC was higher in the control silage until week 18 where both silages had similar values.

The results of the analysis of Volatile fatty acids (VFA) are shown in figure 4.7. Acetic acid was lower for the untreated silage in week 9 and week 12 (8.19 vs. 4.83 g kg^{-1} and 16.69 vs. 12.46 g kg^{-1} in week 9 and week 12 for the untreated and inoculated silage respectively). No major differences were observed after week 15. Propionic acid and butyric acid remained lower in the control silage during all sampling time (except for butyric acid in week 18)

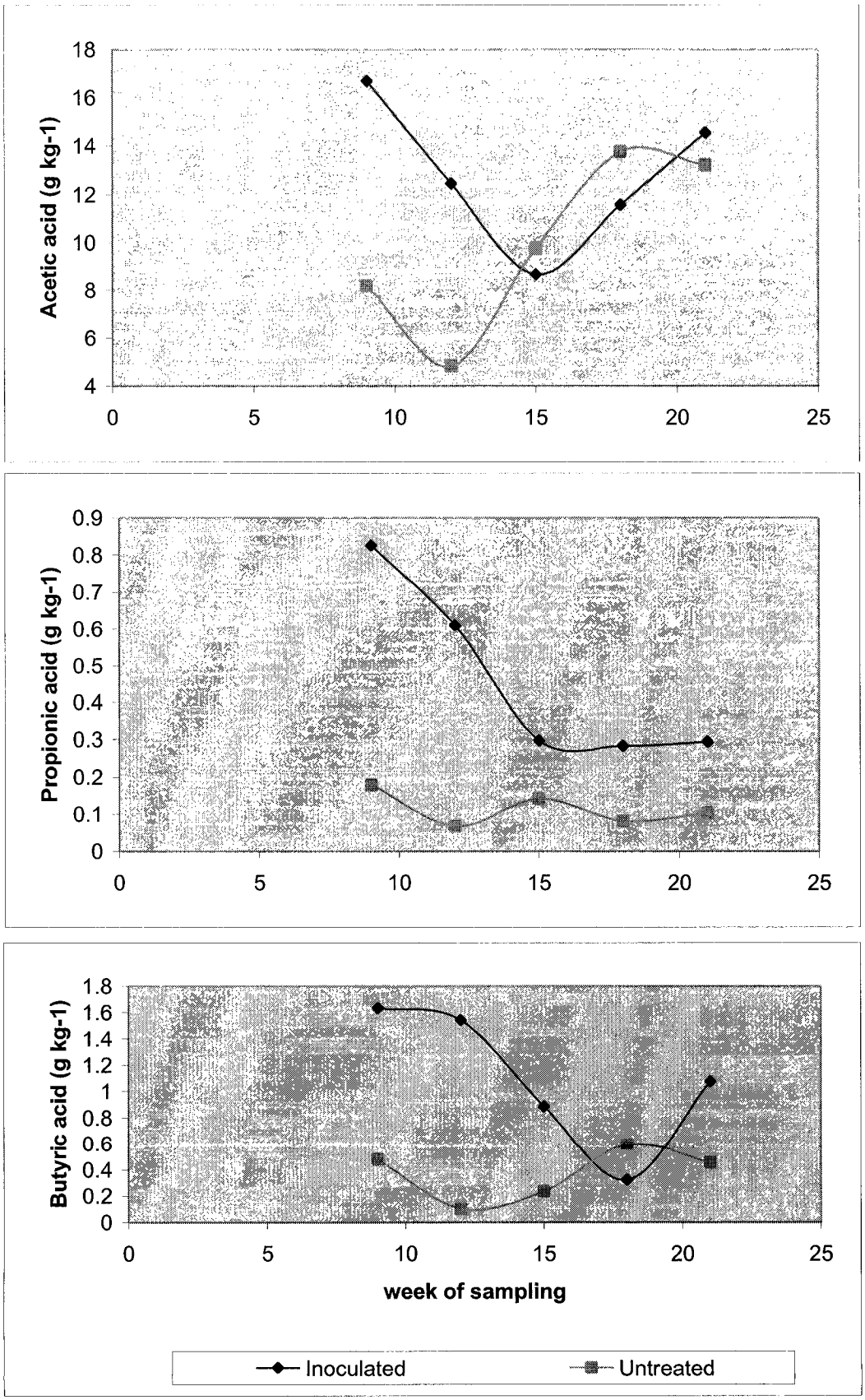
It is clear form the above results that the inoculated silage had overall superior fermentation parameters than untreated silage and that differences in the composition between the two silages were highest during the early sampling weeks. According to previous studies (Mustafa and Seguin, 2002), fermentation is expected to stabilize within 45 days, and therefore the improvement in composition of the control silage at later sampling date is not likely due to an extended fermentation during a longer time. These changes might be attributed to several factors. The fermentation might not be equal in all parts of the silo and that in the lower portion of the tower the fermentation might be superior possibly because of higher packing density and / or infiltration of air in the higher portion of the silage and the metabolism of lactic acid produced. In that case, the inoculant was useful in improving the fermentation at the upper layers of the silo and obtained more homogenous silage.

Figure 4.6 – Changes in pH, WSC and lactic acid in the tower silos



DM: Dry matter; WSC: Water soluble carbohydrates

Figure 4.7 – Changes in acetic, propionic and butyric acid in the tower silos



Higher DM losses, Maillard products, higher pH, lower lactic and other organic acids (namely acetic acid) are all indication of aerobic deterioration. When aerobic spoilage occurs, yeasts could degrade lactic acid and other compounds to produce CO_2 and H_2O, which contribute to DM losses. The degradation of these compounds is exothermic and causes the temperature within the silage to rise therefore increasing the Maillard product in the silage. The degradation of acids leads to rise in the pH.

Our results tend to indicate that aerobic spoilage happened in the early weeks (week 9 and 12) and this deterioration was more significant in the untreated than the inoculated silage. The higher spoilage in the early weeks of the study could be explained by the unloading rate of the silage. Although the rate was not calculated in this experiment, few animals were on trial during the early weeks, which indicates low unloading rate, and that the silage had time to spoil. Since our tower silos were top unloading type, lower unloading rates, mean that the surface of the silage was exposed to air for longer time before being unloaded. The higher aerobic spoilage in the untreated silage compared with the inoculated agrees with our previous results in the aerobic stability trial, where inoculation significantly improved aerobic stability.

The reasons for the lower propionic and butyric acid in the control compared with the inoculated silage are unclear. Although these differences are consistent throughout the sampling weeks, there are not of important magnitude. For both silages propionic acid was lower than 0.1% DM, acetic acid ranged between 0.5 and 2% DM through all the sampling period. These results comply with the normal values for alfalfa silages ensiled at 45-55% DM (Kung, 2000). Butyric acid values of the control silage are again representative of a normal fermentation (< 0.1 %DM) during all sampling weeks, however the butyric acid value of the inoculated silage were slightly above that range in the first two sampling weeks (0.16 and 0.15 % DM for week 9 and 12 respectively). Lactic acid values were higher, and pH was lower than the normal (2 to 4% DM and 4.7 to 5.0 for lactic acid and pH, respectively) for the treated silage during all sampling weeks, and both silages (treated and untreated) showed higher lactic acid and lower pH than the normal during the last two weeks of sampling (week 18 and week 21). Overall

both silages showed normal to slightly above normal quality when compared with high DM (44 55 %DM) alfalfa silage (Kung, 2000).

4.4.2 Effect of feeding inoculated silage on total tract digestibility

Results of the digestibility trial are shown in Table 4.4. Inoculation had no effect on DM intake. Total tract digestibility of DM (average 65%), CP (average 64.8%), NDF (average 60.4%), ADF (62.1%) and GE (69.2%) were similar for both diets. These results are in agreement with other studies, which used PIONEER inoculants. Phillip et al. (1990) inoculated alfalfa with PIONEER brand 1174® and found no differences in total tract nutrient digestibilities between inoculated and untreated alfalfa silage except that of fiber, which was improved as a result of inoculation. The lack of difference in fiber digestibility between inoculated and untreated alfalfa silage in the present study may be due to the fact that untreated and inoculated alfalfa silages were fed as part of total mixed diet (40% of the diet) while in the study of Phillip et al. (1990) alfalfa silages were fed alone. It is possible that the effect of inoculant on fiber digestibility of alfalfa silage has been diluted by feeding it as part of a total mixed diet.

McAllister et al. (1995) treated barley silage with PIONEER 1174® inoculants and found no effect of inoculation on DM, ADF, NDF and CP digestibilities when the silage was offered with concentrates to ram lambs. Sharp et al. (1994) inoculated perennial ryegrass with Pioneer 1188® and found no effects on apparent digestibility of organic matter, or CP when the treated silage was fed alone or with concentrates to Jersey heifers. Fellner et al. (2001) treated high moisture ear corn with a mixture of *Lactobacillus plantarum* and *Enterococcus faecium* (Pioneer 1189®). Control and inoculated silages were fed as part of a complete diet (75% of the diet DM basis). No effect on DM, organic matter, and ADF digestibilities were found.

Other studies using inoculants from different sources gave different results. Gordon (1989) inoculated ryegrass with ECOSYL® and found significant increase in DM, energy and CP digestibilities when inoculated silage was fed to sheep. However, when silages

were mixed with concentrates and fed to cows, inoculation had no effect on nutrient digestibilities. Mayne (1990) inoculated perennial ryegrass with ECOSYL® and found increased DM, energy and nitrogen digestibilities when treated silage was fed to sheep. When the silages were fed with concentrates to cows, inoculation led to improvement in DM digestibility. Keady et al. (1994) inoculated perennial ryegrass with *Lactobacillus plantarum* (ECOSYL ICI®). When the silage was fed to growing cattle, improvements in DM, energy, CP and NDF digestibilities were reported. When fed as part of a ration (1 kg concentrate per head), improvements in organic matter, and nitrogen digestibilities were observed. Keady and Steen (1995) observed similar results to those of Keady et al. (1994) when perennial ryegrass was inoculated with ECOSYL®. O'Kiely (1996) inoculated a grass mixture with *Lactobacillus plantarum MTD1* (ECOSYL ICI®) and found improved DM digestibility when treated silage was fed to steers. Few studies found no improvement in digestibilities upon inoculation with ECOSYL® (Rooke and Kafilzadeh, 1994).

Different strains of LAB used or different selection criteria for the strains could explain these observations. Other studies used inoculants from different sources and obtained variable results regarding digestibility. Martinsson (1992) used a mixture of *Streptococcus faecium*, *Lactobacillus plantarum* and *Pediococcus sp.* on grass. Inoculation increased DM, organic matter and energy digestibility of silage when fed to sheep. Mayne (1993) inoculated perennial ryegrass with *Lactobacillus plantarum* and found reduced digestibility in term of DM, organic matter, energy and CP in 1st regrowth, and no effects on second regrowth (high DM, low WSC) when the silage was fed to sheep. Keady and Murphy (1996) used an inoculant, which consisted of a mixture *Lactobacillus plantarum*, *Pediococcus acidilactici* and *Lactococcus lactis lactis* on perennial ryegrass and found no effects on DM, organic matter, NDF, and CP digestibility when the inoculated silage was fed with concentrates to cows. Charmley et al. (1996) inoculated grass and wheat crops with *Lactobacillus casei*, *Lactobacillus plantarum* and *Streptococcus lactis*. Inoculation decreased DM, organic matter, CP, ADF and NDF of grasses, and ADF and NDF of wheat when the silages were fed to sheep. It

appears that the improved nutrient digestibility as a result of inoculation is greatly affected by the source of inoculant.

Table 4.4 – Intake and total tract nutrient digestibilities of cows fed diets containing inoculated and untreated alfalfa silage

	Alfalfa silage		SEM
	Inoculated	Untreated	
Dry matter intake (kg d^{-1})	20.1	20.1	3.05
Digestibility coefficient (%)			
Dry matter	65.6	65.6	2.26
Crude protein	64.4	65.2	1.37
Neutral detergent fiber	59.4	61.4	2.09
Acid detergent fiber	61.6	62.5	2.26
Gross energy	69.2	69.2	2.15

4.5 Study V - Performance of dairy cows fed inoculated alfalfa silage

4.5.1 Dry matter intake

Dry matter intake of dairy cows was not affected by inoculation (average 20.2 kg day^{-1}, Table 4.5). Our results are in accordance with other studies, which found no improvement in DM intake of lactating cows (Ahrens et al., 1981; Kung et al., 1987; Kent et al., 1988; Fredeen et al., 1991), lambs (Phillip et al., 1990) or heifers (Phillip et al., 1992) fed inoculated alfalfa silage. Our results are also in agreement to other studies that showed no effect of inoculated grass silages (Keady et al., 1994; Keady and Murphy, 1996; Mayne, 1993; Keady and Murphy, 1997; O'Kiely, 1996; Charmley et al., 1996) corn silage (Fellner et al., 2001; Wardynski et al., 1993) and barley silage (McAllister et al. 1995) on DM intake. However, some researchers have reported improved DM intake when inoculated grass (Gordon et al. 1989; Mayne 1990; Sharp et al. 1994, Rooke and Kafilzadeh 1994, Martinsson 1992, Keady and Steen 1995; Petit et Flipot 1990; Meeske et al. 1999), and corn (Kung et al. 1993) silages were fed to ruminants.

The increased DM intake of inoculated silages has been attributed to improvement in fermentation parameters (Petit and Flipot, 1990; Sharp et al., 1994) and / or enhancement in NDF digestibility (Gordon et al., 1989; Mayne, 1990; Martinsson, 1992; Keady and Steen, 1995; Meeske et al., 1999). However, others provide no explanation (Rooke and Kafilzadeh, 1994). In our experiment the inoculant failed to have significant effect on NDF digestibility of the ration (Table 4.3).

4.5.2 Weight gain

Inoculation had no significant effects on body weight gain. Our results are similar to the findings of many researchers (Kent et al. 1988; Kung et al. 1993; Sharp et al 1994; Mayne 1990; Martinsson 1992; Kung et al. 1987; Keady and Murphy 1997).

Table 4.5 – Performance of dairy cows fed diets containing untreated and inoculated alfalfa silage

	Alfalfa silage		SEM	P>F
	Untreated	Inoculated		
Dry matter intake (kg d^{-1})	21.1	19.3	0.87	0.86
Yield				
Milk (kg d^{-1})	43.2	41.1	1.45	0.33
4% FCM (kg d^{-1})	41.5	39.0	2.4	0.26
Fat (kg d^{-1})	1.5	1.3	0.10	0.40
Protein (kg d^{-1})	1.1	1.3	0.22	0.5872
Lactose (kg d^{-1})	2.1	1.9	0.75	0.2081
Composition				
Fat (%)	3.76	3.63	0.20	0.67
Protein (%)	2.64	3.13	0.48	0.47
Lactose (%)	4.63	4.77	0.051	0.098
Milk urea nitrogen (mg dL^{-1})	13.8	13.6	0.50	0.83
Milk efficiency				
Milk/feed (kg kg^{-1})	2.1	2.2	0.05	0.24

FCM: Fat Corrected Milk

4.5.3 Milk yield

Effects of feeding inoculated alfalfa silage on milk yield are shown in Table 4.5. Inoculation had no significant effects on total and 4% fat corrected milk yield. Since we obtained no effect on DM intake total tract nutrient utilization, differences in metabolisable energy intake and milk production between untreated and inoculated alfalfa silage treatment were not expected. In that sense the results obtained are expected and explainable.

Several studies reported positive (Kent el al., 1988; Gordon et al., 1989; Mayne 1990; Martinsson 1992; Kung et al. 1993) or no effects (Mayne, 1993; Keady and Murphy, 1996 Keady and Murphy, 1997) of silage inoculation on milk yield. Improvement in milk yield was for most part attributed to increased metabolisable energy intake, either due to increased dry matter intake (Kung et al., 1993; Mayne, 1990; Gordon et al., 1989; Martinsson, 1992) or improved dry matter digestibility (Martinsson, 1992).

Treatment by parity interaction was reported for milk efficiency (Table 4.5). When parities were analyzed separately, no significant differences between untreated and inoculated alfalfa silage diets were observed for cows in parity two and parity three. However, it is interesting to point out that milk efficiency was better ($P < 0.05$) for parity one cows fed inoculated alfalfa silage than for those fed the untreated alfalfa silage diet. The reason for improvement in milk efficiency for cows in parity one and not in parity two or three is unclear. It might be possible that inoculation increased DM digestibility for primparous but not for multiparous cows. Improved efficiency of metabolisable energy utilization could be another explanation. Metabolisable energy efficiency could be enhanced by the ability of the inoculant to increase ruminal pH and the concentrations of iso-acids (Fellner et al., 2001) or the concentration of propionate in the rumen (Keady et al., 1994).

4.5.4 Milk composition

Milk composition was similar for cows fed untreated and inoculated alfalfa silage diets (Table 4.5). Our results agree with other studies that found no effect of inoculation on milk composition, whether inoculation improved (Kent et al., 1988; Mayne, 1990; Martinsson, 1992; Kung et al., 1993), or failed to enhance animal performance (Kung et al., 1987 Kent et al., 1988; Fredeen et al., 1991; Mayne, 1993; Keady and Murphy, 1997).

However, in few studies, inoculation with ^{Ho}LAB increased milk fat % (Mayne 1990), milk protein % (Kung et al., 1987; Keady and Murphy, 1996) or overall milk composition (Gordon, 1989). Mayne (1990) inoculated first regrowth of perennial ryegrass with *Lactobacillus plantarum* (Ecosyl®) and found an improvement in milk fat % of dairy Friesian cows in early lactation. Keady and Murphy (1996) inoculated perennial ryegrass with a mixture of *Lactobacillus plantarum, Pediococcus acidactili* and *Lactobacillus lactis lactis* and enzymes and fed the silage to lactating dairy cows 26 days in lactation. The authors reported increased milk protein concentration due to inoculant treatment and associated this increase to the higher propionate and VFA content of rumen liquor when cows were fed the treated silage. Gordon (1989) found higher milk fat, protein and lactose % of dairy Friesian cows in early lactation fed ryegrass inoculated with *Lactobacillus plantarum* (Ecosyl®).

5 General conclusion

Some of the most important purposes of silage inoculation are: increase acidification rate during the initial phase of fermentation; limit microbial and enzymatic deterioration and prevent extensive proteolysis. The objectives of the first experiment were to test the effects of inoculation with multiple strains of *Lactobacillus plantarum* on the ensiling characteristics of high DM alfalfa silage. Inoculation improved fermentation by reducing pH and increasing lactic acid production. However, inoculation increased proteolysis of alfalfa protein during early stages of ensiling, and resulted in slightly higher proteolysis after 45 days of ensiling. The higher proteolysis rate may be attributed to higher microbial activity in the inoculated high DM alfalfa silage. As the evolution in pH, lactic acid and true protein showed, the addition of *Lactobacillus plantarum* induced fermentation and proteolysis simultaneously.

It is believed that homolactic fermentation, while enhancing silage quality may reduce its aerobic stability (Kung et al., 1991; Weinberg et al., 1993). The objectives of our second study were to determine the effects of inoculation on the aerobic stability of alfalfa. After 21 days of aerobic exposure, inoculation resulted in higher aerobic stability as indicated by a lower rise in silage temperature and pH. The higher deterioration of the control silage might be partly attributed to the differences in initial chemical composition between the two silages. The control silage was less acidic and had more residual water soluble carbohydrates, both factors have been shown to decrease aerobic stability (Weinberg et al., 1993; Cai et al., 1999). Compared with other studies with alfalfa silage (Kung et al., 1991), both treatments gave silages with fairly high aerobic stability.

The objectives of the third study were to determine the effects of inoculation on *in situ* ruminal degradability. The improvement in fermentation characteristics as a result of inoculation in the first study was not reflected on ruminal *in situ* nutrient degradability which agree with other studies (Hristov and McAllister, 2002). In the fourth study we determined the effects of inoculation on the total tract digestibility of a total mixed ration containing alfalfa silage. Compared with the control, inoculation had no effect on DM,

protein, fiber and energy digestibility. Inoculation with Sila-Bac 11H50® does not seem to affect the degradability of the silage nor the digestibility of the ration that contains the silage. The results of the *in situ* study suggested that the failure to report any improvement in total tract digestibility in the fourth study is not likely to be the result of a dilution factor.

The objectives of the fifth study were to determine the effects of feeding inoculated alfalfa silage on the performance of dairy cows in early lactation. Feeding the inoculated silage did not show any benefits in terms of DM intake, milk yield or milk composition. The lack of effect on milk yield is consistent with the inability of the inoculant to improve DM intake and digestibility. Studies that reported improvements in milk yield have attributed it to higher intake of metabolisable energy (Mayne, 1990; Martisson, 1992).

Based on the results of this research the following conclusions can be made:

- Inoculation of high DM alfalfa with Sila-Bac 11H50® have improved the fermentation rate in the mini silos, but it did not have important effects on the extent of proteolysis after 45 days of ensiling.
- Inoculation of alfalfa silage with Sila-Bac 11H50® improved the aerobic stability of the alfalfa silage.
- It seems that inoculation with Sila-Bac 11H50® improved the quality of silage in the tower silos during the earliest sampling weeks, but the quality of the control and inoculated silage were similar in the later sampling weeks.
- Inoculation failed to improve ruminal degradability of the alfalfa silage. When diets containing inoculated and untreated alfalfa silage were fed to dairy cows, no improvement in total tract nutrient digestibility was observed as a result of inoculation.
- Feeding the same diets to lactating cows did not result in any improvement in DM intake or milk yield.

Future considerations

- The analysis of the silage in the tower silos showed clearly that the silage quality was not homogeneous throughout the sampling weeks. It would be interesting to test the effect of inoculation on the ensiling characteristics of silage in the tower silos at different dept levels.
- Inoculation with multiple strains of *Lactobacillus plantarum* (Sila-Bac 11H50®) clearly improved the fermentation parameters in the mini-silos, however, unexpectedly it also lead to higher proteolysis rate. It would be interesting to test the proteolytic activity of different strains of *Lactobacillus plantarum* inoculants and see whether these can affect the overall proteolysis in the silage.

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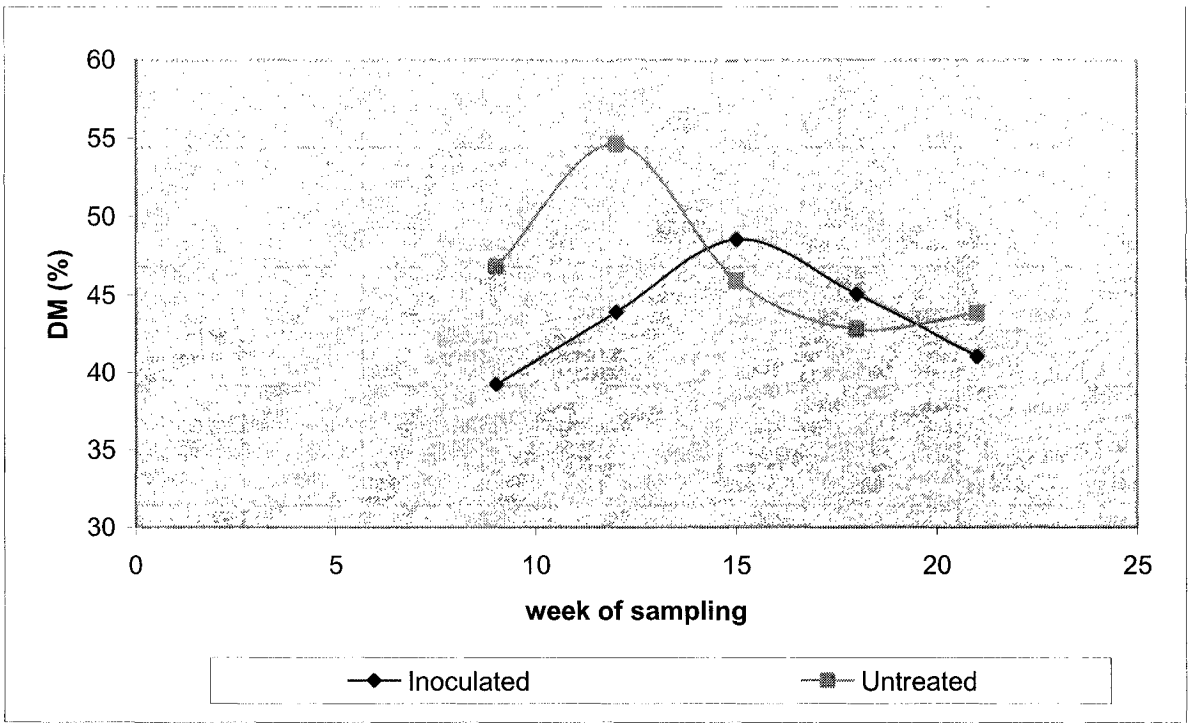
Appendix

In general, Sila-Bac® treated silage had lower NDF compared with the control (Figure 4.9). When the silages NDF were corrected for their protein content, the inoculated silage still tended to have lower values (fig 4.10). Differences in ADF and protein corrected ADF were negligible except for week 15 (fig 4.9 and fig 4.10). When the tower silage NDF and ADF were compared to those of the wilted forage, the silages (Sila-Bac® and control) had higher values at all time (except for NDF values on week 18). At week 9, NDF were 117% and 119 % and ADF 142 % and 140% times those of the wilted forage for Sila-Bac® and control silage respectively. However at week 21 the silages NDF and ADF decreased. NDF measured was 108 % and 107% and ADF 127% and 128% times that of the fresh forage for Sila-Bac® and the control silage respectively.

These observations suggest higher DM losses as CO_2 probably at the earlier weeks of the experiment. When the silage samples were compared with the wilted forage, ADF increase was more important than NDF increase. This is probably due to hemicellulose broken down during long fermentation periods (Dewar et al. 1963). When hemicellulose was calculated (protein corrected NDF- protein corrected ADF) the control silage was higher during all but week 21 (fig 4.10).

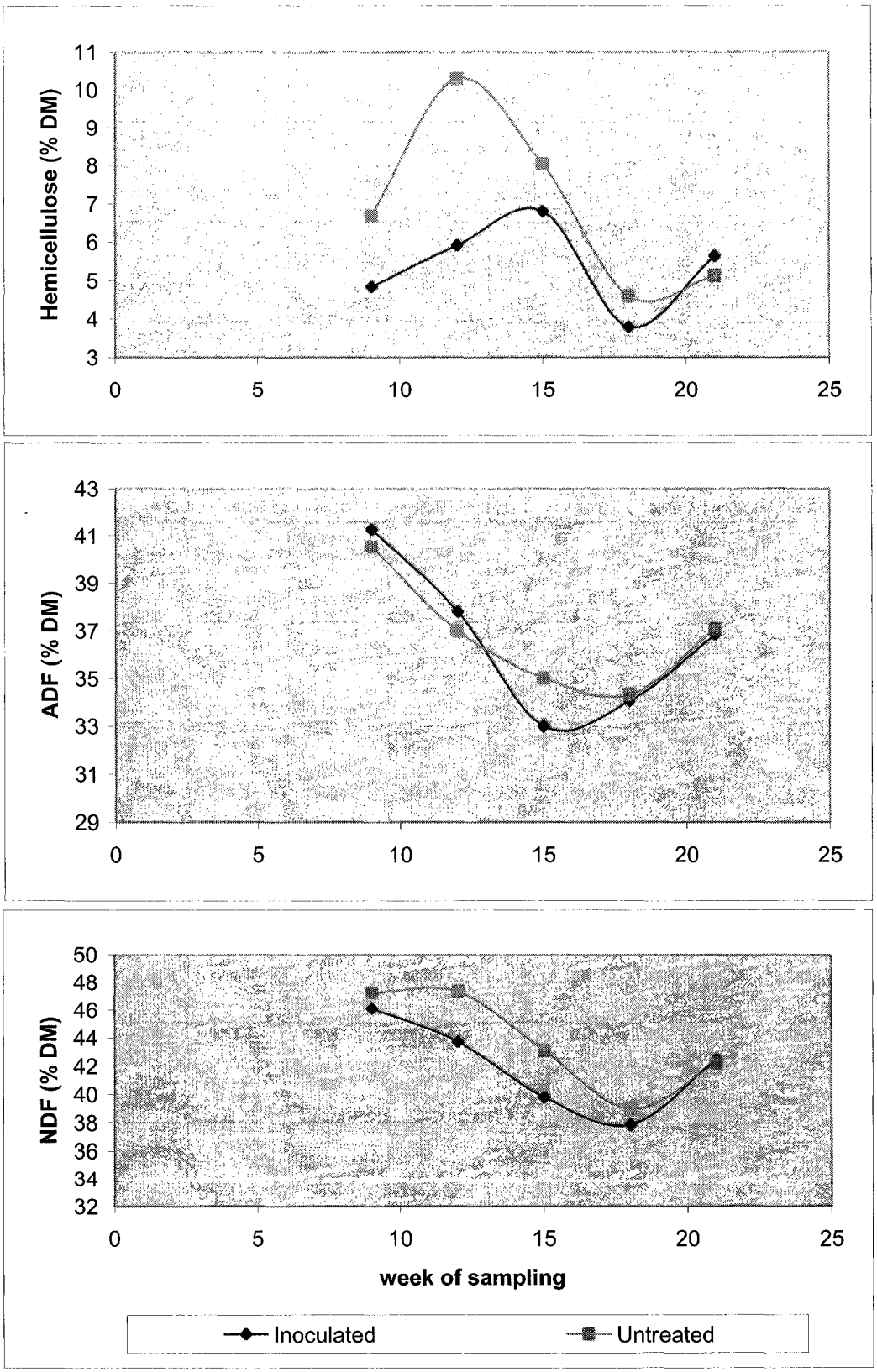
ADICP was 139% and 177% that of the wilted forage for the Sila-Bac® and the control silage at week 9. ADICP values were 131% and 132% that of the wilted forage at week 21 suggesting higher Maillard products at the earlier weeks of fermentation.

Figure 1 – Changes in DM in the tower silos



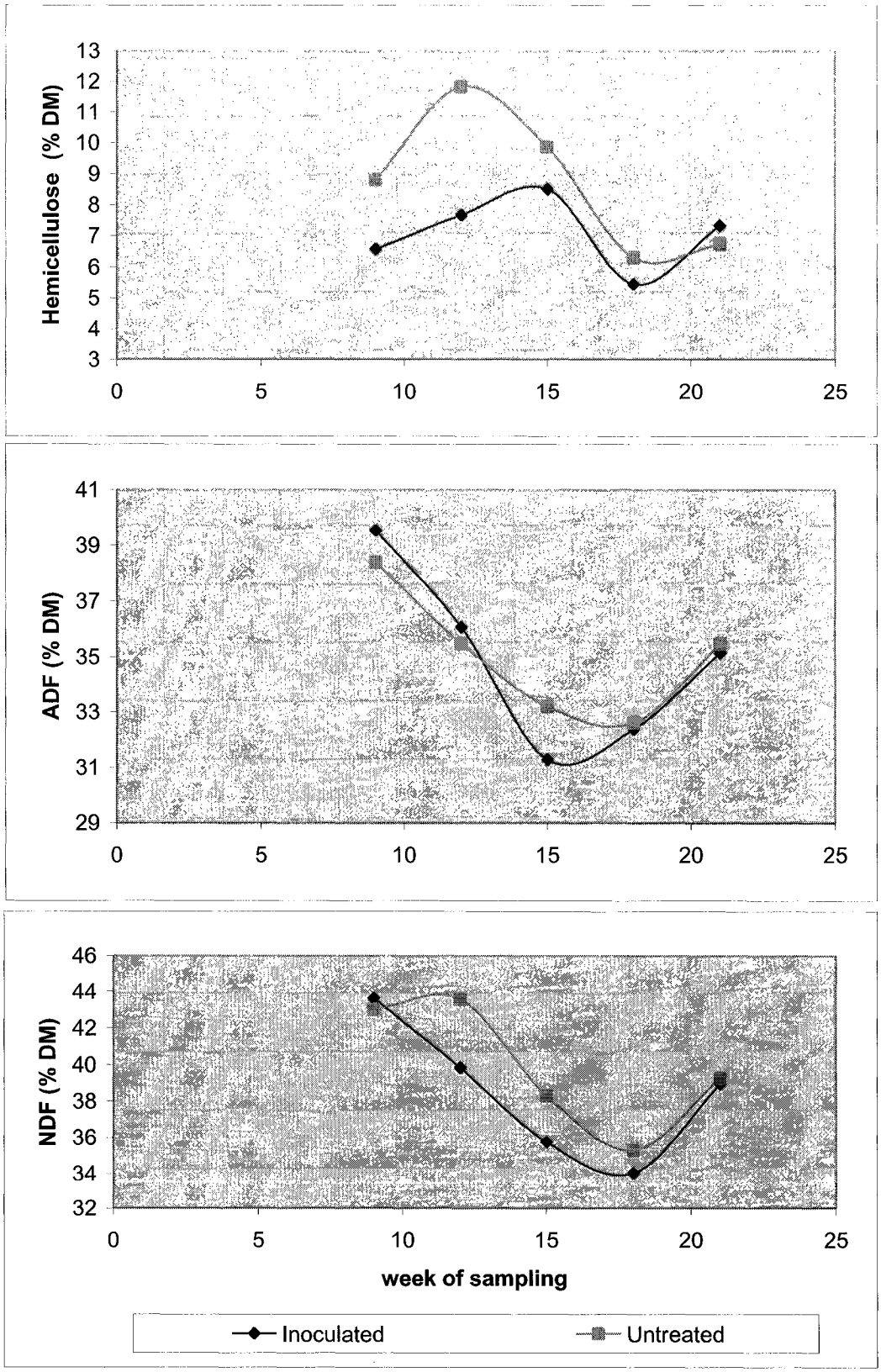
DM: Dry matter

Figure 2 – Changes in NDF, ADF and hemicellulose in the tower silos



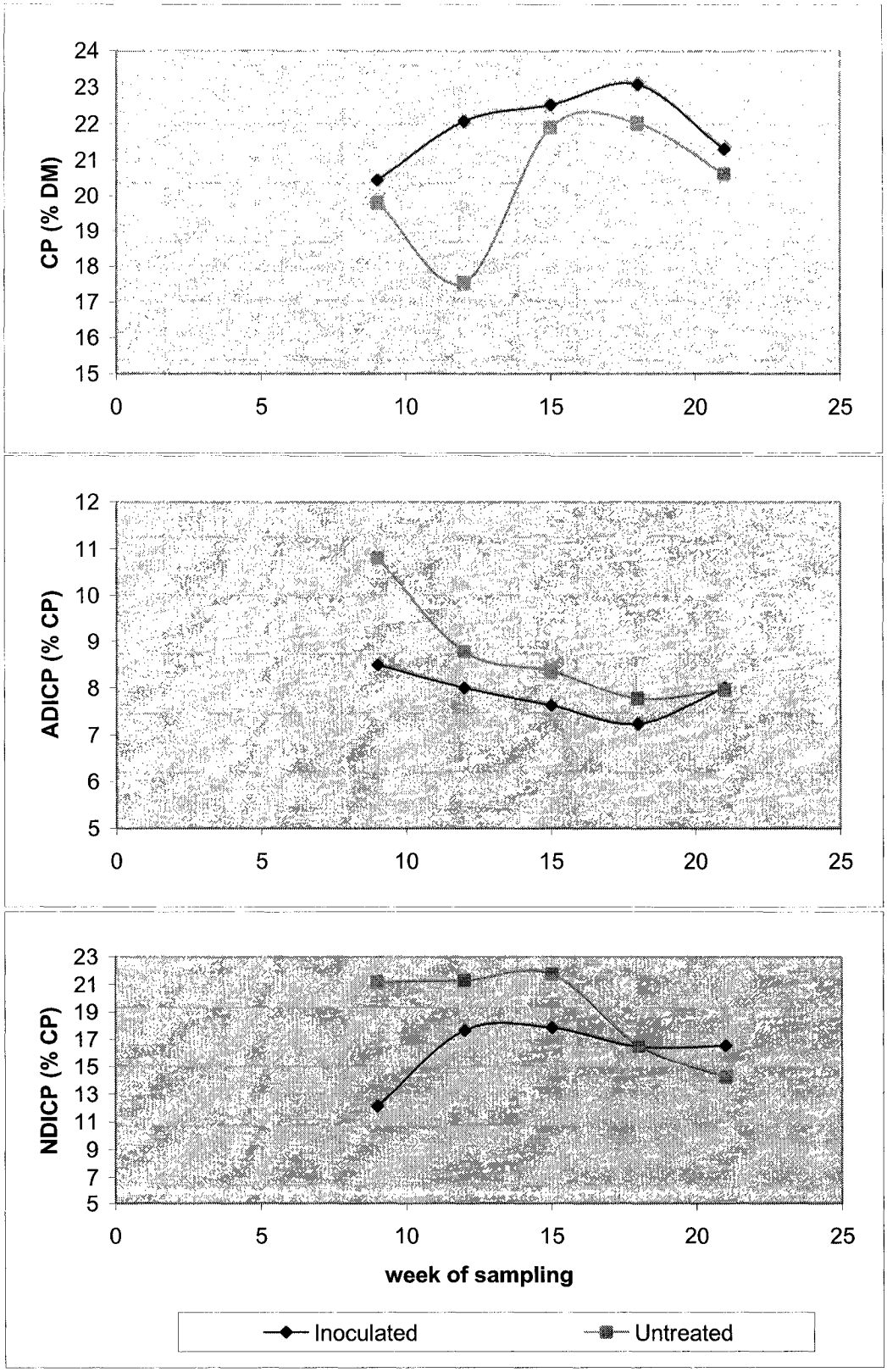
DM: Dry matter; ADF: Acid detergent fiber; NDF: Neutral detergent fiber

Figure 3 – Changes in NDF, ADF and hemicellulose (corr. for protein) in the tower silos



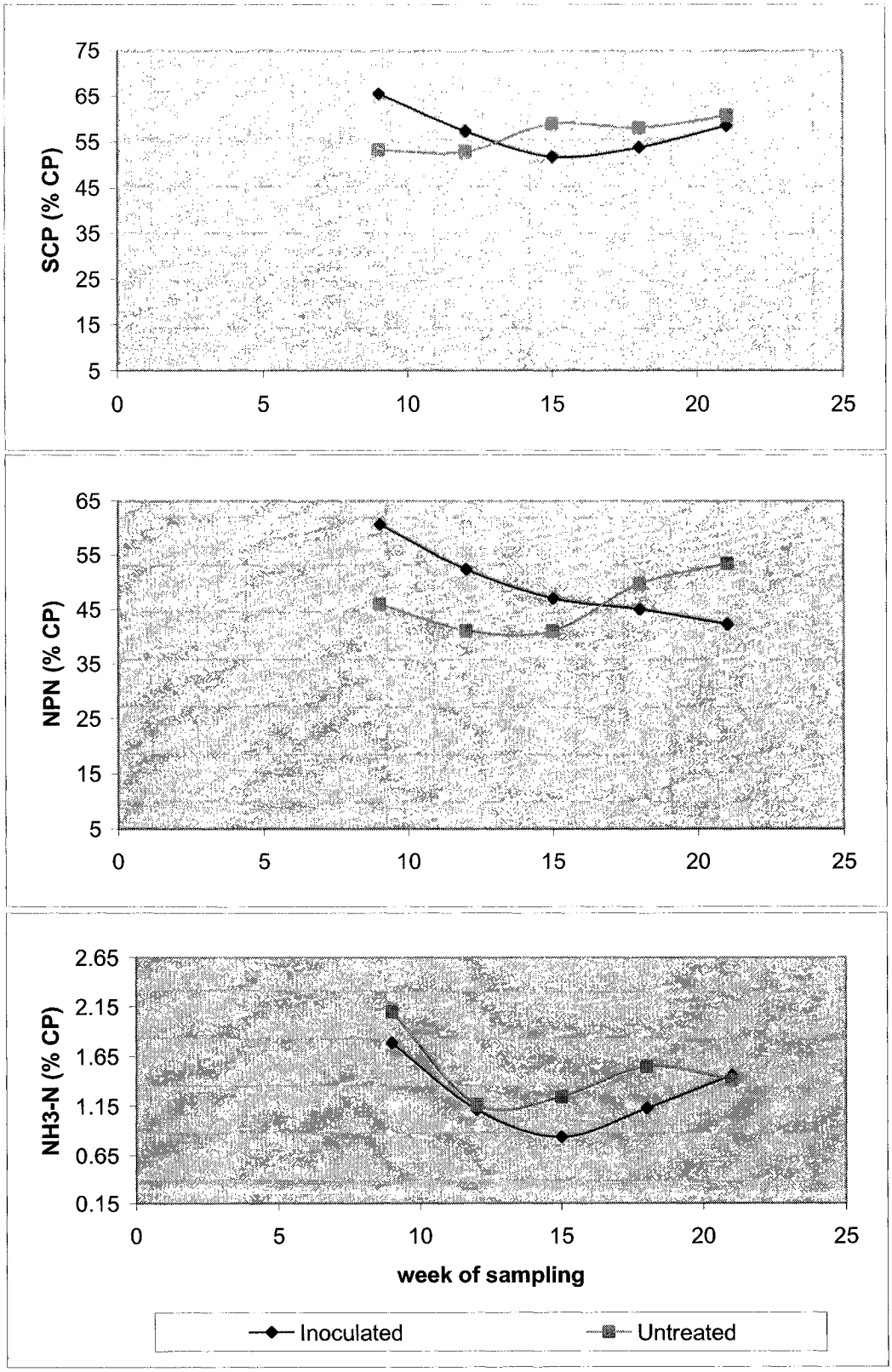
DM: Dry matter; ADF: Acid detergent fiber; NDF: Neutral detergent fiber

Figure 4 – Changes in CP, ADICP and NIDCP in the tower silos



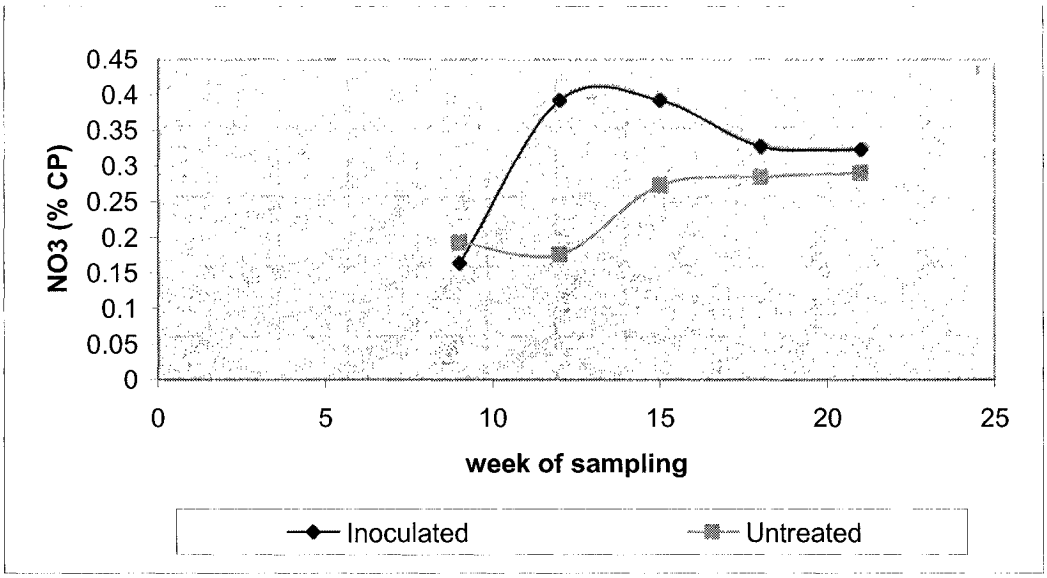
DM: Dry matter; CP: Crude protein; ADICP: Acid detergent insolube crude protein; NDICP: Neutral detergent insoluble crude protein

Figure 5 – Changes in SCP, NPN and NH3 in the tower silos



CP: Crude protein; SCP: Soluble crude protein; NPN: Non protein nitrogen

Figure 6 – Changes in NO3 in the tower silos



CP: Crude protein