# Whole-Crop Pea-Oat Silages in Dairy Production

Effects of Maturity Stage and Conservation Strategy on Fermentation, Protein Quality, Feed Intake and Milk Production

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Cover: Pea-oat bi-crop at the pod fill-soft dough stage (left). Harvesting pea-oat bi-crop silage with a precision chopper at Röbäcksdalen (middle). Swedish Red cow eating pea-oat bi-crop silage (right). (Photo: T. Rondahl)

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### Tomas Rondahl

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# Whole-Crop Pea-Oat Silages in Dairy Production: Effects of Maturity Stage and Conservation Strategy on Fermentation, Protein Quality, Feed Intake and Milk Production

### Abstract

The thesis summarises and discusses six studies, presented as three papers, concerning harvest and treatment of pea-based silages for use in dairy production. The studies were performed at the Swedish University of Agricultural Sciences Research Farm at Röbäcksdalen, Umeå, Sweden ( $63^{\circ}35'N$ ,  $20^{\circ}45'$  E) in 2000 – 2004, using Swedish Red cows housed in a tie-stall barn. Maturity stages and silage treatments (acid addition or wilting) were compared for effects.

A laboratory silo experiment with whole-crop pea silages revealed that proteolysis was reduced at later maturity stages, and that both wilting and acid addition reduced proteolysis during ensilage. In the other studies, pea-oat bi-crop silages (seed rate 80:20) were produced. In the first study (18 cows), treatment did not change the intake of silage cut when peas were at the flat pods stage, and acid-treatment was preferable for harvesting at desired maturity stages. In the second study (30 cows), including a 7-day *in vitro* apparent digestibility study (15 cows), wilting to  $\geq 250$  g kg<sup>-1</sup> dry matter, then adding 6 l acid tonne<sup>-1</sup> fresh matter, resulted in good quality silages. Silages harvested when peas were at the pod fill, and oats at the early dough stage gave the overall best intake, digestibility and milk production. Finally, a production experiment (48 cows), including a 7-day *in vitro* apparent digestibility are pea-oat bi-crop silage can replace, and improve, the effect of high-quality grass-clover silage on silage intake, diet digestibility and milk production.

Thus, it is recommended to harvest pea-oat bi-crop silage (seed rate 80:20) when the peas are at the pod fill stage, wilt it to  $\geq 250$  g kg<sup>-1</sup> dry matter and then add 6 1 acid tonne<sup>-1</sup> fresh matter. This silage can replace high-quality grass-clover silage (11.3 MJ metabolisable energy) in diets to high-yielding dairy cows, and mixed peaoat and grass-clover silage (0.50:0.50) has a concentrate-sparing effect.

Keywords: acid treatment, Avena sativa, concentrate-sparing, dairy cows, grass-clover, intake, maturity stage, pea-oat, Pisum sativum, silage, whole-crop, wilting

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# Dedication

To the farmers

You don't concentrate on risks. You concentrate on results. No risk is too great to prevent the necessary job from getting done. Chuck Yeager

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# List of publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

- I T. Rondahl, J. Bertilsson and K. Martinsson. 2007. Protein fractions and chemical composition of whole-crop pea silages; effect of maturity stage, conservation strategies and pea cultivar (manuscript).
- II T. Rondahl, J. Bertilsson, E. Lindgren and K. Martinsson. 2006. Effects of stage of maturity and conservation strategy on fermentation, feed intake and digestibility of whole-crop pea-oat silage used in dairy production, *Acta Agriculturae Scandinavica Section A, Animal Science*, 56, pp. 137-147.
- III T. Rondahl, J. Bertilsson and K. Martinsson. 2007. Mixing whole-crop pea-oat silage and grass-clover silage; positive effects on intake and milk production of dairy cows, *Grass and Forage Science*, 62 (Proof version, in Press).

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# Abbreviations

| ADF   | acid detergent fibre                        |
|-------|---|
| AIA   | acid-insoluble ash                          |
| CNCPS | Cornell Net Carbohydrate and Protein System |
| СР    | crude protein                               |
| DM    | dry matter                                  |
| ECM   | energy corrected milk                       |
| FC    | fermentation coefficient                    |
| FM    | fresh matter                                |
| ME    | metabolisable energy                        |
| Ν     | nitrogen                                    |
| NDF   | neutral detergent fibre                     |
| NPN   | non-protein nitrogen                        |
| VFA   | volatile fatty acids                        |
| WSC   | water-soluble carbohydrates                 |
|       |   |

# 1 Thesis at a glance

|                                | -   |   | -   |   |   |   |                                  |
|--------------------------------|---|---|---|---|---|---|----------------------------------|
| Year                           | Type of<br>study                                    | Experiment<br>Set-up Variables <sup>A</sup>   | Cultivar  | Silages<br>Treatment  | Silages<br>Treatment Maturity stage   | Main conclusions and recommendations  | Paper                            |
| 2000                           | Laboratory<br>silo                                  | 10-kg silos Protein fractions<br>Ensiling (CNCPS)<br>~100 d                           | Timo (pea)<br>Capella (pea)                       | Acid <sup>B</sup> 6 or<br>wilting                                     | 1. Pod set<br>2. Pod swell<br>3. Full pods  | Capella peas are prone to bird predation<br>6 I formic acid tonne <sup>-1</sup> FM insufficient to prevent volatile<br>fatty acid formation   | Pilot<br>study<br>(in<br>thesis) |
| 1000                           | Laboratory<br>silo                                  | 10-kg silos Protein fractions<br>Ensiling (CNCPS)<br>~103 d Effluent                  | Timo (pea)<br>Capella (pea)                       | Acid <sup>c</sup> 4, 6, 8<br>or wilting<br>Acid $6^{c}$ or<br>wilting | 1. Pod set<br>2. Pod swell<br>3. Full pods  | Acid4 insufficient, acid6 sufficient for ensiling<br>Acid6 and wilting reduced proteolysis<br>Proteolysis was reduced at later maturity stage   | Ι                                |
| 1007                           | Feed intake (3<br>x 3 Latin<br>square; 3 x 21<br>d) | 18 cows Intake<br>3 silages Milk production<br>Big bales Live weight                  | <u>Bi-crop</u> :<br>Capella (pea):<br>Svala (oat) | Acid <sup>c</sup> 6 or<br>wilting<br>Acid <sup>c</sup> 12             | <ol> <li>Flat pods &amp;<br/>middle milk</li> <li>Full pods &amp; ripe</li> </ol> | Intake similar for acid6 and wilted, highest for acid12<br>Acid preferable for reliable harvest at particular maturity<br>stage<br>Extensive lodging of the crop after flat pod stage   | II<br>(Exp 1)                    |
| 2002                           | Feed intake (3<br>x 3 Latin<br>square; 3 x 28<br>d) | 30 cows Intake<br>3 silages Milk production<br>Bunker silos Live weight               | <u>Bi-crop</u> ":<br>Nitouche<br>(pea):Belinda    | Wilting to<br>250 g kg <sup>-1</sup>                                  | 1. Flat pods & early<br>milk<br>2. Pod fill & late                                | I. Flat pods & early       Wilting to 250 g kg <sup>-1</sup> DM and acid treatment yields good milk         nilk       silage and facilitates harvesting at desired maturity stage         2. Pod fill & late       Recommended maturity stage for harvest : peas = pod | II<br>(Exp 2a)                   |
|                                | Digestibility<br>(7 days)                           | 15 cows Faecal grab<br>3 silages samples<br>Bunker silos Intake & Refusals            |   | DM +<br>acid <sup>c</sup>   | milk to early dough<br>3. Full pods & late<br>dough                               | milk to early dough fill, oats = late milk to early dough, based on intake, milk<br>3. Full pods & late yield, milk composition, N use efficiency and diet<br>dough digestibility   | II<br>(Exp 2b)                   |
| 2003                           | Production (3<br>x 2 Factorial<br>design; 9 w)      | 48 cows Intake<br>3 silages + Milk<br>h/l conc <sup>D</sup> Live weight               | <u>Bi-crop</u> :<br>Nitouche<br>(pea):Belinda     | Wilting to $250 \text{ g kg}^{-1}$<br>DM + Acid <sup>E</sup>          | 1. Pod fill & early<br>to soft dough  | Pea-oat bi-crop silage can replace grass-clover silage of<br>high nutritional quality in rations to dairy cows<br>A mixed ration of hea-oat hi-cron and orses-clover silage   | E                                |
| 1                              | Digestibility<br>(7 days)                           | 18 cows Faecal grab<br>3 silages + samples<br>h/1 conc <sup>D</sup> Intake & Refusals | <u>Grass-clover</u><br>s                          | Wilting<br>overnight +<br>Acid <sup>E</sup>                           | 1. First-cut  | of high nutritional quality has a concentrate-spring effect<br>and can be recommended for high-yielding dairy cows  |                                  |
| <sup>A</sup> Incl <sup>i</sup> | udes chemica  | l and fermentation analysis o   | of forages and s                                  | ilages <sup>B</sup> Formi   | ic acid [850 g kg <sup>-1</sup> ].  | <sup>A</sup> Includes chemical and fermentation analysis of forages and silates <sup>B</sup> Formic acid [850 g kg <sup>-1</sup> ], expressed as 1 tonne <sup>-1</sup> FM <sup>c</sup> PR.OENS <sup>TM</sup> (Perstorn Speciality                                       | Speciality                       |

<sup>•</sup>Includes chemical and fermentation analysis of forages and silages <sup>•</sup>Formic acid [850 g kg<sup>-1</sup>], expressed as 1 tonne<sup>-1</sup> FM <sup>•</sup> PROENS<sup>1,m</sup> (Perstorp Speciality Chemicals AB, Perstorp, Sweden), 1 tonne<sup>-1</sup> FM, [formic (600 – 660 g kg<sup>-1</sup>) and propionic acid (230 –290 g kg<sup>-1</sup>)] <sup>D</sup> Bi-crop, grass-clover, and 0.50:0.50 (w/w DM basis) mixed silages + 7 (l) or 10 (h) kg concentrate <sup>E</sup> PROMYR<sup>TM</sup> (Perstorp Speciality Chemicals AB), 1 tonne<sup>-1</sup> FM, [formic acid (420 – 490 g kg<sup>-1</sup>) propionic acid (170 – 230 g kg<sup>-1</sup>) and ammonia (50 – 90 g kg<sup>-1</sup>)] <sup>F</sup> Pea-oat bi-crop seed rate 80:20 (200 kg:50 kg)

# 2 Introduction

According to EU legislation (EU regulation no. 1804/1999; Council for the European Union, 1999), the use of 100 % organically produced feedstuffs has been mandatory since 2005 for all European organic dairy producers. The challenge for dairy production in northern Europe is to match the energy and nutrient requirements of high-yielding cows with crops that can be grown in an organic crop rotation under northern European conditions. A particular challenge is the production of protein crops. In parts of Scandinavia, including northern Sweden, the low average summer temperatures and short growing season limit the selection of crops that can be cultivated. However, harvest yields comparable to those in more favourable regions can be obtained using grass (e.g. Phleum and Festuca spp.), barley (Hordeum vulgare), oats (Avena sativa) and peas (Pisum sativum). Interest in home-grown protein crops has increased (Wilkins and Jones, 2000, Frank and Swensson, 2002), especially in organic farming (Mogensen et al., 2004), and field peas are being cultivated by many farmers in Sweden, and other countries, as a replacement for expensive protein supplements, such as imported soy beans, in animal feeds. At the same time, increasing the cultivation of peas improves crop rotation, reduces the need for nitrogen (N)-fertilization and diminishes the overproduction of cereals (Lunnan, 1989, Olesen et al., 2007).

Dairy cows utilise feed crude protein (CP) much more efficiently than other ruminant livestock (Broderick, 2005). Despite this, 2 to 3 times more N is excreted in manure than in milk, thus inefficient N utilisation increases the need for supplemental protein and contributes to environmental pollution (Broderick, 2005). Some silage-based diets can cause large N losses due to poor utilisation of the N fractions (Givens and Rulquin, 2004). This is a problem in grass and legume silages in particular, and the way these silages are produced needs to be re-evaluated. N utilisation in the rumen can be enhanced by improving the forage supply of carbohydrates (Dewhurst et al., 2000, Givens and Rulquin, 2004). Furthermore, a decreased proteolysis during ensilage results in improved efficiency of silage protein utilisation and thus reduced N losses (Charmley, 2001). Methods shown to decrease proteolysis include effective wilting and rapid acidification (Carpintero et al., 1979, Charmley, 2001).

Bi-crops of various grain legumes and cereals have received much attention because of their high yields (Kristensen, 1992, Salawu et al., 2001a). In particular, bi-crops with short-stem pea varieties or mixtures with high grain to straw ratios (Salawu et al., 2002a) are considered to have a good balance of energy and protein contents (Anil et al., 1998). In northern Sweden bi-crops of peas with oats or barley can be grown as nurse crops for grassland reseeds, but it is difficult to fully exploit the benefits of peas. When peas are sown as a major component in a mixture, the crop may lodge severely, becoming very difficult to handle and smothering any undersown grasses. Therefore, the choice of companion cereal cultivar is important (Gilliland and Johnston, 1992, Salawu et al., 2001a). One anticipated advantage of feeding bi-crop silages of cereal and legumes is an improvement in the efficiency of nutrient utilization due to the possible synchronous supply of readily fermentable energy and protein in the rumen (Adesogan et al., 2002).

Legume-cereal bi-crops compete well with conventional grass silages because they are associated with higher intakes of N, digestible protein and digestible dry matter (DM) (Kristensen, 1992, Adesogan et al., 2002, Salawu et al., 2002a). However, it can be difficult to determine the best time to harvest whole-crop forages to optimise their nutritive value without compromising yield.

### 2.1 Protein quality in silage

### 2.1.1 Protein fractions

Feed protein is often discussed in terms of soluble and insoluble crude protein (CP). Soluble CP is rapidly degraded to ammonia in the rumen, and insoluble CP is either more slowly degraded in the rumen, or escapes ruminal degradation altogether (Charmley et al., 1995). Feed protein can be partitioned into three fractions: non-protein N (NPN or fraction A), true protein (fraction B) and unavailable N or bound true protein (fraction C) (Pichard and Van Soest, 1977, Van Soest et al., 1981). Fraction A is fully soluble in water and is completely degraded in the rumen. Fraction C contains protein associated with lignin, tannin-protein complexes and Maillard products that cannot be degraded by ruminal bacteria and does not provide amino acids postruminally (Krishnamoorthy et al., 1982). Fraction B, the true protein fraction, is further sub-fractionated into three fractions: B1, B2 and B3 (Van Soest et al., 1981, Krishnamoorthy et al., 1983). Fraction B1 proteins are rapidly degraded in the rumen. Some of the fraction B2 is degraded in the rumen, and some escapes to the gut. Most of fraction B3 escapes degradation in the rumen. In the Cornell Net Carbohydrate and Protein System (CNCPS) a submodel, based on standard chemical analysis methods, partitions CP in feedstuffs into these five protein fractions (Sniffen et al., 1992). In this system, fraction A and B1 constitute the soluble protein fraction, and fraction B2, B3 and C constitute the insoluble protein fraction (Figure 1).



*Figure 1.* The partitioning of feed protein. Feed protein is partitioned into non-protein N, true protein and unavailable true protein. Non-protein N is also known as protein fraction A, which is completely degraded in the rumen. True protein is sub-fractionated into three fractions. Fraction B1 is rapidly degraded in the rumen, and in the Cornell Net Carbohydrate and Protein System it constitutes the soluble protein fraction together with fraction A. Some of fraction B2 is degraded in the rumen, and some escapes to the gut and is digested there. Most of fraction B3 escapes to the gut and is digested there. The unavailable true protein (fraction C) is neither degraded nor digested. (Reviewed in Sniffen et al., 1992)

### 2.1.2 Proteolysis in forage and during ensilage

After harvest, a large proportion of the forage true protein is hydrolysed by plant proteases to peptides and amino acids i.e. proteolysis occurs (Kemble, 1956, Heron et al., 1989, Charmley et al., 1995, Winters et al., 2000). Within 2 to 5 days, plant protease activity declines to non-measurable levels, however, microbial fermentation continues and breaks down amino acids and other N compounds (Ohshima and McDonald, 1978, Muck, 1988, Winters et al., 2000, Givens and Rulquin, 2004). There is less proteolysis in later harvested forage (Papadopoulos and McKersie, 1983, McKersie, 1985, Muck, 1987). The amount of protein hydrolysed during ensiling is largely dependent upon the rate of acidification, and the "proteolytic potential", i.e. the total protease activity, the protein availability and the protein susceptibility, factors influenced by growth environment and crop management (McKersie, 1985). Proteolysis continues during ensiling; within 24 h of the start of fermentation the protein content can drop from 800 g kg<sup>-1</sup> total N to less than 600 g kg<sup>-1</sup> total N, and by the end of ensilage to 300 g kg<sup>-1</sup> total N or less (McDonald et al., 1991, Givens and Rulquin, 2004). The rapid degradation of soluble CP in the rumen results in an accumulation of ruminal ammonia and inefficient incorporation of degraded N into microbial protein (Broderick, 1995, Charmley et al., 1995). In addition, a large proportion of the water-soluble carbohydrates (WSC) available in fresh forage is consumed during ensilage and is not available as an energy source in the rumen (Givens and Rulquin, 2004).

### 2.1.3 Reduction of proteolysis

Proteolysis in forage and silage must be reduced in order to increase the utilisation of forage CP. To achieve this, plant protease activity after harvest and during ensilage must be minimised. The main factors affecting proteolysis are DM content, temperature, pH and plant species (Figure 2) (Muck, 1988, McDonald et al., 1991). In forages with DM contents > 400 g kg<sup>-1</sup>, proteolysis is much reduced (McDonald et al., 1991). Wilting decreases plant protease activity, although substantial proteolysis still occurs during the wilting process, especially in early harvested forages (Papadopoulos and McKersie, 1983, Muck, 1987, Cavallarin et al., 2006). Good drying conditions are necessary for efficient inhibition of proteolysis (Dawson et al., 1999). If the wilting period is extended, or occurs under wet conditions, proteolysis in the forage can increase rather than decrease



*Figure 2.* Factors that affect proteolysis in forages harvested for silage production. During wilting, the cultivar, maturity stage and weather conditions all influence the wilting efficiency and thereby the extent of proteolysis. The plant protease activity changes during crop maturation, which affects the extent of proteolysis both during wilting and ensilage. During ensilage, a rapid drop in pH is important to decrease plant protease activity, and can be achieved both through efficient lactic acid bacteria (LAB) fermentation (epifytic LAB or from inoculant additives) and through acid addition. (Reviewed in McDonald et al., 1991)

(Carpintero et al., 1979, Muck, 1987, Owens et al., 1999). Wilting efficiency can be improved by maceration of the crop (Charmley, 2001). However, this is not a feasible method when harvesting whole-crop peabased forages, since maceration results in unacceptable field losses. Plant proteases have high temperature optima, therefore increased temperatures during ensiling will increase their activity (Brady, 1961). However, in correctly harvested and ensiled forage, there should be little respiration and therefore little temperature increase. Acidification inhibits plant proteases by rapidly reducing the pH (Brady, 1961, Finley et al., 1980, McKersie, 1985, Chamberlain and Quig, 1987). For effective inhibition of proteolysis, the pH reduction must occur quickly, therefore the addition of acids is more effective than natural fermentation (Chamberlain and Quig, 1987). Nevertheless, although quick acidification to a pH below 4.0 reduces proteolysis, it is not completely inhibited (Muck, 1988, McDonald et al., 1991, Broderick, 1995). For wetter crops, a rapid decline in pH is more important than a low final pH (Muck, 1988, McDonald et al., 1991, Broderick, 1995). If a rapidly decreasing pH is to be achieved by fermentation, an anaerobic environment is essential, as is the presence of sufficient numbers of lactic acid bacteria and adequate substrate for the bacteria (Muck, 1988, McDonald et al., 1991, Broderick, 1995). Finally, proteolysis parameters, and the effects of wilting and maturity stage on proteolysis, differ between plant species (Papadopoulos and McKersie, 1983). For instance, Papadopoulos & McKersie (1983) showed that alfalfa silage undergoes a high degree of proteolysis, whereas red clover silage undergoes a low degree of proteolysis during wilting and ensiling.

### 2.1.4 Reducing proteolysis

The most important factors influencing the amount of feed CP that will be degraded in the rumen are the chemical parameters of the feed CP (NRC, 2001). The two most important considerations are the proportional concentrations of NPN and true protein, and the physical and chemical characteristics of the true protein fractions (NRC, 2001). In terms of improving the protein fraction distribution, the main aim of harvesting and ensiling is to obtain low concentrations of protein fractions A and C in the silage. Much work has been directed towards reducing the proportion of soluble proteins in silage (Charmley, 2001). Methods that reduce the solubility of CP includes heat treatment (Charmley, 2001), rapid acidification (Charmley, 2001), efficient wilting (Muck, 1987), and harvesting at later maturity stages (Papadopoulos and McKersie, 1983, Cavallarin et al., 2006). Silage based on coloured-flowered or variegated peas, with higher tannin contents, can have increased contents of rumen escape proteins, i.e. fractions B and C (Hart, 2005), and increased tannin contents decrease the proportion of NPN (Broderick, 1995). Addition of formic acid, formaldehyde or tannic acid to pre-wilted alfalfa forage of about  $330 \text{ g kg}^{-1}$  DM has been shown to reduce fraction A and increase fraction B1 contents compared to pre-wilted forage ensiled without any acid addition (Guo et al., 2007). Furthermore, all three additives decreased the fraction B2 content, the addition of formaldehyde or tannic acid increased fraction C contents, but only formic acid increased fraction B3 contents (Guo et al., 2007). If the treatments were combined, fraction B3 content was increased, and when tannic acid was included in the combination, the fraction B2 content was increased in the cited study.

### 2.2 Whole-crop pea-based silages in dairy production

The forage quality of grasses and legumes declines with age due to reductions in the leaf-to-stem ratio, in conjunction with a decline in the stem component's nutritive value. Therefore, the strategy for obtaining good quality forage is to harvest when the leaf-to-stem ratio is high, e.g. grasses at boot stage and legumes at the beginning of flowering. In contrast, the quality of grain legumes (e.g. peas), whole-crop cereals, and their bicrops, do not decline with age in the same manner as grasses and legumes, because of the additional influence of the grain and pod yields (Salawu et al., 2001a). This makes it more difficult to determine the appropriate harvest time for these crops to use in dairy herd feeds. Pea whole-crop quality tends to decline less than cereal whole-crop quality, so the cereal component's maturity stage can be more important when deciding the appropriate time for harvesting bi-crops (Salawu et al., 2001a). In addition, grain legumes and cereals can only be harvested once each growing season, and planting seeds must be purchased for each harvest. From the farmers' perspective, this increases the importance of the DM yield of these crops compared to that of grasses and legumes, which can be harvested several times each season.

Barley, wheat, and oats are the most frequently used cereals in bi-crops (Brundage and Klebesadel, 1969, Chapko et al., 1991, Salawu et al., 2001a). The cereal type has been shown to affect both the nutritional value and yield of the crop; but there is conflicting evidence regarding which of the cereals oat or barley is most favourable (Chapko et al., 1991, Jedel and Helm, 1993, Khorasani et al., 1993). Barley has an efficient initial growth period and higher leaf-area index than pea, and thus could suppress early pea plant growth (Lunnan, 1989), while wheat needs a long, warm growth period and is not a good choice in northern Sweden. Oats have a longer harvest window than barley (Juskiw et al., 2000), they are less competitive than barley when drilled simultaneously with peas (Lunnan, 1989), and several varieties are suitable for growing in northern Sweden.

### 2.2.1 Forage and dry matter yields

In northern Sweden, the yields of whole-crop pea forages range from 3.5 to 6.0 tonnes DM ha<sup>-1</sup>, and the yields of pea-oat bi-crop forages from 4.7 to 6.8 tonnes DM ha<sup>-1</sup>, when the proportion of pea is between 330 and 460 g kg<sup>-1</sup> (Ericson and Norgren, 2003). In southern Sweden, whole-crop pea forage yields are similar, ranging from 3.8 to 6.5 tonnes DM ha<sup>-1</sup> (Åman and Graham, 1987). Dry matter yields of whole-crop peas are similar at different maturity stages due to the increase in DM content with maturity (Fraser et al., 2001). In bi-crops, forage yields and CP concentration can be affected by

the seeding rates of the individual components. In pea-cereal bi-crops an increase in the pea component seeding rate can improve the CP concentration, but does not affect the forage yield (Carr et al., 1998). Conversely, the cereal component seeding rate can affect the forage yield but not the CP concentration, and if sown at rates higher than cereal sole-crop seeding rates, forage production is maximised (Carr et al., 1998). Dry matter yields of pea-wheat bi-crops generally increase with maturity (Salawu et al., 2001a).

### 2.2.2 Nutritional content

According to the Swedish standards for feed evaluation no reliable methods have been described, as yet, for calculating the energy content of wholecrops such as those available for grasses. Currently, the energy content of whole-crops is estimated by combining tabulated digestibility coefficients and energy factors of CP, crude fat and carbohydrates (Spörndly, 2003). Starch content influences metabolisable energy (ME) content (expressed as MJ kg<sup>-1</sup> DM), but a correction for starch content is only included when it is greater than 200 g kg<sup>-1</sup> DM (Spörndly, 2003). According to this method, the average ME content of Swedish pea silages is 12.2, and the ME content of whole crop cereal silages ranges between 9.1 and 9.8 (Spörndly, 2003). According to Kristensen (1992), pea silages have calculated ME contents > 11, low fibre contents and high digestibility, while whole-crop cereal silages have ME contents between 9.4 and 10.7. The amount of biologically fixed N is highest in monoculture peas, but appreciable quantities are fixed in the mixtures even at N rates of 80 kg ha<sup>-1</sup> (Lunnan, 1989). Pea forages have higher CP and in vitro digestible organic matter in DM, and lower neutral detergent fibre (NDF) and acid detergent fibre (ADF) than wheat (Salawu et al., 2001a), and higher CP contents than oats (Faulkner, 1985). However, adding pea to wheat, oat or barley increases forage CP concentration and decreases NDF and ADF (Brundage and Klebesadel, 1969, Chapko et al., 1991, Salawu et al., 2001a). Mustafa & Seguin (2004) found that whole-crop pea silage has similar forage yields, but higher CP and lower NDF contents than pea-cereal silages and that the in vitro DM digestibility of pea silage is higher than that of pea-cereal mixtures at earlier harvest, but the difference is reduced at later harvest (Mustafa and Seguin, 2004). Pea-oat mixtures have significantly lower NDF- and higher CP-contents than pea-barley mixtures, although the latter generally produces more forage (Chapko et al., 1991).

# 2.2.3 Changes in botanical composition and nutritional contents during maturation

In pea plants, the most dramatic changes in the distribution of chemical constituents occur during the pea filling process, a rapid exponential phase of pea growth (Åman and Graham, 1987). Nutrients, especially carbohydrates and protein, are translocated from the vegetative parts of the plant to the pea grains, while the leaves and stems gain cellulose, hemicellulose and lignin. Nonetheless, the gross chemical composition of the whole crop remains remarkably constant, with the exception of the transformation of soluble sugars to starch, and the increased content of cell walls (Åman and Graham, 1987). In forage harvested at early pod fill stage, the leaf component constitutes the largest part of the total plant dry weight (Trevino et al., 1987). At later harvests, the fully developed peas constitute around half the whole crop (Åman and Graham, 1987), and after an initial increase the CP content remains relatively uniform, as does the lignin content (Brundage and Klebesadel, 1969, Åman and Graham, 1987). In pea-oat bi-crop forage, the CP content declines during maturity (Jaster et al., 1985), as does the protein solubility (Åman and Graham, 1987). Conversely, the DM, CP, starch, NDF contents and in vitro digestible organic matter in DM of pea forages increase with maturity, and in pea-wheat bi-crops, the CP, starch, WSC, NDF and ADF contents are influenced by both the maturity stage and the pea-wheat proportion (Salawu et al., 2001a). Pea-wheat bi-crops have higher digestibility and greater aerobic stability when harvested with the peas at pod swell stage and wheat at early milk stage, than bi-crops harvested at later stages (Salawu et al., 2002b).

### 2.2.4 Harvesting and conservation strategies

The choice of harvest time affects the methods available for harvesting, which in turn can affect field losses. In general, peas must be handled with some care since mechanical manipulation increases the risk of both soil contamination and field losses. Disc mowers with conditioner have been shown to be unsuitable for harvesting pea forages at the flat pods maturity stage or later, but nevertheless they still are often used for this purpose (Rodhe and Thylen, 1991). It is not advisable to use rotating discs, since there is an increased risk of shattering peas and increasing field losses. Furthermore, lodged crops should be cut against the lodging direction to decrease field losses (Rodhe and Thylen, 1991). Harvesting whole-crop pea silages as big bales with a crimper mower has been found to result in greater field losses than harvesting them with a Haldrup harvester (Fraser et al., 2001).

Whole-crop cereals and grain legumes with 300 to 500 g kg<sup>-1</sup> DM are generally easy to ensile. Water soluble carbohydrate levels are strongly correlated with DM contents of silage, and should therefore always be sufficient for effective lactic acid fermentation (Kristensen, 1992). In practice, DM contents should be  $>300 \text{ g kg}^{-1}$  at the start of ensilage. Wholecrop peas have low DM contents until senescence, and the peas and pods are difficult to wilt (Åman and Graham, 1987). Whole crop cereals, which generally have DM contents of 300-500 g kg<sup>-1</sup>, can be directly harvested, but pure crops of grain legumes often need to be wilted in order to raise their DM contents above the 300 g kg-1 threshold, and thus ensure favourable silage fermentation (Kristensen, 1992). If wilting is not an option to increase the DM content of whole-crop peas prior to ensiling, satisfactory fermentation of wet whole-crop peas can still be achieved at earlier maturity stages when there is still free sugars available in the crop (Åman and Graham, 1987, Fraser et al., 2001), at the increased risk of effluent losses if the DM content is <250 g kg<sup>-1</sup> (McDonald et al., 1991). Addition of inoculants or acids to whole-crop pea-based forages can improve fermentation (Salawu et al., 2001b) and reduce proteolysis (Adesogan and Salawu, 2002, Adesogan and Salawu, 2004). Formic acid-treatment also seems to improve aerobic stability compared to inoculants (Salawu et al., 2001b, Adesogan and Salawu, 2004).

### 2.2.5 Intake and digestibility

The voluntary intake of legumes has long been recognized to be higher than that of grasses of equal digestibility (Thornton and Minson, 1973, Waghorn et al., 1989, Beever and Thorp, 1996, Salawu et al., 2002a, Bertilsson and Murphy, 2003). The current consensus is that NDF content (Dado and Allen, 1995, Mertens, 1997, Varga et al., 1998) and NDF digestibility (Oba and Allen, 1999) are the main limiting factors of silage DM intake by ruminants. However, several other factors have been suggested to affect DM intake. These include fermentation acids (Huhtanen et al., 2002), silage ammonia N content (Wright et al., 2000, Huhtanen et al., 2002), CP content (Rook and Gill, 1990, Steen et al., 1998, Broderick, 2003), DM content (Rook and Gill, 1990, Steen et al., 1998, Wright et al., 2000), and ruminal ammonia levels (Charmley, 2001).

The rate of degradation, CP content, ruminal degradability and total tract digestibility are all higher for whole-crop pea silages than for pea-wheat bicrop silages (Salawu et al., 2002b). Furthermore, intakes of bi-crop silages are higher than those of grass silages at similar concentrate amounts (Salawu et al., 2002a, Adesogan et al., 2004). Pea silage has higher DM, CP and NDF rumen degradability than barley silage, and similar DM degradability to alfalfa silage (Mustafa et al., 2000).

### 2.2.6 Milk yield and milk composition

The effects of feeding pea silage or bi-crop pea-cereal silage compared to cereal silage (Mustafa et al., 2000) or grass silage (Salawu et al., 2002a, Pursiainen and Tuori, 2006) on milk yield and composition are very varied. In one study, pea silage gave similar milk yield, milk composition and milk urea N as barley or alfalfa silages (Mustafa et al., 2000). However, milk yields were increased by feeding cows pea silage rather than whole-crop barley silage (Kristensen, 1992), and feeding cows a pea-wheat bi-crop silage rather than a grass silage can increase milk fat contents and reduce polyunsaturated fatty acid contents (Salawu et al., 2002a). On the other hand, similar milk yields and composition were obtained when a pea-wheat bi-crop silage was used in a diet with 4 kg concentrate as when grass silage was used in a diet with 8 kg concentrate in a study by Adesogan et al. (2004), and Pursiainen and Tuori (2006) concluded that two-thirds of wilted, moderate quality grass silage can be replaced by pea-barley intercrop silage in diets fed to high-yielding dairy cows without adverse effects on their feed intake or milk vield.

# 3 Objectives

The overall objective of the studies presented in this thesis was to provide information that could be used to advise farmers on the use of pea-based silage in diets for high-yielding dairy cows. This was to be achieved by

- 1. determining the optimum maturity stage and treatment for reducing proteolysis
- 2. determining the maturity stage and treatment that yields the best silage, measured by silage intake, diet digestibility and milk production
- 3. determining whether optimally harvested pea-based silage can replace, or improve the effect of, high-quality grass-clover silage in dairy cow rations

The specific objectives of the experiments detailed in each paper were:

- to examine if appropriate choices of maturity stage, treatment and pea cultivar can reduce proteolysis in whole-crop pea silages (I)
- to determine the effects of acid-treatment and wilting on the chemical composition and intake of pea-oat bi-crop silages (II, Experiment 1)
- to use an optimised treatment of pea-oat bi-crops and identify the maturity stage that provides the highest silage intake, diet digestibility, N-use efficiency and milk production (II, Experiment 2)
- to harvest and treat pea-oat bi-crop silage in an optimal manner and determine if such silage can replace, or improve the effect of, a high-

quality grass-clover silage by comparing the silages alone and in a mixture (0.50:0.50 on a DM basis) in terms of silage intake, diet digestibility, N-use efficiency and milk production (**III**)

Since the results of the experiments were to provide the basis of advice offered to farmers, it was important to use crops and conservation strategies that are readily available for farmers, and to use machines and storage methods that are available on most farms where grass-based silages are harvested.

## 4 Pilot study

At the start of the work with this thesis, one important hypothesis was that the proteolysis during ensilage of pea whole-crops could be reduced by manipulating maturity stage and preservation treatment. This was a prerequisite for the later studies, in which optimal harvest strategies for pea silages to use in diets for dairy cows were to be determined. Therefore, a pilot study was performed to test if this hypothesis was feasible, and to confirm that the experimental set-up was appropriate.

Pilot study title: Maturity stage and preservation treatment can change the extent of proteolysis in whole-crop pea silages.

### 4.1 Pilot study introduction and aims

Field peas are protein crops that can be cultivated and processed for feeding cattle on most farms in Sweden. However, a disadvantage with pea protein is that, to a large extent, it is digested in the rumen. If degradation of pea protein in the rumen could be decreased, peas could provide more of the dietary protein requirements for cattle. Furthermore, if its N utilization was improved, the risk of N leakage into the environment would be reduced. By developing new and improved harvesting and conservation strategies for pea crops it might be possible to improve the protein availability to ruminants and thus reduce N losses. The aims of this pilot study were to determine whether the extent of proteolysis in pea silages can be manipulated by 1) harvesting at different maturity stages, 2) using either of two preservation treatments, and 3) choosing different pea varieties. The results and difficulties experienced were taken into account in the subsequent phasing and planning of the follow-up studies underlying this thesis.

### 4.2 Pilot study materials and methods

### 4.2.1 Harvest and ensiling

Two varieties of peas, one variegated (*Pisum arvense* L., cv. Timo) and one white flowered (*Pisum sativum* L., cv. Capella) were drilled on  $2^{nd}$  of June, 2000, at the Swedish University of Agricultural Sciences (SLU) Research Farm at Röbäcksdalen, Umeå, Sweden (63°35′N, 20°45′ E). Each cultivar was drilled on a separate 360 m<sup>2</sup> plot (fertilized with around 40 tonnes ha<sup>-1</sup> of slurry in October in the previous year), at a seed rate of 250 (Timo) or 240 kg ha<sup>-1</sup> (Capella). The seed rate was adjusted to give  $10^6$  germinal seeds ha<sup>-1</sup>.

The crops were cut with a Haldrup 1500 plot harvester (J. Haldrup a/s, Løgstør, Denmark) at three different maturity stages: pod set, pod swell and full pods (Knott, 1987). Each crop was either acid-treated with 6 litres tonne<sup>-1</sup> FM formic acid [850 g kg<sup>-1</sup>] or wilted to a DM content > 300 g kg<sup>-1</sup>. At each harvest, the crop was divided in half; one half was placed in a thin layer on a clean plastic sheet for wilting, the other half was immediately chopped into lengths of 3-5 cm with a simple straw chopper (Ysta-sjuan, Ysta-maskiner, Sweden), acid-treated and ensiled in laboratory silos. To ensure a good distribution of the acid, it was diluted to double weight in water before being added to the crop by intermittent spraying with a washbottle and thorough mixing of the forage. The wilted forage was manually tedded once or twice a day. During the day, if the weather conditions were good, wilting was done outdoors, otherwise the wilting forage was kept indoors in a garage equipped with an electric fan. When the desired DM content was reached, the wilted forage was chopped in the same manner as the acid-treated forage and ensiled in laboratory silos.

Following each treatment, about 10 kg FM of all forage samples were manually compacted into laboratory silos fabricated from PVC-tubing (height 0.60 m, diameter 0.30 m), equipped with one layer of plastic tubing and a valve in the bottom to remove effluent. The plastic tubing was compressed to evacuate air and sealed by twisting. A 10-kg sandbag was put on top of the sealed tubes to prevent air from re-entering the silo. Effluent was drained from the silos twice within the first 20 d of ensiling. The silos were incubated for  $102 \pm 1$  days at room temperature. At opening, each silo was emptied on a clean plastic sheet and the silage was inspected for signs of malfermentation.

### 4.2.2 Sampling

At all cuts, forage samples were collected daily to determine the change in DM contents. To calculate DM content, the samples were chopped,

weighed, dried at 145°C for 2 h and weighed again. Forage samples were collected by pooling 5-7 grab samples from random parts of the chopped forage into 1-litre samples, which were collected after discarding the top and bottom 0.05 m of the silage stack. Forage and silage samples were immediately frozen and stored at -20 C° until analysis.

### 4.2.3 Chemical analysis

Chemical composition (DM, ash, CP and WSC) and fermentation parameters – buffer capacity, pH, lactic acid, volatile fatty acids (VFA), 2,3butandiol, ammonia-N and ethanol contents – were determined at the Kungsängen Research Centre Laboratory of the Swedish University of Agricultural Sciences, Uppsala, Sweden. Analysis of protein fractions was performed according to the Cornell Net Carbohydrate and Protein System (CNCPS; Sniffen et al., 1992). All analyses were performed as described in the Materials and Methods section of this thesis.

### 4.2.4 Statistical analysis

Minitab 14 software for Windows was used for all statistical analyses. The effect of cultivar was not included because the Capella results were excluded (see Pilot study results section). The effects of maturity stage and treatment were analysed using the GLM procedure, and post-hoc Tukey paired comparisons, according to the model

$$y_{jkr} = \mu + \alpha_i + \gamma_k + (\alpha \gamma)_{ik} + e_{r(ijk)}$$

where  $y_{ik}$  = the response variable;  $\alpha_i$  = maturity stage;  $\gamma_k$  = treatment; ( $\gamma$ )<sub>ik</sub> = maturity stage×treatment interactions; r = number of replicates;  $e_{ik}$  = random errors assumed to be NID(0, $\sigma^2$ ). The effects of maturity stage were confirmed with Scheffés F-distributed contrasts.

### 4.3 Pilot study results

Birds predated the Capella planted seeds shortly after drilling and consequently a large part of this crop consisted of weeds. The Capella results were therefore excluded from the analysis and the remainder of the study. There was very little or no bird predation of the Timo planting seeds. The weather conditions were very different at each of the three cuts (Figure 3, weather data supplied by SMHI, 2007). Notably, high humidity impeded wilting at the second cut, despite the otherwise good weather conditions, and this also influenced the results.





All the direct-harvested forages had DM contents < 150 g kg<sup>-1</sup> and relatively high buffer capacities. Wilting reduced the buffer capacity of the forages (Table 1). Within each treatment, the WSC and CP contents of the silages were similar at all maturity stages (Table 2). All silages had high contents of acetic acid and butyric acid, and the acid-treated silages had higher contents of propionic acid than the wilted silages (Table 3). At pod swell, the wilted silage had a higher CP content than the acid-treated silages.

More proteolysis occurred, detected as increased contents of protein fractions A+B1, during ensilage than during wilting, and the most extensive proteolysis occurred in the pod set silages (Figure 4, Table 4). For cuts harvested at the pod swell and full pod stages, the level of proteolysis that occurred during ensilage of the acid-treated silages was similar to that which occurred during wilting. Since proteolysis continued in the wilted silages, these silages had higher fraction A+B1 contents than the acid-treated silages. The protein fraction B2 content was mostly decreased during ensilage of wilted forage at pod set and pod swell. In all silages, proteolysis decreased the fraction B3 content.

### 4.4 Pilot study's implications

There were differences in the chemical composition of the pea forages used in this study, and after ensilage there were significant between-maturity stage and between-treatment differences in the silages' DM contents and protein fraction distribution. Thus, it seems to be possible to manipulate the proportions of protein in desired fractions by adjusting the selected forage variables.

The extent of proteolysis decreased with each maturity stage, in accordance with published observations that proteolytic enzyme activity decreases during senescence (Papadopoulos and McKersie, 1983, McKersie, 1985, Muck, 1987). More proteolysis occurred in the wilted than the acid-treated silages, and most of the proteolysis of the wilted forages occurred during their ensilage. For a substantial reduction in proteolysis, it is necessary to increase the DM content to  $\geq 400$  g kg<sup>-1</sup> (McDonald et al., 1991), or reduce the pH to  $\leq 4.0$  (McKersie, 1985, Broderick, 1995). The full pod wilted silage had the highest DM content, and consequently the least proteolysis.

|  |                  | g kg-1        |                          | g kg <sup>-1</sup> DM |                                  | mequiv 100 g <sup>-1</sup> DM | g <sup>-1</sup> DM               | Prot               | Protein fractions (g kg <sup>-1</sup> CP)   | kg <sup>-1</sup> CP) | _    |
|--|------------------|---------------|--------------------------|-----------------------|----------------------------------|-------------------------------|----------------------------------|--------------------|---|----------------------|------|
| Treatment                              | Maturity stage   |               | Ash                      | CP                    | WSC                              | buffer capacity               | acity pH                         | I A+B1             | B2  | B3                   | C    |
| Formic acid 6 l tonne <sup>-1</sup> FM | pod set          | 137           | 7 101                    | 226                   | 93                               | 44.9                          | 6.1                              | 470                | 353   | 147                  | 29   |
|  | pod swell        | 136           | 5 92                     | 217                   | 91                               | 37.0                          | 6.1                              | 1 500              | 296   | 173                  | 30   |
|  | full pods        | 146           | 64                       | 212                   | 113                              | 42.0                          | 5.9                              | ) 540              | 272   | 156                  | 31   |
| Wilted                                 | pod set          | 380           | 91                       | 227                   | 108                              | 34.4                          | 6.2                              | 2 500              | 397   | 78                   | 25   |
|  | pod swell        | 322           | 2 80                     | 235                   | 94                               | 27.4                          | 6.2                              | 2 550              | 305   | 112                  | 32   |
|  | spod lluj        | 394           | 4 79                     | 217                   | 69                               | 31.9                          | 9.9                              | 550                | 299   | 105                  | 46   |
|  | Motivity stores  |               |                          |                       | g kg <sup>-1</sup> DM            | DM                            |                                  |                    | g kg <sup>-1</sup> N                        |                      |      |
|  | iviatuilly stage | Lactic acid   | Acetic acid              | Propio                | Propionic acid                   | Butyric acid                  | 2,3-Butandiol                    | Ethanol            | $\mathrm{Am}	ext{-}\mathrm{N}^{\mathrm{A}}$ | Hq                   | Т    |
| Formic acid 6 l tonne <sup>-1</sup> FM | pod set          | $34 \pm 9.0$  | $50 \pm 4.8$             | 8.7 ±                 | $8.7 \pm 0.21$                   | $34 \pm 9.0$                  | $1.5 \pm 0.25$                   | $36 \pm 3.7$       | $156 \pm 25.8$                              | $4.8\pm0.18$         | 0.18 |
|  | pod swell        | $47 \pm 9.0$  | $24 \pm 4.8$             | 6.3 ±                 | $6.3 \pm 0.21$                   | $13 \pm 9.0$                  | $1.3 \pm 0.25$                   | $26 \pm 3.7$       | $77 \pm 25.8$                               | $4.2 \pm 0.18$       | 0.18 |
|  | full pods        | $55 \pm 9.0$  | $16 \pm 4.8$             | 5.4 ±                 | $5.4 \pm 0.21$                   | $15 \pm 9.0$                  | $1.2 \pm 0.25$                   | $24 \pm 3.7$       | $55 \pm 25.8$                               | $3.9 \pm 0.18$       | 0.18 |
| P <sup>B</sup>                         |                  | 0.517         | 0.00                     | <0>                   | <0.001                           | 0.221                         | 0.888                            | 0.391              | <0.001                                      | 0.065                | 65   |
| Wilted                                 | pod set          | $46 \pm 12.8$ | $15 \pm 6.9 \star \star$ | 4.5 ± (               | $4.5 \pm 0.30 \star \star \star$ | $12 \pm 12.6$                 | $6.4 \pm 0.36 \star \star \star$ | $15 \pm 5.2 \star$ | $76 \pm 36.5$                               | $4.9 \pm 0.26$       | 0.26 |
|  | pod swell        | $98 \pm 10.4$ | $16 \pm 5.6$             | 2.6±(                 | $2.6 \pm 0.25 * * *$             | $4 \pm 10.4$                  | $2.3 \pm 0.29$                   | $13 \pm 4.2$       | $121 \pm 29.8$                              | $4.2 \pm 0.21$       | 0.21 |
|  | full pods        | $90 \pm 9.0$  | $18 \pm 4.8$             | $1.7 \pm ($           | $1.7 \pm 0.21 * * *$             | $5 \pm 9.0$                   | $2.0 \pm 0.25$                   | $13 \pm 3.7$       | $123 \pm 25.8 \star$                        | $4.3 \pm 0.18$       | 0.18 |
| P <sup>B</sup>                         |                  | 0.095         | 0.982                    | <0>                   | <0.001                           | 0.853                         | <0.001                           | 0.809              | 0.035                                       | 0.290                | 90   |

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Table 3. Chemical composition of Timo whole-crop pea silages in the **pilot study**.

|  | Maturity stage | g kg <sup>-1</sup>              |                 | $g kg^{-1} DM$ |                        |  |
|--|----------------|---------------------------------|-----------------|----------------|------------------------|--|
|  | Maturity stage | DM                              | Ash             | WSC            | CP                     |  |
| Formic acid 6 l tonne <sup>-1</sup> FM | pod set        | $137 \pm 3.5$                   | $106\pm2.7$     | $0.75 \pm 1.7$ | $247 \pm 6.4$          |  |
|  | pod swell      | $158 \pm 3.5$                   | $90\pm2.7$      | $2.50\pm1.7$   | $228\pm6.4$            |  |
|  | full pods      | $180 \pm 3.5$                   | $54\pm2.7$      | $6.25 \pm 1.7$ | $232\pm6.4$            |  |
| $\mathbf{P}^{A}$                       |                | < 0.001                         | < 0.001         | 0.277          | 0.346                  |  |
| Wilted                                 | pod set        | $355 \pm 5.0 \star \star \star$ | $94\pm3.8$      | $10.0\pm2.4$   | $262\pm9.1$            |  |
|  | pod swell      | 317 ± 4.1***                    | $82\pm3.2$      | $2.00\pm1.9$   | $263 \pm 7.4 \bigstar$ |  |
|  | full pods      | 361 ± 3.5***                    | $75\pm2.7\star$ | $2.25\pm1.7$   | $251\pm6.4$            |  |
| $\mathbf{P}^{A}$                       |                | < 0.001                         | 0.035           | 0.153          | 0.659                  |  |

 $^{A}P$  = effect of maturity stage within each treatment \*, \*\*, \*\*\* = p < 0.05, p < 0.01, p < 0.001 compared to acid-treated silage at same harvest number

Table 4. Protein fraction content of Timo whole-crop pea silages in the **pilot study**.

|                        | Maturity stage |                                 |                         |                |                        |              |
|------------------------|----------------|---------------------------------|-------------------------|----------------|------------------------|--------------|
| Treatment              | Waturity stage | А                               | B1                      | B2             | B3                     | С            |
| Formic                 | pod set        | $62 \pm 2.6$                    | $571 \pm 20.0$          | 277 ± 13.1     | 46 ± 11.2              | $44\pm4.6$   |
| acid 6 l               | pod swell      | $86\pm2.6$                      | $484 \pm 20.0$          | $272 \pm 13.1$ | $112 \pm 11.2$         | $46\pm4.6$   |
| tonne <sup>-1</sup> FM | full pods      | $55\pm2.6$                      | $500\pm20.0$            | $310 \pm 13.1$ | $105 \pm 11.2$         | $30 \pm 4.6$ |
| $P^{A}$                |                | < 0.001                         | 0.100                   | 0.317          | 0.020                  | 0.198        |
| Wilted                 | pod set        | 57± 3.7                         | $688 \pm 28.1 \bigstar$ | 146 ± 18.5***  | $78 \pm 15.9$          | $31\pm 6.5$  |
|                        | pod swell      | $128 \pm 3.0 \star \star \star$ | $589 \pm 23.0 \bigstar$ | 197 ± 15.1*    | $48 \pm 13.0 \bigstar$ | $38 \pm 5.3$ |
|                        |                | 117 ±                           |                         |                |                        |              |
|                        | full pods      | 2.6***                          | $518 \pm 20.0$          | $276 \pm 13.1$ | 52 ± 11.2*             | $37 \pm 4.6$ |
| P                      |                | < 0.001                         | 0.011                   | 0.002          | 0.547                  | 0.796        |

<sup>A</sup>P = effect of maturity stage within each treatment **\***, **\*\***, **\*\*\*** = p < 0.05, p < 0.01, p < 0.001 compared to acid-treated silage at same harvest number



Timo whole crop peas cultivated and ensiled in 2000, using 6 l formic acid tonne<sup>-1</sup> FM on direct-harvested forage (acid silage) or wilting to about 300 g  $kg^{-1}$  DM (wilted forage and wilted silage). The changes in protein fraction content reflect the proteolysis that occurred during wilting and/or ensiling of direct-harvested forages. Fractions are expressed as % of total CP content. Figure 4. Pie plots of protein fraction contents, determined according to the Cornell Net Carbohydrate and Protein System (CNCPS). Pilot study with

There were problems with poor fermentation in some of the silages, especially the direct-harvested, acid-treated pod set silage. The pod set forage had high buffering capacity and low DM content, hence the silage it yielded had all the signs of malfermentation: low lactic acid contents, high acetic acid, butyric acid, ethanol contents and high pH. At DM contents lower than 150 g kg<sup>-1</sup>, as was the case for all of the direct-harvested forages, a pH reduction to 4.0 does not guarantee inhibition of clostridial activity (McDonald et al., 1991). Peas have relatively high lysine contents (McDonald et al., 1991, Spörndly, 2003), and the amounts of butyric acid relative to the other fermentation acids indicated that the butyric acid resulted mainly from lysine deamination, caused by proteolytic clostridia and enterobacteria, rather than from saccarolytic clostridial activity (McDonald et al., 1991). There were also indicators of heterofermentative lactic acid fermentation; high acetic acid and ethanol contents (McDonald et al., 1991). Acetic acid fermentation results in slower, and more inefficient, pH reduction than lactic acid fermentation (Weissbach, 1996). Although the fermentation quality improved with crop maturation, there were still some problems with malfermentation in the silages. At the end of the experiment it was discovered that air had leaked into some of the silos with detrimental effects, and these leakages were remedied before using the silos in subsequent experiments.

Chamberlain & Quig, (1987) showed that the success of adding formic acid as a preservative depends upon the level of acid added. In their study, addition of 2 or 6 l tonne<sup>-1</sup> FM resulted in good fermentation of perennial ryegrass, but addition of 4 l tonne<sup>-1</sup> FM produced badly fermented silage (Chamberlain and Quig, 1987). In order to determine the optimal level of formic acid addition to produce well-preserved whole-crop pea silage, it is obviously necessary to study the effects of different acid levels.

One of the aims of the pilot study was to compare the chemical composition and protein fraction distribution of two pea varieties. However, the white-flowered Capella was subjected to extensive bird predation after drilling, resulting in a crop with a high proportion of weeds that could not be used in the study. Bird predation is known to be a potential problem when cultivating peas (Åman and Graham, 1987), but high tannin contents negatively influence the palatability of pea seeds (Min et al., 2003), and thus presumably since Timo has higher tannin contents than Capella (Daveby, 1997), it was less susceptible to predation.

### 4.5 Pilot study conclusions

The conclusions of the pilot study were:

- > proteolysis in whole-crop pea silages decreased at later maturity stages
- wilting resulted in more proteolysis than acid-treatment, but most of the proteolysis occurred during ensilage of the wilted forage
- ➤ treatment with 6 1 formic acid tonne<sup>-1</sup> FM was not appropriate for effective fermentation
- bird predation of pea seeds can be substantial and must be prevented
- the laboratory silos needed to be improved to prevent air leaking into them

Based on these conclusions, a number of measures were taken during the preparation of the experiments in **Paper I**. In the phrasing of hypothesis, we suggested that the proteolysis that occurred during ensilage of wilted forage might be reduced if the DM content is  $\geq 400 \text{ g kg}^{-1}$  rather than  $\geq 300 \text{ g kg}^{-1}$ . Furthermore, at least two levels of acid addition should be compared in order to acquire better knowledge about the effectiveness of acid addition in ensilage of unwilted whole-crop pea forages. As the formic acid was inappropriate, the acid additive was changed. Also, bird predation should be prevented if possible. Finally, the sealing of the laboratory silos was improved.
# 5 Materials and Methods

# 5.1 Weather conditions (I-III)

The weather conditions during the cultivation periods were very varied (Figure 5, weather data supplied by SMHI, 2007). An important aspect was that from August onwards the occurrence of low night temperatures, dewfall and high humidity all increased. These weather conditions are very detrimental to the wilting of crops.

## 5.2 Crops: cultivation, harvest and treatments (I-III)

All the crops used in the experiments were grown at the Swedish University of Agricultural Sciences (SLU) Research Farm at Röbäcksdalen, Umeå, Sweden (63°35′N, 20°45′ E). The crops, timing of cuts (maturity stages and weeks after drilling) and treatments used for preservation are shown in table 5.

The pea crops used in **I** were drilled in 2001, on May  $15^{\text{th}}$  at seed rates of 260 kg ha<sup>-1</sup> on 1000 m<sup>2</sup> plots. No fertilisers were used. The plots were covered with fleece sheets until the shoots were about 0.05 m long to prevent bird predation, and thereafter an acoustic scarecrow was used. The crops were cut with a Haldrup 1500 plot harvester (J. Haldrup a/s, Løgstør, Denmark) and ensiled in 10 kg laboratory silos (for details see **I**) for 103 days.

The pea-oat bi-crops used in **II** were drilled on June 6<sup>th</sup> 2001 (**Experiment 1**), and on June 3<sup>rd</sup> 2002 (**Experiment 2**), at seed rates of 250 kg ha<sup>-1</sup> with a seed ratio of 80:20 pea:oat. Just prior to drilling, slurry was spread at 30 tonne ha<sup>-1</sup>. The first cut was performed using a disc mower (Kverneland Ta 339) with conditioner. Subsequent cuts were performed



Figure 5. Average daily precipitation (solid lines) and temperature data (dashed lines) for the cultivation seasons 2000-2003 (SMHI, 2007) (Pilot study, I-**III**). Arrows indicate drilling date. Harvest dates are indicated by boxes: 2000 grey boxes for Timo and Capella whole-crop peas (**Pilot study**); 2001 grey boxes for Timo whole-crop peas (**D**), white boxes for Capella whole-crop peas (**D**), black boxes for Capella-Svala pea-oat bi-crops drilled on  $6^{th}$  June (**II**); 2002 and 2003 black boxes for Nitouche-Belinda pea-oat bi-crops (**IL**, **III**); 2003 striped box for gress-clover ley.



<sup>A</sup> Peas and oats were used as bi-crop <sup>B</sup> Acid additions are given as 1 tonne<sup>-1</sup> FM <sup>C</sup> Wilting of crop  $\geq$  250 g kg<sup>-1</sup> DM <sup>D</sup> Pea-oat bi-crop: 6 1 tonne<sup>-1</sup> FM, grassclover: 4 l tonne<sup>-1</sup> FM

using a disc mower (Lely Optimo 205 or Kuhn GMD 700 HD), but without a conditioner in order to reduce field losses from threshing. In **Experiment 1**, the cuts were harvested with a combined round baler and wrapper (Krone Combi Pack Multi Cut 1500V) equipped with a reduced set of knives to minimise field losses. Direct-harvested bales were wrapped in four layers of plastic film, and after four days of fermentation the effluent was drained by cutting the film. The bales were then wrapped with another six layers of plastic film. The wilted silage was wrapped with eight layers of film. All bales were stored upright on a sand bed for at least five months. In **Experiment 2**, a precision chop forage harvester (Taarup 602B) was used for collection, and all silages were stored for a minimum of 90 days in concrete bunker silos with a capacity of 12 tonnes DM.

The pea-oat bi-crop used in **III** was drilled on  $6^{th}$  of June 2003 at a seed rate of 80:20. Slurry was spread at 30 tonne ha<sup>-1</sup> just prior to drilling. The grass-clover crop was a three-year-old ley with timothy, meadow fescue and red clover. About 70 kg N ha<sup>-1</sup> was applied before the cut. A disc mower (Kverneland Ta 339) with conditioner was used to cut the grass-clover ley, and the pea-oat bi-crop was cut with a small plated disc mower (JF SB 2400) with 2.4 m working width and no conditioner. For pick-up a precision-chop forage harvester (Taarup 602B) was used for both crops, and both silages were stored for a minimum of 60 days in concrete-walled bunker silos with a capacity of 40 tonnes DM.

### 5.3 Animals and experimental design (II and III)

In all experiments, Swedish Red dairy cows of varying parities were used (Table 6). The cows belonged to the Swedish University of Agricultural Sciences (SLU) Research Farm at Röbäcksdalen, Umeå, Sweden, were housed in a tie-stall barn and fed individually. Silage was fed at 0600 h and 1500 h, with refusals recorded each morning. In addition, a commercial concentrate was fed according to production level three or four times daily. Furthermore, all cows were fed a commercial mineral and vitamin supplement daily to meet the recommended requirements (Spörndly, 2003). The cows were milked twice daily.

In the feed intake trials in **II**, the cows were fed each of the silages *ad libitum* according to a 3×3 Latin Square design. In **Experiment 1** (**II**), the cows were blocked according to production level. Each period was 21 d, comprising 14 d for diet adaptation and 7 d for experimental data recording. In **Experiment 2a** (**II**) the cows were blocked according to production level, parity and date of parturition. Each period was 28 d long, with 14 d

for diet adaptation and 14 d for recording data. In the feed intake trial in **III**, the cows were fed each of the silages *ad libitum* in a continuous trial in a factorial design. The cows were grouped into eight blocks on the basis of lactation number, parturition date and milk yield in the pre-experimental weeks and each cow within each block was randomly allocated to one of the six diets. The pre-experimental period was two weeks and the experimental period was seven weeks.

|                        |    |         |     | Mean values |           |                   |        |                   |      |  |  |
|------------------------|----|---------|-----|-------------|-----------|-------------------|--------|-------------------|------|--|--|
|                        | No | o of co | ows | Days c      | of lactat | tion <sup>1</sup> | Milk y | ECM) <sup>2</sup> |      |  |  |
| Parity (no of calves): | 1  | 2       | ≥3  | 1           | 2         | ≥ 3               | 1      | 2                 | ≥3   |  |  |
| II Experiment 1        | 4  | 8       | 7   | 171         | 160       | 167               | 26.5   | 33.6              | 33.7 |  |  |
| II Experiment 2        | 9  | 7       | 14  | 107         | 118       | 114               | 30.4   | 34.6              | 35.7 |  |  |
| III                    | 13 | 14      | 21  | 80          | 97        | 75                | 31.2   | 36.1              | 39.2 |  |  |

Table 6. Details on cows included in II and III.

<sup>1</sup> Days of lactation at the start of the adaptation period in each experiment <sup>2</sup>Energy corrected milk (4% ECM) (Sjaunja et al., 1990)

In the *in vivo* apparent digestibility trial in **II** (**Experiment 2b**) fifteen cows from **Experiment 2a** were maintained on the same silage as during the last period of the intake trial. The silages were fed restrictively, at 90 % of the intake of the previous two weeks, during a seven-day period. In the *in vivo* apparent digestibility trial in **III**, 18 cows from three randomly selected blocks were used in a seven-day trial. In both experiments, digestibility was assessed by faecal grab sampling and analysis of an indigestible marker (AIA).

# 5.4 Sampling and Analysis

### 5.4.1 Maturity stages and botanical composition

Pea maturity stages were classified according to Knott (1987), and oat maturity stages according to Zadoks et al. (1974). The botanical composition of each of the crops used in **II** and **III** was determined immediately before each cut, from six samples collected from randomly located,  $0.5 \text{ m}^2$  plots along a diagonal across each field. Samples were cut with scissors 0.01 m above the ground, leaving lodged plant parts on the field, to imitate the stubble height at harvest.

### 5.4.2 Forage samples

Forage samples were collected from random parts of the chopped forage in **I**, from randomly selected 0.5×1.20 m plots in the swath in **II** (**Experiment** 

1), and from random parts of each wagon in **II** (**Experiment 2**) and **III**. In **I** and **II** (**Experiment 1**), the samples were pooled into 1-litre subsamples then stored at -20 °C until analysis. These samples were thawed prior to buffer capacity and pH determinations in **I** (Table 7), then dried overnight (**I** and **II**, **Experiment 1**) in a forced-air oven at 60°C before being milled through a 1 mm screen in a hammer mill before subsequent analyses (Table 7). In **II** (**Experiment 2**) and **III**, the samples were mixed, reduced and dried at 60°C overnight and stored until analysis. Prior to analysis, the samples were milled through a 1 mm screen in a hammer mill.

Table 7. Analyses performed on forage samples.

|                | Dry matter <sup>1</sup> | Crude protein <sup>1</sup> | $Ash^{1}$ | Water soluble carbohydrates <sup>2</sup> | $\operatorname{Starch}^2$ | Neutral detergent<br>fibre <sup>3</sup> | Buffer capacity <sup>4</sup> | $\mathrm{pH}^{\mathrm{5}}$ | <b>CNCPS</b> <sup>6</sup> |
|----------------|-------------------------|----------------------------|-----------|--|---------------------------|---|------------------------------|----------------------------|---------------------------|
| Paper I        | Х                       | Х                          | Х         | Х  | -                         | -                                       | Х                            | Х                          | Х                         |
| Paper II Exp 1 | Х                       | Х                          | Х         | Х  | Х                         | Х                                       | -                            | -                          | -                         |
| Paper II Exp 2 | Х                       | Х                          | Х         | Х  | -                         | Х                                       | -                            | -                          | -                         |
| Paper III      | Х                       | Х                          | Х         | Х  | Х                         | Х                                       | -                            | -                          | -                         |

<sup>1</sup> (Murphy et al., 2000)

<sup>2</sup> (Larsson and Bengtsson, 1983), starch not determined in grass-clover silage (III)

<sup>3</sup> (Chai and Uden, 1998)

<sup>4</sup>(Playne and McDonald, 1966)

<sup>5</sup> pH was determined with a Metrohm 654 pH meter

<sup>6</sup> Cornell Net Carbohydrate and Starch (CNCPS) method (Sniffen et al., 1992) by Dairy One (Ithaca, NY, USA)

#### 5.4.3 Effluent samples

In **I**, effluent samples were collected from acid-treated Timo silos on days 4 and 20 of ensilage then stored at -20 °C until analysis. The pH of the thawed samples was determined with a Metrohm 654 pH meter. Effluent samples were not collected for analysis in the other studies.

### 5.4.4 Feed samples

Representative 1-litre silage samples were collected from each silo in **I** after discarding the top and bottom 0.05 m of the silage stack. In **II** and **III**, silage samples were collected daily throughout the feeding period and were stored at -20°C until submission for analysis. Prior to analysis, frozen feed samples from each three-week (**II**, **Experiment 1**) or four-week (**II**, **Experiment 2**) period were ground in a mincing machine and representative samples for

each period were collected. In **III**, the silage samples were pooled on an equal weight basis to obtain representative samples for consecutive twoweek periods during the trial. In the *in vivo* apparent digestibility experiments (**II**, **Experiment 2b**; **III**), samples were pooled to obtain one representative sample for each experimental week. The frozen samples were thawed prior to buffer capacity and pH determinations (Table 8), then dried overnight in a forced-air oven at 60°C and milled through a 1 mm screen in a hammer mill before subsequent analyses (Table 8). In **III**, the ME content of the grass-clover silage was determined according to Lindgren et al. (1979) (Lindgren, 1979).

Concentrate samples were collected daily throughout all production and digestibility experiments and stored dry until analysis. Prior to analysis, samples were pooled in the same way as described for the silage samples. Mineral supplement samples were collected daily in the *in vitro* apparent digestibility experiment, stored dry and pooled into one sample.

#### 5.4.5 Faecal samples

On days three to seven of the 7-day *in vivo* apparent digestibility trials (**II**, **Experiment 2; III**), rectal grab samples of faeces were collected at 0900 h and 1400 h and stored at -20°C. When the collection period was completed, the faecal samples from each cow were thawed, mixed and reduced to a single sample, which was stored at -20°C until analysis. Sub-samples were freeze-dried and milled (1-mm screen), and then their DM content, ash, NDF, starch and AIA concentrations were determined as described for feed samples. The total production of faeces was calculated from the total intake of AIA and faecal concentration of AIA (Van Keulen and Young, 1977). Digestibility was calculated as the proportion of components in the feeds found in the faeces.

#### 5.4.6 Statistical methods

In **I**, all statistical analyses were performed using Minitab 14 software for Windows. The effects of maturity stage, treatment and pea cultivar were analysed by the GLM procedure, and post-hoc Tukey paired comparisons. The significance of the observed effects of variations in the acid level and maturity stage were confirmed by Scheffés F-distributed contrasts.

In **II**, the SPSS software package for Windows was used to analyse differences in chemical composition between silages with one-way ANOVA and post-hoc Tukey paired comparisons. Statistical analyses of all other results were performed in the SAS System for Windows (version 5.1.2600) using the MIXED procedure. Carry-over effects from the previous

treatments and the interaction between period and treatment were tested initially, but were not found to be significant (P > 0.10) and these three interaction terms were excluded from the used model.

Table 8. Analyses performed on feed samples.

|                               | Dry matter | Crude protein | Ash   | Water soluble carbohydrates | Starch       | Neutral detergent fibre | CNCPS | pH <sup>2</sup> | Lactic acid | Volatile fatty acids | Ethanol | Ammonia-N |   | Acid-insoluble ash <sup>9</sup> |
|-------------------------------|------------|---------------|-------|-----------------------------|--------------|-------------------------|-------|-----------------|-------------|----------------------|---------|-----------|---|---------------------------------|
| Paper I (silages)             | X          | <br>X         | <br>X | X                           | ، · · د<br>– |                         | X     | X               | X           | X                    | X       | X         |   | ە <sup>ت</sup> «                |
| Paper II Exp 1 & Exp 2a:      |            |               |       |                             |              |                         |       |                 |             |                      |         |           |   |                                 |
| Silages                       | Х          | Х             | Х     | Х                           | Х            | Х                       | -     | Х               | Х           | Х                    | Х       | Х         | Х | _                               |
| Concentrates                  | Х          | Х             | Х     | -                           | -            | -                       | -     | -               | -           | -                    | -       | -         | - | -                               |
| Paper II Exp 2b:              |            |               |       |                             |              |                         |       |                 |             |                      |         |           |   |                                 |
| Silages                       | Х          | Х             | -     | -                           | Х            | Х                       | -     | -               | -           | -                    | -       | -         | - | Х                               |
| Concentrates                  | Х          | -             | -     | -                           | -            | -                       | -     | -               | -           | -                    | -       | -         | - | Х                               |
| Mineral supplement            | -          | -             | -     | -                           | -            | -                       | -     | -               | -           | -                    | -       | -         | - | Х                               |
| Paper III                     |            |               |       |                             |              |                         |       |                 |             |                      |         |           |   |                                 |
| Silages                       | Х          | Х             | Х     | -                           | Х            | Х                       | -     | Х               | Х           | Х                    | Х       | Х         | Х | -                               |
| Concentrate                   | Х          | Х             | Х     | -                           | -            | -                       | -     | -               | -           | -                    | -       | -         | - | -                               |
| Paper III Digestibility trial |            |               |       |                             |              |                         |       |                 |             |                      |         |           |   |                                 |
| Silages                       | Х          | Х             | -     | -                           | Х            | Х                       | -     | -               | -           | -                    | -       | -         | - | Х                               |
| Concentrates                  | Х          | Х             | -     | -                           | Х            | Х                       | -     | -               | -           | -                    | -       | -         | - | Х                               |
| Mineral supplement            | _          | -             | -     | -                           | -            | -                       | -     | -               | -           | -                    | -       | -         | - | Х                               |

<sup>1</sup>(Murphy et al., 2000)

<sup>2</sup> (Larsson and Bengtsson, 1983), starch not determined in grass-clover silage (III)

<sup>3</sup> (Chai and Uden, 1998)

<sup>4</sup>Cornell Net Carbohydrate and Starch (CNCPS) method (Sniffen et al., 1992), by Dairy One (Ithaca, NY, USA)

<sup>5</sup> determined with a Metrohm 654 pH meter

<sup>6</sup> (Andersson and Hedlund, 1983)

<sup>7</sup> analysed by direct distillation

<sup>8</sup> In vitro digestible organic matter (Lindgren, 1979)

<sup>9</sup> (Van Keulen and Young, 1977)

In **III**, the SPSS software package was used to analyse differences in chemical composition between diets with one-way ANOVA and post-hoc Tukey paired comparisons, and differences in body condition score were analysed with the Mann-Whitney test. Statistical analyses of all other data were performed in the SAS System for Windows using the GLM procedure. The response variables were the mean responses during the seven-week experimental period, and the covariates were the mean responses during the two-week pre-experimental period. When effects on daily intake were analysed, the precision was high without covariates so covariates were excluded from the model. When effects on milk production were analysed, covariates were included in the model since they improved its precision.

# 6 Results and discussion

# 6.1 Protein solubility in and fermentation of whole-crop pea silages (Pilot study and I)

#### 6.1.1 Protein solubility in whole-crop pea silages

The single most important factor affecting proteolysis in pea silages was found to be the maturity stage (**I**). In pea crops, proteolysis is reduced in crops harvested from the pod filling stage or later, due to the translocation of protein to the pea seeds and improvements in the fermentation pattern (Cavallarin et al., 2006). The proteolysis in pea silages, evaluated as the change in fraction A+B1 content of silages compared to the content of the direct-harvested forage, decreased with maturity stage (pod set, pod swell or full pods) in all compared treatments (**Pilot study**; **I**). In contrast, increasing the level of acid added to Timo forages – to 4, 6 or 8 1 PROENS<sup>TM</sup> kg<sup>-1</sup> FM, hereafter referred to as acid4, acid6 and acid8 (**I**) – did not reduce the proteolysis in pea silages from the same maturity stage, although there was a decrease in the fraction A content (**I**). Wilting reduced the fraction B3 contents. It proved very difficult to ensure efficient wilting due to the prevailing weather conditions (**Pilot study**; **I**).

In Timo, there was more proteolysis during wilting than during ensilage at the pod set stage (**I**). At the pod swell and full pod stage, there was more proteolysis during ensilage of the wilted forage than during wilting or ensilage of acid-treated, direct-harvested forage. In Capella, there was more proteolysis during ensilage of acid-treated, direct-harvested forage than during wilting or ensilage of wilted forage at the pod set and the full pod stage. At the pod swell stage, there was more proteolysis during ensilage of the wilted Capella forage than during wilting or ensilage of acid-treated, direct-harvested forage. The extent of proteolysis during ensilage of directharvested forages was lower in Timo than in Capella at the pod set stage, but similar at the pod swell and full pod stages. The final content of soluble proteins did not differ between the wilted Timo and Capella silages at the pod set and the pod swell stage, but at the full pod stage, the wilted Capella silage had a lower content of soluble proteins than the wilted Timo. In the acid6-treated silages, the final content of soluble proteins was lower in Timo than in Capella at the pod set and pod swell stages. At the full pod stage, the final content of soluble proteins was similar in the acid6-treated silages.

#### 6.1.2 Fermentation of whole-crop pea silages

Both the Timo and Capella direct-harvested forages were difficult to ensile when cut at pod set (I). At this maturity stage, the DM contents were lower than 150 g kg<sup>-1</sup> and the fermentation coefficient (FC) was very low (30 or less). The FC indicates whether sufficient substrates are available to promote lactic fermentation and to obtain anaerobic, butyric acid-free silage (Weissbach, 1996). The risk of obtaining unstable silage increases when the FC is < 45 (Weissbach, 1996). The FC values of the wilted silages of all maturity stages and both varieties were > 45, and they had high lactic acid contents, low acetic acid contents, and low or non-detectable butyric acid contents, which are indicative of good fermentation and stable, anaerobic silage (I). However, a stable pH was never reached in the silages obtained from acid4- and acid6-treated direct-harvested Timo forages cut at pod set, in which the FC was about 20. In the acid4 silage, high acetic acid, butyric acid and ethanol contents, together with a low content of lactic acid, indicated that secondary fermentation had occurred (McDonald et al., 1991). Ensiling unwilted pea silage at pod set stage will result in intense fermentation, and a high likelihood of butyric acid fermentation (Borreani et al., 2006).

## 6.2 Producing pea-oat bi-crop silages (II, III)

Pre-set requirements for the experiments underlying this thesis were that the methods and techniques used should be available to most farmers. Thus, the machines used were of types that are available to average farmers, and all cultivars used were commercially available in Sweden at the time of the experiments (Table 9), as were the acid additives used (PROENS<sup>™</sup> or PROMYR<sup>™</sup>, Table 5).

#### 6.2.1 Choice of cereal cultivar

The choice of cereal cultivar in the pea-cereal bi-crops was influenced by the northern location of the cultivations. The main mono-crop cereal cultivated in northern Sweden is barley, and for the benefit of crop rotation, another cereal should be chosen for the bi-crop. Oats have a longer harvest window than barley (Juskiw et al., 2000), they are less competitive than barley when drilled simultaneously with peas (Lunnan, 1989), and several varieties are suitable for growing in northern Sweden. However, these varieties mature faster than the peas and were therefore not suitable as components of bi-crops, as found in **II** (**Experiment 1**). It was better to use an oat cultivar with slower maturation and stiffer straw (Table 9) as a component of the bi-crop (**II**, **Experiment 2**; **III**).

Table 9. Description of pea and oat cultivars used in **II** and **III**. Data from the Field Research Unit (FFE), Swedish University of Agriculture, Uppsala, Sweden (FFE, 2007).

| Cultivar              | Morphological<br>type |       |        | Stem/straw<br>length (cm) |      | Time to<br>maturity<br>(days) | Protein<br>content (g<br>kg <sup>-1</sup> DM) |
|-----------------------|-----------------------|-------|--------|---------------------------|------|-------------------------------|---|
| Capella <sup>A</sup>  | Semi-leafless         | White | Yellow | 66                        | Low  | 106                           | 25.9  |
| Nitouche <sup>B</sup> | Semi-leafless         | White | Green  | 78                        | High | 112                           | 23.2  |
| Svala <sup>B</sup>    | -                     | -     | -      | 91                        | Low  | 104                           | 10.9  |
| $Belinda^{B}$         | -                     | -     | -      | 92                        | High | 112                           | 10.5  |

<sup>A</sup> Average for years 2002–2006 <sup>b</sup> Average for years 1998–2002

#### 6.2.2 Yield and protein contents

Desirable properties for good pea-cereal bi-crop silages include good yields and protein contents that are higher than, or at least comparable to, those of grass silages. The yield of pea-cereal bi-crop forages is strongly influenced by the pea:cereal seeding rate and the extent of crop lodging (Gilliland and Johnston, 1992, Carr et al., 1998). The experiments were not set up to compare yield and field losses rigorously, but estimated yields were calculated from botanical composition data (Table 10). The botanical composition of the crop depends on the seeding rate at drilling, and the establishment of the cultivars included. A large proportion of peas (800 g kg <sup>1</sup> total seeds) was used (**II**, **III**) to increase the CP content (Carr et al., 1998), to obtain a bi-crop with quality that was approximately the average of pure cultures of the two components (Kristensen, 1992), and to reduce the need for N-fertilisation (Lunnan, 1989, Berntsen et al., 2004). The botanical composition was similar in the first two bi-crop experiments (II) despite cultivar changes, but differed between II (Experiment 2) and III, in which the cultivars were the same (Table 10). In **II** (Experiment 2), the oats were

afflicted by oat aphids (*Rhopalosiphum padi*), which reduced the oat yield. Although the combination of Nitouche and Belinda (**II**, **Experiment 2**; **III**) resulted in higher estimated yields than the combination of Capella and Svala (**II**, **Experiment 2**) (Table 10), these experiments were not set up to compare yields, and the weather conditions varied during each cultivation year. Furthermore, the growth of pea crops, and their subsequent contribution to the botanical composition of the final crop, is unpredictable and one of the potential drawbacks of pea-cereal bi-crops (Gilliland and Johnston, 1992).

|      |                |                       |   | Botanic | al com              | position | Calculated             |
|------|----------------|-----------------------|---|---------|---------------------|----------|------------------------|
|      |                | Cultivars             | Maturity stage                                    | (g      | kg <sup>-1</sup> Dl | yield    |                        |
| Year | Paper          | (pea + oat)           | (pea & oat)                                       | Peas    | Oats                | Weeds    | (kg ha <sup>-1</sup> ) |
| 2001 | II             | Capella +             | Flat pods & middle milk                           | 690     | 270                 | 40       | 4200                   |
|      | (Exp.1)        | Svala                 | Full pods & ripe                                  | 830     | 160                 | 10       | 4900                   |
| 2002 | II<br>(Exp. 2) | Nitouche<br>+ Belinda | Flat pods & early milk<br>Pod fill & late milk to | 670     | 260                 | 70       | 7300                   |
|      | · · · /        |                       | early dough                                       | 820     | 160                 | 20       | 9800                   |
|      |                |                       | Full pods & late dough                            | 880     | 100                 | 20       | 11500                  |
| 2003 | III            | Nitouche<br>+ Belinda | Pod fill & early to soft<br>dough                 | 520     | 440                 | 40       | 7800                   |

Table 10. Botanical compositions and calculated yields of pea-oat bi-crops cultivated at the Röbäcksdalen research farm, Swedish University of Agriculture, Umeå, Sweden, 2001-2003 (**II**, **III**)

In **II** (**Experiment 1**) the Capella:Svala bi-crop began lodging at the flat pods to pod fill stage, and when the full-pod crop was cut, the lodging was extensive. The ripe Svala probably exacerbated this. The change to a pea cultivar with stiffer stems, Nitouche, and an oat cultivar that ripened much later, Belinda, appeared to reduce lodging (**II**, **Experiment 2**). Nitouche has the additional benefit of being resistant to root rot (*Aphanomyces euteiches*).

The CP contents of the experimental silages were similar to (or higher than) those of the average grass silages obtained by farmers in northern Sweden (Spörndly, 2003) (Figure 6).



*Figure 6.* Crude protein (CP) content of the silages used in **II** and **III** compared to the average CP content of grass silages from farms in Northern Sweden (Spörndly, 2003). Treatments and maturity stages as indicated, acid addition given in l tonne<sup>-1</sup> FM.

#### 6.2.3 Optimal treatment for ensilage

In northern Sweden, an additive, either bacterial or acid-based, is used prior to ensiling grass forages in 90% of cases for silo storage, and 25% of cases for baled silage (Eriksson, 2007). In the studies this thesis is based upon, the utility of wilting as a treatment was found to be limited by its dependence on good weather conditions at harvest, especially if the intention is to harvest the crop at a particular maturity stage (**I**; **II**, **Experiment 1**). It is important to recognise that high precipitation and unfavourable wind conditions are not the only weather factors that can adversely affect ensilage; high humidity, low night-temperatures and dewfall can also have strongly negative effects, as manifested in the extended wilting of crops harvested in August and September (**I**; **II**, **Experiment 1**), when the weather conditions appeared to be acceptable in other respects (Figure 5).

The rate of wilting affects both the quality of the silage (Dawson et al., 1999, Wright et al., 2000) and intake responses (Yan et al., 1998). However, because the intake of wilted silage is usually related to the rate of crop drying in the field, i.e. the prevailing weather conditions at harvest (Dawson et al., 1999), and heavy dew deposits frequently occur during August in northern Sweden, we anticipated that the wilting efficiency would be different at each harvest time. Therefore, these unpredictable environmental effects could

have interfered with the effect of maturity stage on the quality of the silage (Yan et al., 1998, Dawson et al., 1999).

The parameters that were considered particularly important when choosing the treatments were silage contents of WSC and ammonia-N: WSC because it is a good energy source for rumen microbes and improves ruminal N use efficiency (Dewhurst et al., 2000, Givens and Rulquin, 2004), and ammonia-N because it reduces dairy cow intake of silage (Charmley, 2001, Huhtanen et al., 2002). Both wilting and the acidtreatments efficiently reduced pH and resulted in silages of good hygienic quality, with no butyric acid, low acetic acid contents ( $< 20 \text{ g kg}^{-1} \text{ DM}$ ) and low ethanol contents (< 15 g kg<sup>-1</sup> DM) (II, Experiment 1). However, the acid-treated silages had higher WSC contents and lower ammonia-N contents. The acid-treated silages also had higher starch contents, indicating that the pea seed losses were lower in them than in the wilted silages. However, the CP contents did not significantly differ between the treatments, probably because much of the protein still remained in the pods, flowers, leaves and stems (Åman and Graham, 1987). These results regarding silage quality, together with the practical experiences previously discussed, prompted the decision to exclude wilting as a sole treatment in the later experiments i.e. II (Experiment 2) and III.

The use of an acid additive was chosen rather than bacterial inoculants for four main reasons: acid additives have a well-documented inhibitory effect on proteolysis (Carpintero et al., 1979, Nagel and Broderick, 1992, Vagnoni et al., 1997), they are effective in forages with a wider range of DM contents, their effectiveness is not dependent on the WSC contents (McDonald et al., 1991), and they can improve aerobic stability (McDonald et al., 1991, Salawu et al., 2001b, Adesogan and Salawu, 2002). Bacterial inoculants can reduce proteolysis in silages, but their effects on fermentation and aerobic stability vary depending on the buffer capacity and WSC concentration of the forages (Davies et al., 1998, Fraser et al., 2001, Adesogan and Salawu, 2004). The use of PROMYR<sup>TM</sup> is not approved for use in organic production by KRAV (an incorporated association for organic production in Sweden, authorised to carry out inspections of organic production in Sweden), and was used in III due to a misunderstanding at harvest. The main drawback with PROMYR<sup>TM</sup> in this experiment is that the ammonia in it affects the interpretation of data regarding ammonia-N contents in the silage.

It proved difficult to wilt the forages effectively (**I**; **II**, **Experiment 1**), and there were problems with bird predation of pea seeds during wilting (**II**, **Experiment 1**). However, the effectiveness of the acid treatments was

impaired by the low forage DM contents (I; II, Experiment 1). The approach chosen in the later experiments was to wilt the forage to DM contents of at least 250 g kg<sup>-1</sup> FM, since very little effluent is produced from herbage ensiled with this DM content (McDonald et al., 1991), and then treat it with at least 6 l acid tonne<sup>-1</sup> FM (II, Experiment 2; III). This treatment ensured that the crop could be cut at the desired maturity stage; only a day and a half of acceptable weather was required for successful harvest. The combination of wilting followed by rapid acidification can, by reducing the solubility of the protein, have positive effects on the protein fraction of silage, improve N utilisation and increase silage intake (Charmley, 2001, Huhtanen et al., 2002).

#### 6.2.4 Optimal harvest stage

Frequently, both the cereal and pea maturity stages are used to define the appropriate maturity stage for harvesting pea-based bi-crops. However, since changes in quality during maturity are more pronounced in cereals than in peas, it has been suggested that it is sufficient to consider the cereal component's maturity stage (Salawu et al., 2001a). One of the reasons for including a high proportion of peas in the seeding rate in the experiments this thesis is based upon (II, III) was to increase the influence of the pea cultivar on the chemical composition of the silage (Kristensen, 1992). Therefore, the pea maturity stage was considered the main parameter for selecting the optimal maturity stage for harvesting the pea-oat bi-crops (II, Experiment 2). This also takes into account the risk of lodging, which is not the case when the maturity stage of the cereal is the sole parameter for deciding the appropriate maturity stage (Salawu et al., 2001a). Based on experiences from the previous studies (I; II, Experiment 1), the crops should be cut when the pea maturity has passed the pod set stage, because proteolysis was increased in pea silages harvested at this stage (I). However, harvest should not occur at very late maturity stages since the risk of adverse weather conditions is then increased (II, Experiment 1). Based on this, the pea-oat bi-crops in Experiment 2 (II) were harvested when the peas were at the flat pod, pod fill and full pod stages.

At the full pod stage, the peas of the pea-oat bi-crop were afflicted with powdery mildew, which increased their DM contents, but otherwise did not seem to have any adverse effect on fermentation, silage quality, feed intake or animal production (**II**, **Experiment 2**). Powdery mildew is an infection that is most prevalent when the days are warm and dry, but the nights are cool (Cousin, 1997), as they were during this experiment (Figure 5). As in the comparison of the wilting and acid-treatments, the two most important parameters of chemical composition were considered to be WSC (Dewhurst et al., 2000, Givens and Rulquin, 2004) and ammonia-N (Charmley, 2001). At all three maturity stages, pH was efficiently reduced and the silages were of good hygienic quality (Huhtanen et al., 2003), with traces of butyric acid, acetic acid contents < 15 g kg<sup>-1</sup> DM and ethanol contents < 10 g kg<sup>-1</sup> DM (II, Experiment 2). The pod fill silage had an intermediate WSC content, not differing significantly from either the higher content of the flat pods silage or the lower content of the full pod silage, and the ammonia-N contents were similar in all three silages. In terms of overall performance, the best silage was obtained from the pod fill forage. Although DM intakes were highest for the full pod silage, the flat pods and pod fill silages had better digestibility and N efficiency than the full pod silage. However, intake of pod fill and full pod silages stimulated higher milk, milk fat and milk protein yields than flat pods silage. Based on these results, the recommended maturity stage for harvesting pea-oat bi-crop silage is when the peas are at the pod fill stage (Knott, 1987), and the oats are in the late milk to early dough stage (Zadoks et al., 1974).

In the subsequent experiment (III), the pea-oat bi-crop was cut when the peas were at the pod fill and the oats at the early to soft dough stage, but PROMYR<sup>™</sup> was used instead of PROENS<sup>™</sup>. The resulting silage was of good fermentation quality (Huhtanen et al., 2003), with no butyric acid, acetic acid content of 9 g kg<sup>-1</sup> DM and an ethanol content of 4 g kg<sup>-1</sup> DM. The ammonia-N contents of the pod fill silages from II (Experiment 2) and III were not comparable due to the different additives used. In III, the concentrate levels fed (per cow and day) were 7.0 kg (6.2 kg DM) and 10.0 kg (8.8 kg DM), compared to 8.0 kg (7.2 kg DM) in II (Experiment 2). Therefore, it was difficult to compare the results, but some observations were made: the digestibility was almost identical; the DM, starch and NDF intakes were higher in III (Figure 7); and the milk yield, protein yield and N efficiency were reduced when feeding 7.0 kg concentrate but increased when feeding 10.0 kg concentrate in III. Thus, harvesting pea-oat bi-crops when the peas are at pod fill and the oats at late milk to soft dough does, indeed, appear to yield high quality silage that is appropriate for dairy cows. An additional benefit of harvesting pea-oat bi-crops when the peas are at the pod fill stage is that they have seldom lodged at this stage (II, Experiment 2) unless unsuitable cultivars have been chosen (I; II, Experiment 1).



*Figure* 7. The intake (**A**), digestibility (**B**), and effect on milk yield, milk fat and milk protein content (**C**) of the pod fill pea-oat bi-crop silages in **II** (**Experiment 2**) and **III**. Means  $\pm$  s.e.m. DM = dry matter, NDF = neutral detergent fibre, CP = crude protein, OM = organic matter.

# 6.3 Use of pea-oat bi-crop silage in diets of high-yielding dairy cows (III)

Most studies performed with pea-based silages in the diets of dairy cows have used cows of low or intermediate yield, i.e. about 20 kg day<sup>-1</sup> (Kindesjö, 1984, Urbanski and Brzóska, 1996, Salawu et al., 2002a, Adesogan et al., 2004) and few published studies have described the effects of using pea-based silages in diets of intermediate or high-yielding dairy cows (Mustafa et al., 2000, Pursiainen and Tuori, 2006, Rondahl et al., 2006, Rondahl et al., 2007). Nevertheless, these studies have shown that pea-based silages can be used in diets for dairy cows of any yield level, but there are still doubts about their suitability for high-yielding dairy cows, for two main reasons. Firstly, the high proportion of soluble proteins in peas increases the N losses unless the protein degradability in the rumen can be reduced. Secondly, most of the pea starch is rapidly degradable in the rumen (Yu et al., 2002), and the risk of rumen acidosis and other ailments is increased when intakes of rapidly-degradable starch are high (Nocek, 1997). Cereals have very high contents of rapidly-degradable starch (Robinson and McQueen, 1989), further increasing the doubts about including pea:cereal silages in diets of high-yielding dairy cows.

#### 6.3.1 Silage intake and milk production

The feed intakes of acid-treated and wilted silages were similar, and the highest intake was of the silage treated with 12 l tonne<sup>-1</sup> FM, harvested when the peas were at the full pod stage and the oats were ripe (**II**, **Experiment 1**). The extremely high level of acid added to this silage restricted the fermentation to a minimum and no WSC was consumed during fermentation. Instead, there was an increase in the WSC content after ensilage, presumably because the addition of high amounts of organic acids can produce reducing sugars from hemicelluloses by acid hydrolysis (pH 4) during storage (Dewar et al., 1963). There were no immediately obvious adverse effects on the health of the animals fed this high-level acid silage. However, such high additions of acids would not be economically justifiable in routine practice.

The voluntary intake and digestibility of pea-oat bi-crops varied according to the maturity stage, therefore the stage of crop maturity at harvest also affected milk production (**II**, **Experiment 2**). As already mentioned, the flat pod and pod fill silages had higher digestibility than full pod silage, as also evidenced by the higher N-use-efficiency of cows eating these silages. On the other hand, voluntary intakes of the pod fill and full pod silages were higher than that of the flat pod silages. This resulted in better production and higher yields of milk, milk fat and milk protein from the pod fill and full pod silages. The starch intake appeared to be of greater importance for the milk protein yield than the NDF intake, since the NDF intake was similar for all silages.

#### 6.3.2 Pea-oat bi-crop silage compared to grass-clover silage

Cows that were fed a 0.50:0.50 (DM basis) mixture of pea-oat bi-crop silage and grass-clover silage in combination with 7 kg concentrate day<sup>-1</sup> had similar milk yields to cows fed 10 kg concentrate day<sup>-1</sup> and either the mixture or the component silages alone (**III**). These findings suggest that a mixed ration of pea-oat bi-crop and grass-clover silage has a concentratesparing effect. The pea-oat silage could replace the grass-clover silage at both concentrate levels, although the milk yields decreased when cows were fed either of the component silages with 7 kg concentrate day<sup>-1</sup>. The silage DM intake did not significantly differ between the diets, although there were some between-diet differences in NDF intake and NDF digestibility (**III**). The NDF intake did not increase as NDF digestibility increased, as has been suggested previously (Allen, 2000). However, the NDF digestion rate and the NDF amount in the diet can affect intake (Miller et al., 1990). Both silages could be characterised as low bulk silages, with low NDF contents compared to those used in similar studies (Salawu et al., 2002a, Adesogan et al., 2004). Legumes have a higher rumen passage rate than grasses (Waghorn et al., 1989, Hoffman et al., 1998, Salawu et al., 2002a), and this could explain the similarity of the DM intake of the diets (Allen, 2000). However, rapid physical breakdown of legume leaves (i.e. pea plants and red clover) can be very important for DM intake. Efficient chewing or ruminating increases the rumen passage rate of legume leaves. This, together with the high protein content of legumes, enables more feed protein to reach and be digested in the intestines of cows fed legumes than cows fed grasses (Waghorn et al., 1989).

The relatively low CP (144 g kg<sup>-1</sup> DM) and high starch (207 g kg<sup>-1</sup> DM) contents of the pea-oat bi-crop silage made it suitable for mixing with the grass-clover silage to manipulate the ratio of CP and starch (III), as suggested by Dewhurst et al., 2000. The diet with pea-oat bi-crop silage + 10 kg commercial concentrate improved N efficiency and maintained milk yields. However, the diet with mixed silage + 7 kg concentrate resulted in similar N efficiencies and milk yields to all three diets that included 10 kg concentrate. The best way to enhance N-use efficiency in milk production is to manipulate the rumen ratio of degradable protein and fermentable energy sources, e.g. non-structural carbohydrates (Castillo et al., 2001, Huhtanen and Shingfield, 2005). Furthermore, N losses can be reduced without any adverse effect on milk production by feeding mixtures of energy sources that include starches with low degradability, and by balancing the diets according to the animals' requirements and level of milk production (Castillo et al., 2001). It is important to ensure there is an adequate supply of fermentable carbohydrates in the diet (Salawu et al., 2002a). The pea-oat bi-crop silages resulted in degradable-starch intakes that were higher than the recommended 200 g kg<sup>-1</sup> DM of total feed intake (Nocek, 1997). Overfeeding of rapidly-degradable starch can cause rumen acidosis (Nocek, 1997), but no such symptoms were detected in any of the cows fed the diets containing the pea-oat bi-crop silages.

# 7 Conclusions

The main overall conclusion from the studies presented in this thesis is that the optimal time for harvesting pea-oat bi-crop silage is when the peas are at the pod fill stage and the oats are between the late milk to soft dough stage, and the optimal treatment is wilting to  $\geq 250$  g kg<sup>-1</sup> DM, then adding 6 1 acid tonne<sup>-1</sup> FM. Furthermore, when pea-oat bi-crop silage is mixed with high-quality grass-clover silage in diets supplied to high-yielding dairy cows the effect of the grass-clover silage is improved, and there is a concentratesparing effect.

In more detail, the conclusions were:

- whole-crop, direct-harvested pea silages have low DM contents and reduced ensilability when harvested at the pod set stage, and an acid addition of 8 l tonne<sup>-1</sup> FM was necessary to reach a stable pH during their ensilage (I). At the pod swell and full pod stages, an acid addition of 6 l tonne<sup>-1</sup> DM was sufficient for stable fermentation and reduction of proteolysis (I)
- the extent of proteolysis differed between the two pea cultivars variegated Timo and white-flowered, semi-leafless Capella; there was more proteolysis in acid-treated Capella silages harvested at the pod set and pod swell stages, but less proteolysis in wilted Capella harvested at the pod swell and full pod stages than in corresponding Timo silages (I)
- although wilting can reduce proteolysis during ensilage (I), efficient wilting was difficult to achieve due to the frequent occurrence of adverse weather conditions (I; II, Experiment 1)

- to reduce the risk of pea-oat bi-crop lodging it is recommended that a pea cultivar with stiff stalks and an oat cultivar with stiff stems are selected e.g. white-flowered, semi-leafless Nitouche peas and Belinda oats (II). Furthermore, the maturity rate of the two cultivars should be considered carefully to reduce the risk of the oats being too ripe at the time of harvest (II)
- ➤ wilting to ≥ 250 g kg<sup>-1</sup> DM and treatment with 6 l acid tonne<sup>-1</sup> FM ensures good ensiling of whole-crop pea-oat silage and facilitates harvesting at a desired maturity stage (II, Experiment 2)
- based on silage intake, diet digestibility, N-use efficiency and milk production, the recommended maturity stage for harvesting pea-oat bicrop silage is when the peas are at the pod fill stage, and the oats are in the late milk to soft dough stage (II, Experiment 2; III)
- pea-oat bi-crop silage can replace high-quality grass-clover silage in diets of high-yielding dairy cows; cows fed pea-oat bi-crop silage had similar DM intakes and milk yields to those fed high-quality grass silage, and higher N-use efficiency (III)
- a 0.50:0.50 mixture of pea-oat bi-crop and grass-clover silage has a concentrate-sparing effect; feeding high-yielding dairy cows a diet with a 0.50:0.50 mixture (DM basis) of pea-oat and grass-clover silage and 7 kg concentrate resulted in the same milk yield as diets that included 10 kg concentrate and either each silage alone or the mixed silage (III).

# 7.1 Advice for farmers

The following advice, based on the results presented in this thesis, presumes that the farmers use an 80:20 seed rate (200 kg:50 kg) for pea-oat bi-crops.

The aim should be to harvest pea-oat bi-crops when the peas are at the pod fill stage and the oats are in the late milk to soft dough stage. However, if the peas mature to the full pod stage, the resulting silage will still be of good nutritional quality. Therefore, the harvest window for pea-oat bi-crops is widened, and the crop can be cut when the weather is appropriate.

- Pea-oat bi-crops can be cut with a small-plated disc mower without conditioner.
- Wilting the pea-oat bi-crop to about 250 g kg<sup>-1</sup> DM and then adding 6 l acid kg<sup>-1</sup> FM will ensure good ensiling.
- A forage with about 300 g kg<sup>-1</sup> DM can be ensiled as big bales, but if the DM content is about 250 g kg<sup>-1</sup> DM it is recommended that bunker silos are used due to the increased amount of effluent.
- The resulting pea-oat bi-crop silage can be used in diets to both intermediate- and high-yielding cows. It can replace high-quality grassclover silage. However, it is even more beneficial to mix pea-oat bi-crop and grass-clover silage 0.50:0.50 on a DM basis, as this has a concentratesparing effect.

# 8 Future prospects

### 8.1 Immediate future

It would be very interesting to determine the extent of proteolysis in the silages used in **II** and **III**. The main question to address is whether the extent of proteolysis was correlated with silage intake, diet digestibility, N-use efficiency and milk yield. Furthermore, the analysis should also assess whether the effects of treatment and maturity stage in pea-oat bi-crops (**II**) were similar to those observed in whole-crop peas (**I**), and if there were major differences in the effects of PROENS<sup>TM</sup> (**II**) and PROMYR<sup>TM</sup> (**III**) on proteolysis.

Recently, a common feed evaluation system (called NorFor) was suggested for Sweden, Norway, Denmark and Iceland (Mehlqvist and Gustafsson, 2005). This system was introduced to the Swedish market in 2007 by the Swedish Dairy Association. As the energy content of wholecrops is difficult to estimate, with consequent difficulties in feed evaluation, it would be very interesting to analyse the feeds used in both **II** (**Experiment 2**) and **III** according to the NorFor feed evaluation system. Since we already have the animal production data, this could be a retrospective study to test the accuracy of the NorFor system for evaluating pea-oat silages in feed rations for high-yielding dairy cows.

## 8.2 Looking ahead

The beneficial effects of mixing pea-oat bi-crop silage with grass-clover silage are of such potential interest that we would like to explore them in more detail. In addition, the notorious unpredictability of pea crops regarding yield and botanical composition (Gilliland and Johnston, 1992)

necessitates repeated studies to verify the concentrate-sparing effects of peaoat bi-crop silages in mixtures with grass-clover silages. The pea-oat:grassclover mix diets used in **III** appeared to result in stable milk production rates, similar to those of cows fed grass-clover silage with 10 kg concentrate day<sup>-1</sup>, and it would be very beneficial for the farmer if this stability persisted throughout most of the lactation period. Therefore a longer production study would be warranted. This would also yield more reliable data on body weight and body score index changes. No significant changes in these parameters were detected in the studies reported here, but it was not determined whether this was because the studies were too short, or if there were really no changes in them. The new loose-housing barn at Röbäcksdalen has better facilities for weighing the cows than the previous tie-stall barn where the studies underlying this thesis were performed.

In Paper **III** we suggested that the grass-clover silage supplied enough WSC to the diets to increase microbial protein synthesis rates, i.e. rumen synchrony (Dewhurst et al., 2000), which would explain the lack of difference in N-use efficiency between the mixed and grass-clover silages. Furthermore we observed that the pea-oat bi-crop silages resulted in starch intakes higher than the recommended 200 g kg<sup>-1</sup> DM of total feed intake (Nocek, 1997), but that there were no detrimental health effects in the cows fed them. A production and digestibility study incorporating determinations of blood N metabolite contents and N excretion in urine and faeces would further elucidate the N-use efficiency.

Finally, the new loose-housing barn at Röbäcksdalen has increased the possibilities for production experiments on dairy cows at the department. It incorporates one warm and one cold housing area, allowing comparative studies on forage intake effects on milk production at different environmental temperatures. There are also balconies in both housing areas, enabling researchers to easily observe cows and register their behaviour, e.g. feeding and resting patterns. Cows fed pea-wheat bi-crop silages have been found to spend more time at the feed bunks and have more meals than cows fed grass silages (Salawu et al., 2002a). It would be very interesting to determine if this was valid not only for pea-oat bi-crop silages, but also for mixed diets such as the one used in **III**.

# 9 Populärvetenskaplig sammanfattning

Ett stort problem för både ekologisk och konventionell mjölkproduktion är som kan tillgodose mjölkkornas att producera fodermedel stora proteinbehov. Konventionella mjölkproducenter kan använda proteinfoder baserat på importerade sojarestprodukter, men ekologiska producenter måste antingen odla själva eller köpa godkända ekologiska alternativ. EU:s ekologisk kompletteringsförordning om animalieproduktion (nr. 1804/1999), som antogs 1999, beslutade att från 2005 skall alla djur inom ekologisk animalieproduktion utfodras enbart med ekologiskt producerat foder. Dessutom efterfrågar konventionella mjölkproducenter alternativa, egenodlade proteinfodermedel, eftersom importerade sojabaserade fodermedel stiger alltmer i pris. De vanligast förekommande egenodlade svenska proteinfodren är ärtor, åkerbönor och raps. Av dessa är det bara ärtor som kan odlas och färdigställas på gårdar över i stort sett hela Sverige. Ärtor liksom övriga baljväxter är självförsörjande när det gäller kväve. De klassas som proteinfodermedel eftersom de innehåller mer protein än spannmål och har liksom de senare högt stärkelseinnehåll. Till ensilage odlas de sällan i renbestånd eftersom avkastningen kan variera mellan år och grödan tenderar att lägga sig med ökad mognad. En liggande gröda är svårskördad, och fältförlusterna kan därför bli omfattande. För att minska risken för att ärtplantor lägger sig odlas ärtor ofta i blandning med spannmål. En grönfoderblandning av ärt och spannmål får ofta också en bättre balans mellan energi och protein om den utfodras som ensilage till mjölkkor. Årtbaserade ensilage äts gärna av mjölkkor, det har dessutom förhållandevis låg andel fiber (NDF), vilket ger potential till stort foderintag.

En nackdel med ärtor är att ärtprotein är lättlösligt, vilket innebär att en stor andel av det lättlösliga proteinet omvandlas till ammoniak, peptider, aminosyror mm i vommen och kväveöverskottet förloras via urin och träck. Detta resulterar i stora kväveförluster, vilket dels innebär att kon utnyttjar fodret optimalt, dels att kväveutsöndringen till miljön blir onödigt hög. Genom att stabilisera ärtproteinet kan man minska dess nedbrytning i kons förmagar. Istället passerar det vidare till tarmkanalen, där det kan tas upp och utnyttjas av kon till mjölkproduktion. Därigenom utnyttjas fodret bättre av kon, och kväveutsöndringen till miljön minskar.

# 9.1 Avhandlingens syfte

Syftet med denna avhandling kan indelas i tre delar:

- 1. att undersöka olika konserveringsmetoder och mognadsstadiers inverkan på proteinnedbrytningen i ärtensilage
- 2. att fastställa optimal skördetidpunkt och konserveringsmetod för en ärt-havregröda till grönfoderensilage för utfodring av mjölkkor
- 3. att fastställa om en optimalt skördad och konserverad ärt-havregröda ger ett grönfoderensilage vars nutritionella kvalitet är jämförbar med ett bra vallensilage, eller utgör en bra komponent till fullfoderblandning tillsammans med ett bra vallensilage så att det lämpar sig för utfodring till högavkastande mjölkkor

Dessa studier skulle utmynna i konkreta råd till lantbrukare angående utnyttjande av ärt-havre ensilage till mjölkkor. Lantbrukarens självförsörjning av proteinfodermedel skulle därmed kunna öka, och behovet av att köpa importerat proteinfoder minska. Därutöver får lantbrukaren ytterligare en gröda att använda i växtföljden. Eftersom ärtgrödor har kväve-fixerande egenskaper minskar behovet av kvävegödsling eftersom ärtgrödan kommer att tillföra kväve genom kvävefixering.

# 9.2 Studierna som ingår i avhandlingen

Alla grödor som användes i avhandlingen var odlade vid Sveriges Lantbruksuniversitets försöksgård Röbäcksdalen i Umeå under åren 2000-2003. Korna som ingick i de olika försöken tillhörde Röbäcksdalens besättning med kor av Svensk Röd mjölkras. Samtliga maskiner, tillsatsmedel och växtsorter som användes var sådana som var tillgängliga för lantbrukare då försöken genomfördes. För att slå ärt-baserade helgrödor efter baljsättning bör en rotorslåtter utan krossaggregat användas för att minska risken att ärtfröna ska tröskas ur genom att grödan dubbelslås (Figur 8). I studierna har ärt-havre ensilage med framgång ensilerats både som storbalar (experiment 1, studie II) och i plansilo (experiment 2, studie II, och i studie III) (Figur 9).



*Figure 8.* Rotorslåttermaskin med små rotortallrikar och utan krossaggregat. Foto: T. Rondahl.



*Figure 9*. Till vänster rundbalspress med inplastare (användes i experiment 1, studie II). Till höger packning av ärt-havre ensilage i plansilo (användes i experiment 2, studie II och i studie III). Innan inläggning i plansilo hade grödan exakthackats, se omslagsbild. Foto: T. Rondahl.

I den första studien, som var ett försök med mini-silor, studerades om ärtgrödans mognad samt behandlingen av ärtgrödan innan ensilering kunde påverka ärtproteinets löslighet. Under ensileringen bryter växtenzymer ned protein så att andelen lättlösligt protein i ensilaget ökar, om man jämför med innehållet i den färska grödan. I studien jämfördes två ärtsorter, Timo, en brokblommig ren foderärt samt en vitblommig matärt, Capella. Dessa skördades vid olika mognadsstadier: blomning (8 respektive 9 veckor efter sådd), baljsättning (10 respektive 11 veckor efter sådd) samt ärtutveckling (12 respektive 14 veckor efter sådd). Varje gröda skördades vid tre tillfällen (mognadsstadier). Vid varje tillfälle delades grödan upp i hälften. Ena hälften ensilerades direkt genom tillsats av 6 l Proens<sup>™</sup> per ton grönmassa (Proens<sup>™</sup> är en blandning av ca 2/3 myrsyra och 1/3 propionsyra). Den andra hälften efter att den skördade grödan fått förtorka till ca 40% ts-halt. Efter ca 100 dagar förvaring i rumstemperatur öppnades silorna och det färdiga ensilaget analyserades. Denna studie baserades enbart på laboratorieanalyser av ärtensilaget. Nedbrytningen av protein minskade ju mer mogen ärtgrödan var – de senare skördade ensilagen hade en lägre andel lättlösligt protein än de tidigare skördade ensilagen. Dessutom kunde både förtorkning och tillsats av syra minska nedbrytningen av ärtproteinet under ensileringen. Även om proteinnedbrytningen var något mindre i Timo än i Capella vid de tidigare skördetillfällena, så var ärtsorterna mycket lika vid sista skörd. Dessa resultat kommer att publiceras som "nytt från institutionen för norrländsk jordbruksvetenskap, ekologisk odling" 2007-2008.

Den andra studien bestod av två experiment under två påföljande år. Till dessa två utfodringsförsök odlades ärt-havre blandningar 80:20 på normal utsädesmängd för ärt respektive havre (200 kg ärt:50 kg havre). I det första experimentet användes Capella (ärt) och Svala (havre), och grödan skördades vid två olika tillfällen: tidig baljsättning (10 veckor efter sådd) och fullmatade baljor (15 veckor efter sådd). Vid första tillfället jämfördes förtorkning till ca 30% ts-halt med direktskörd och tillsats av 6 l Proens<sup>TM</sup> per ton grönmassa. Vid andra tillfället var enda konserveringsmetoden 12 l Proens<sup>TM</sup> per ton pga det mycket blöta vädret. Vid detta tillfälle hade stora delar av grödan lagt sig och det var mycket svårskördat. Medeltalen för foderintaget av dessa tre ensilage var 9 – 10,5 kg ts per ko och dag. Högst intag blev det för det sent skördade ensilaget, medan foderintaget var likvärdigt för de två tidigt skördade ensilagen. För att kunna jämföra ensilage från olika skördetillfällen

I det andra experimentet användes Nitouche (ärt) och Belinda (havre). Nitouche är en mer stjälkstyv sort än Capella och Belinda är en mer stråstyv sort än Svala, dessutom mognar Belinda långsammare än Svala. Dessa sorter valdes i syfte att minska risken för att grödan skulle lägga sig vid senare mognadsstadier, samt för att grödan skulle få bättre ensileringsegenskaper. Grödan skördades vid olika mognadsgrad och förtorkades på fält till ca 25% ts-halt, därefter tillsattes 6 l Proens™ per ton grönmassa. Mognadsstadie vid skörd var platta baljskidor, ärtutveckling och fullmatade baljor för ärt och tidig mjölkmognad, tidig degmognad och sen degmognad för havren. Foderintaget av dessa tre ensilage var från 9,5 till 12,5 kg ts per ko och dag, med högst intag av det senast skördade ensilaget, d.v.s då ärterna hade fullmatade baljor. På motsvarande vis hade kor som åt av det senast skördade ensilaget högst avkastning, i genomsnitt 30,4 kg per ko och dag (energikorrigerad mjölk). Dock var fodrets smältbarhet högst när ärterna var i ärtutveckling och havren i sen degmognad. Lämpligast mognadsstadium för skörd av ärt-havre gröda torde därför vara när ärternas utveckling är mellan

ärtutveckling och fullmatade baljor. Detta ger ett skördefönster på ungefär två veckor, och skörd bör därför kunna ske vid gynnsam väderlek. Resultaten av dessa studier har publicerats i serien "nytt från institutionen för norrländsk jordbruksvetenskap, ekologisk odling" som nr 3 2005.

I den sista studien skördades en ärt-havre gröda enligt den bästa metoden funnen i studie II (se ovan), d.v.s då ärterna var i ärtutveckling med halvmatade baljor. Dock användes 6 l Promyr<sup>™</sup> för syra tillsats per ton grönmassa (en blandning av myrsyra, propionsyra och ammoniak) istället för Proens<sup>™</sup>. Ett vallensilage (timotej, ängssvingel och rödklöver) skördades som förstavall och förtorkades över natt innan 4 1 Promyr<sup>™</sup> tillsattes. Vallensilaget hade hög kvalitet - 11,3 MJ omsättbar energi. Ensilagen utfodrades tillsammans med två olika kraftfodergivor - 7 respektive 10 kg till kor av hög avkastningsnivå (i genomsnitt 30,7 kg energikorrigerad mjölk). Ärt-havre ensilaget kunde ersätta vallensilaget i foderstater till mjölkkor med hög produktionsnivå, oavsett koncentratgiva. Dessutom visade det sig att en 0.50:0.50 fullfoderblandning av de två ensilagen var bättre än då varje ensilage utfodrades för sig. Kor som åt fullfoderblandningen tillsammans med 7 kg kraftfoder per dag producerade lika mycket mjölk som de kor som åt endera ärt-havre ensilage eller vallensilage kombinerat med 10 kg kraftfoder per dag. Härmed kunde man konstatera att fullfoderblandningen av de båda ensilagen hade en så kallad koncentratsparande effekt. Detta innebär att andelen kraftfoder kan minskas i foderstaten utan att korna påverkas negativt avkastningsmässigt. Dessa resultat kommer att publiceras som "nytt från institutionen för norrländsk jordbruksvetenskap, ekologisk odling" 2007-2008.

### 9.3 Slutsats av avhandlingen

Slutsatsen av avhandlingen är att ärt-havre ensilage (utsädesproportion 200 kg – 50 kg) med fördel kan användas till mjölkkor, under förutsättning att det skördas när ärterna är i ärtutveckling (halvmatade till fullmatade baljor), samt att de förtorkas till minst 25 % ts-halt innan 6 l syra per ton grönmassa tillsätts. Denna strategi ger ett skördefönster på ungefär två veckor.

Lantbrukare rekommenderas att:

Skörda ärt-havre gröda när ärterna är i ärtutveckling (halvmatade till fullmatade baljor) och havren i sen mjölkmognad till mjuk degmognad

- En rotorslåtter utan krossaggregat kan användas för att skörda en ärthavre gröda
- En förtorkning av grödan till ca 25 % ts-halt följt av en tillsats av 6 l syra per ton grönmassa gör att grödan kan skördas vid rätt mognadsstadie, när väderleken medger. Syratillsats försäkrar en stabil ensilering
- Gröda med ca 30 % ts-halt kan ensileras som rundbalar, medan en gröda med ca 25 % ts-halt rekommenderas att ensilera i plansilo pga den ökade mängden pressvatten
- Det resulterande ärt-havre ensilaget kan ersätta vallensilage till mjölkkor, eller ännu hellre blandas med vallensilage (av hög kvalitet) 0.50:0.50 i en fullfoderblandning

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