Performance of Forage Maize at High Latitudes

Plant Development, Agronomy and Nutritive Value

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Abstract

Following the development of earlier maturing hybrids the cultivation of forage maize (*Zea mays* L.) in Northern Europe has markedly increased in recent decades. This has raised needs for greater knowledge regarding the performance of forage maize in marginal areas, such as Sweden, under current conditions. Thus, the aim of the studies presented in this thesis was to assess the utility of different techniques for evaluating the agronomic performance and nutritive values for ruminants of maize hybrids grown at high latitudes.

The development of selected maize hybrids was graded during their reproductive growth stages, and the nutritive values of both the whole plants and plant fractions were analyzed using available analytical techniques, *inter alia* chemical, *in vitro, in situ* procedures and near infrared spectroscopy (NIRS). The results showed that longer cultivation times are required by maize hybrids in order to accumulate sufficient crop heat units to reach given developmental stages at high latitudes. Thus, the plants may remain immature with high moisture contents, low agronomic and nutritive values at harvest. Dry matter (DM) yields were highest for relatively late maturing hybrids (FAO 210), but the earliest maturing hybrid (FAO 180) had the highest fractional proportion of kernels and neutral detergent fibre digestibility (NDFD) at harvest. Hence, the DM intake and performance of ruminants should be highest if fed the latter. Since the maize hybrids showed nutritional differences at a given maturity (DM concentration g/kg), the effects of maturation should be addressed in trials designed to compare their potential nutritive performance.

The *in vitro* gas production technique showed potential utility for assessing the nutritional value of forage maize, particularly the contributions of specific morphological fractions to whole plant performance. Increasing maturity reduced the *in vitro* NDFD in all plant fractions, but increased the rate of rumen degradation of organic matter in the whole plants due to increases in starch concentration. This conclusion was corroborated by multivariate analysis of forage nutritive value parameters using the MILK 2006 model.

The evaluation of analytical techniques revealed that most tested techniques gave biased predictions of the investigated parameters and that a new calibration set would most likely improve the validity of the NIRS predictions. Overall, the results from the studies underlying this thesis indicate that the agronomic and nutritional value of forage maize hybrids cultivated at high latitudes will depend on their DM maturity at harvest, and thus on both the site and the hybrid.

Keywords: Crop Heat Units, Digestibility, Dry matter concentration, Dry matter yield, Gas production, Maturity, Morphological fractions, MILK 2006, Neutral Detergent Fibre, Net Energy for Lactation.

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Dedication

To my parents and wife, with gratitude for their love and prayers for my success

Contents

List of Publications

This thesis is based on the work presented in the following papers, which are referred to by the corresponding Roman numerals in the text.

I Z. Mussadiq, M. Hetta, C. Swensson and A–M. Gustavsson (2012). Plant development, agronomic performance and nutritive value of forage maize depending on hybrid and marginal site conditions at high latitudes. *Acta Agriculturae Scandinavica. B–Plant and Soil Science* 62, 420–430.

II M. Hetta, Z. Mussadiq, A–M. Gustavsson and C. Swensson (2012). Effects of hybrid and maturity on performance and nutritive characteristics of forage maize at high latitudes, estimated using the gas production technique. *Animal Feed Science and Technology* 171, 20–30.

III Z. Mussadiq, A–M. Gustavsson, P. Geladi, C. Swensson and M. Hetta (2012). Multivariate models of the predicted milk yield and forage quality depending on growing site, plant maturity, contribution and nutritional quality of morphological fractions (submitted).

IV Z. Mussadiq, S. J. Krizsan, M. Hetta, M. Ramin and P. Huhtanen (2012). Predicting feed value of forage maize hybrids harvested at different maturity and sites (manuscript).

Papers I and II are reproduced with the permission of the publishers.

The contributions of Zohaib Mussadiq to the papers included in this thesis were as follows.

I Planning and executing the experiment jointly with co-authors, statistical analysis of data and writing the manuscript.

II Planning and executing the experiment jointly with co-authors, and actively participating in writing the manuscript.

III Planning and executing the experiment jointly with co-authors, statistical analysis of data and writing the manuscript.

IV Planning and executing the experiment jointly with co-authors, statistical analysis of data and participating in writing the manuscript.

Abbreviations

CHU	Crop heat unit
DM	Dry matter
EFOS	Enzym Fordøjelig Organisk Stof = Enzyme digestible organic matter
FAO	Food and Agriculture Organization of the United Nations
GDD	Growing degree days
GP	Gas production
iNDF	Indigestible neutral detergent fibre
NDF	Neutral detergent fibre
NDFD	Neutral detergent fibre digestibility
NEL	Net energy for lactation
OM	Organic matter
OMD	Organic matter digestibility
PCA	Principal Component Analysis
PLS	Partial Least Squares
RMSEP	Root mean square error of prediction
VOS	Våmvätskelöslig organisk substans = Rumen fluid digestible organic matter

1 Introduction

1.1 Background of the studies

Maize or corn (*Zea mays* L.) is a warm season grass that is being increasingly used for diverse purposes globally, e.g. for human food, ethanol production, biogas, feed for livestock and raw material for producing bio-plastics.

In Scandinavia, maize is mostly cultivated as a whole crop for making silage as forage for dairy cows, mainly due to the climatic conditions preventing its cultivation for other purposes. At high latitudes the maturation of the crop is limited by the short growing seasons with long photoperiods and low temperatures (Struik, 1983), resulting in crops with high moisture and low starch concentrations at harvest (Arnesson *et al*., 2009). Studies and practical experience in Denmark have shown that acceptable maturity for producing silage is reached at dry matter (DM) concentrations of 300-350 g/kg (Mikkelsen *et al.,* 2008). However, despite these limitations, maize grown in Scandinavia may yield forage with high nutritive value relatively cost efficiently, as it gives high DM yields compared to perennial forages and only needs to be harvested once per season. Consequently maize is now a well-established forage crop in southern Scandinavia, and in recent decades the area of land used for its cultivation in Sweden has increased from 2 000 to 17 000 ha (Jordbruksverket, 2010). The main reason for the increase is the introduction of new, earlier maturing hybrids (Arnesson *et al*., 2009). In addition, modelling studies (e.g. Eckersten *et al*., 2012), have shown that maize cultivation could potentially be extended further north, mainly due to changes in climatic conditions. However, no maize hybrids are being bred in Sweden to suit the local climate, thus only internationally developed hybrids are currently available.

Despite the growing interest in its cultivation there have been few scientific investigations of maize performance in northern areas (e.g. Pulli *et al*., 1979) hence there is a need for greater knowledge of factors influencing the performance of forage maize in ruminant nutrition under current conditions at high latitudes. Therefore, key aims of the studies underlying this thesis were to improve knowledge of forage maize performance and feed value in relation to hybrid, maturity and climatic conditions. A further major aim was to assess the utility of available methods for analyzing its performance.

1.2 History

Maize was given its scientific binomial name, *Zea mays* L. in 1748 by the famous Swedish scientist Carl Linnaeus. It has been cultivated for a long time. As detailed in Rebourg *et al*. (2003), maize is thought to have been domesticated from teosintes species of wild grass in Central America about 9000 years ago. Maize was brought to Europe by Columbus following his voyages of discovery in the 15th century and its cultivation subsequently spread to central and southern Europe. A key step in the development of human agriculture was taken several centuries later, when Reihlen at Stuttgart, Germany, first explored the ensilage of forage maize in the 1860s. This practice was rapidly established in the USA, where the first silo was built in 1875 (Bunting, 1978). August Goffart, a French farmer, published a book entitled *The Ensilage of Maize and other Green Fodder Crops*, in 1877, and in 1883 a survey commissioned by the British government reported that maize was the best crop for silage (Seifers, 2000). By the mid-19th century ensiling grass and sugar beets tops had spread beyond the Baltic and Germany to most of Europe (Seifers, 2000). By the end of the 19th century a number of farmers were growing maize regularly in the UK, and in 1901 investigations on maize as fodder were initiated at the UK's South Eastern Agricultural College in Wye (Bunting, 1978). Average yields of maize kernels increased 6-fold in the USA and Canada between 1935 and 2005, a rise attributed to the use of improved hybrids and advances in agronomic practices (Lee & Tollenaar, 2007).

1.3 Botany and physiology of forage maize

Barnes & Nelsen (2003) defined forages as "plant and plant parts that are consumed in many forms by domestic livestock, game animals and a wide range of other animals and insects". Most cultivated forages are members of one of two botanical families: *Poaceae*, the grasses, or *Leguminosae*, the legumes (Barnes & Nelsen, 2003). Maize belongs to the *Poaceae* (*Gramineae*) family. Maize (Figure 1) is a tall grass, producing stems that can range in height from 0.6 to 5 m and consist of phytomers that are divided into nodes, internodes, leaf sheaths, leaf blades and auricles. Morphologically, a maize leaf is long, narrow, undulating and tapers towards the tip. The plants produce enclosed structures, consisting of kernels and cobs, called ears. There can be more than one ear on a single plant, but normally one ear develops most fully and is called the main ear. The maize kernel is a caryopsis consisting of pericarp or skin, germ or embryo and endosperm. Generally, the endosperm, germ and pericarp account for 82%, 10–12% and 6–8% of the total kernel weight, respectively. Endosperm consists of about 90% starch, 10% crude protein, oils, minerals and other constituents (Rooney *et al*., 2004). The starch is an important source of energy in forage maize. Maize kernels have different characteristics depending on the type of maize, examples are flint maize and dent maize, the latter being characterized by the formation of dents on the kernels when they mature.

Plants may also be categorized into different classes on the basis of their photosynthetic carbon dioxide (CO_2) fixing pathways. The two main categories of crop plants are C4 plants, e.g. maize, and C3 plants, e.g. alfalfa and soybeans (MacAdam & Nelsen, 2003). Both categories of pathways have advantages and limitations that affect the adaptability of species to the growing environment. C4 plants have the capacity to raise their intercellular carbon dioxide concentrations by fixing it into a 4-carbon molecule called oxaloacetate, making them efficient users of available sunlight and water resources. However, C4 plants are more sensitive to low temperatures (e.g. $< 10°$ C) than C3 species, which can generally fix CO_2 at near-freezing temperatures (MacAdam & Nelson, 2003).

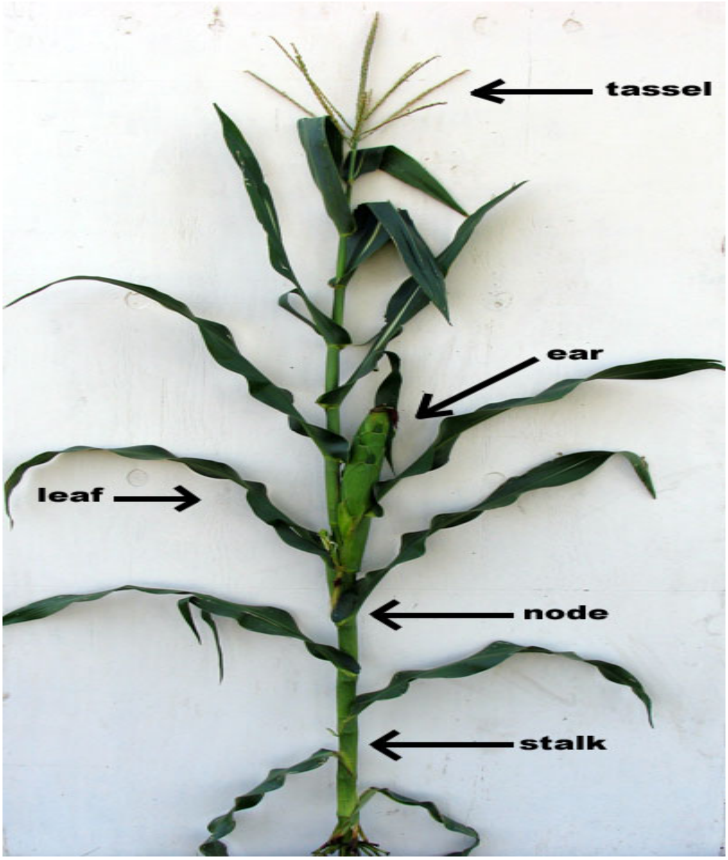

Figure 1. The primary parts of a mature maize plant (Photo: University of Nebraska–Lincoln, 2005)

1.3.1 Temperature responses

Maize growth and development are strongly affected by temperature, especially during the period from planting to silking (Birch *et al*., 1998; OMAFRA, 2009). Three critical temperatures are used to characterize the temperature responses of maize: the base (Tbas), optimum (Topt) and maximum (Tmax) temperatures. Tbas and Tmax are the temperatures below and above which the plants do not grow at all, respectively, while Topt is the temperature at which they grow most rapidly. According to Birch *et al*. (1998) the base, optimum and maximum temperatures, from emergence to tassel initiation, for a range of maize cultivars are 8, 34 and 40 °C, respectively. However the base temperature for C4 grasses e.g. maize, described in MacAdam & Nelseon (2003) is 10° C. Low temperatures during the reproductive phase delay the transportation of available carbohydrates to the ears during grain filling (Jones *et al.,* 1981), and hamper plant growth (Struik *et al.,* 1985). Thus, temperature directly or indirectly affects the rate of DM production (Carr & Hough, 1978). Hence, areas suitable for maize cultivation in Northern Europe, are strongly linked to variation in temperatures (Odgaard *et al*., 2011).

Key climatic parameters for maize production are accumulated thermal units, as the plants develop more rapidly as temperatures increase within the range between Tbas and Topt, then decline with further increases (Stewart *et al*., 1998). Use of these thermal units has proven to be particularly useful in locations such as Canada and northern Europe, where it is important to define as closely as possible both the areas where maize is likely to be grown successfully (Eckersten *et al*., 2012) and the most suitable cultivars for a given locality.

Various models and units have been proposed for describing the cumulative temperature during the growth and development period of maize plants, and the plants' temperature responses. These include: the model presented by Yin *et al*. (1995); crop heat units (CHU), initially used in Ontario, Canada (MAO, 1997; Kwabiah *et al*., 2003); and growing degree days (GDD), proposed by Millner *et al*. (2005). CHUs are most widely used by the farming community and associated organizations in Scandinavia, e.g. the extension service in Denmark (Mikkelsen *et al*. 2008). Cumulative CHUs are calculated from May 1 or the first of three consecutive days with a daily mean temperature above 12.8 ºC in spring until the first day with a minimum temperature of –2º C or lower.

The cumulative sum of CHU during the vegetation period of a region is a useful parameter for selecting hybrids that can be successfully grown. Figure 2 shows the distribution of CHUs in Sweden. The average CHU for a region must exceed the CHU a specific hybrid requires to reach maturity in order to grow it successfully in most years, and the recommended minimum limit for producing forage maize is 2400 CHU (MAO, 1997). The presented map indicates that the coastal regions of southern Sweden are the most suitable for maize production. To avoid the risk of crop failure in the Nordic climate, growers may need to adjust agronomic practices e.g. early planting and selecting appropriate hybrids for sufficient CHU accumulation at the site.

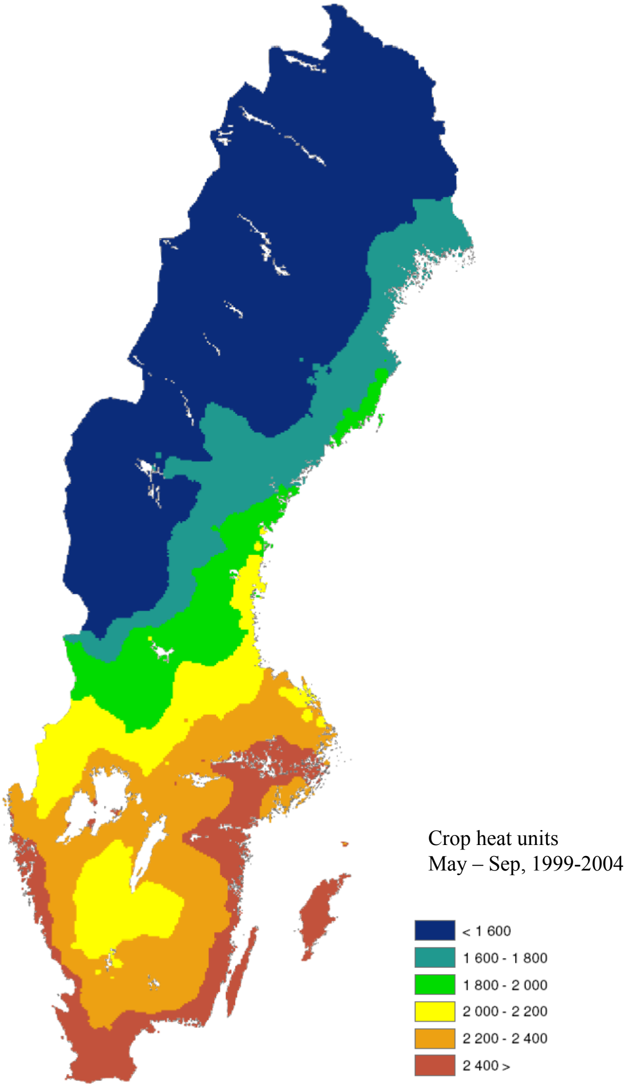

Figure 2. Distribution of crop heat units in Sweden during the forage maize production season (Photo: Gustafsson & Nissen, 2008).

1.4 Plant development and maturation

Plant development and maturation strongly affect DM yields and the DM contributions of different plant fractions to the total plant biomass yield (Phipps & Weller, 1979). Maize has distinct vegetative and reproductive stages, as described in several applied guides for crop management

(OMAFRA, 2009). The vegetative period begins when the seedling emerges and continues until tasseling. Processes during this period include leaf production, root development and growth, stalk formation, and initial formation of reproductive structures (male, tassels, and female, ears). The number of leaves produced by a maize plant is controlled by both genetic and environmental factors (Westgate *et al.*, 2004). The vegetative stages are defined by the number of leaves produced by the plant, and expressed as V1 to Vn (the first to n^{th} leaf stage, respectively) and the last vegetative stage ("tasseling", when tassels appear on the plant) is designated VT. After tasseling, silks appear on the ears, and then the reproductive stage begins with their pollination and ends when starch accumulation in kernels is complete. The associated stages are designated "R1 to R6", described below and partly illustrated in Figure 3, which shows ears of forage maize grown in southern Sweden (Skåne, 56° N) in 2007.

R1 – Silking: Silks (which capture pollen grains and fertilize the ovules, resulting in kernels in the cobs) are visible outside the husks.

R2 – Blister: Kernels are white, filled with clear fluid, with a moisture content of about 85%. Silks have completed their function and become dark and dry.

R3 – Milk: Kernels appear yellowish outside, but contain milky fluid inside. The moisture content is about 80%.

R4 – Dough: Kernels at this stage contain fluids with a doughy consistency and have a moisture content of about 70%.

R5 – Dent: Kernels of dent maize type have visible dents on their tops, and those of all types have a moisture content of about 55%.

R6 – Physiological Maturity: Kernel DM is maximal, a black layer forms at the base of the kernels, their moisture content is about 30–35%, and the plants are fully mature.

1.5 Environmental impacts of forage maize production

Nutrient management in the agricultural sector has received greater attention since the launch of the European Union's Water Quality Directive, which will be fully implemented in 2015. This is of great importance for Sweden, where most dairy production is located around lakes and in coastal areas. Forage producing farms can reduce nutrient losses to the environment by adjusting crop rotations to include annual forages like maize. The need for pesticides to control insects can also be decreased by increasing variation in crop rotations. However more herbicides are required for maize compared to grass/clover. This is important, because although Sweden does not consume large amounts of pesticides, by international standards, it has a very strict pesticide regulatory regime. Furthermore, maize has a low protein content compared to other forages, and thus its use may reduce the N content in the diets of dairy cow and hence N leakage (Huhtanen & Hirstov, 2009). However too low protein in forages will raise a need for protein rich feeds. By using maize in crop rotations on dairy producing farms it is possible to use the manure produced without risking contaminating the green matter with slurry. Including maize silage in ruminant rations may also reduce releases of methane (a potent greenhouse gas) from the livestock sector, an effect that has been highlighted in Denmark (Hvelplund, 2008) where they changed from sugar beet to maize and therefore increased propionate production and decreased methane.

1.6 Forage quality and nutritional value for ruminants

Balasko & Nelsen (2003) defined forage quality as "the physical and chemical characteristics of forage that make it valuable to animals as a source of nutrients and well being". All plants consist of cell walls and cell contents, which are very different from each other. Cell contents are mostly readily digestible compounds, e.g. sugars, starches, proteins and lipids, whereas cell walls have less digestible fibrous constituents, and their amounts, digestibility and digestion rates are the major determinants of the productivity of animals on forage diets (Collins & Fritz, 2003). Maize as forage has high intake rates (Jensen *et al*., 2005) and energy due to starch concentrations (Tomankova & Homolka, 2004). In addition, the starch in maize may be digested more slowly than that of other cereals, e.g. wheat and barley, although there are conflicting reports about its degradation characteristics (Mills *et al*., 1999).

Crop management studies (e.g. Darby & Lauer, 2002a) have shown that plant development and maturation can be manipulated by choice of hybrid (Marton *et al*., 2007) and time of harvest (Wilkinson & Phipps, 1979). The performance of maize crops will depend on the match between the relative maturity rankings of chosen hybrids, which can be expressed in terms of FAO (Food and Agriculture Organisation of the United Nations) classes, and temperatures during the growing period in the region (for details, see below). The harvest timing will also affect the performance, because the relative maturity of plants at harvest is related to their nutritive value (Argillier *et al*., 2000).

1.6.1 Effect of maturity on the nutritive value

Achieving suitable forage crop maturity at harvest is important, due to its impact on forage conservation and nutritive value (Darby & Lauer, 2002a;

Jensen *et al.,* 2005). A robust indicator of plant maturity is the DM concentration of the whole plant (Jensen *et al.,* 2005), which has been used in hybrid evaluation trials to rank the relative maturity of tested hybrids (Schwab *et al.,* 2003; Marton *et al.,* 2007). *In vivo* studies have shown that the digestibility of maize is relatively constant during maturation, due to interactions between starch and fibre availability, (e.g. Di Marco *et al*., 2002; Collins & Fritz, 2003).

To assess the effects of increasing maturation on the nutritional value of forage maize under Scandinavian conditions, a pilot study was conducted in southern Sweden using the hybrid Baxxos (FAO 210), grown in 2007 at Alnarp (55° N; 13° E). The maturity of the maize was graded on several occasions (illustrated by the changes in kernels shown in Figure 3) according to Canadian standards (MAO, 2002), and the chemical composition of plants sampled on each occasion was analyzed (Table 1) (Swensson *et al*. 2008).

Table 1 Composition of forage maize in percentage of dry matter (DM) at indicated maturities in a pilot study in Alnarp (55° N; 13° E).

Date (Maturity)	DM	NDF	Sugar	Starch
2007–08–09 Blister	15	56	24	5
2007–08–16 Milk	18	55	23	4
2007–09–06 Dough	23	49	18	17
2007–09–23 Dent	33	43	11	31

NDF= neutral detergent fibre.

Figure 3. Development of the maize ears (Baxxos, FAO 210) in the pilot trial at Alnarp, 2007 (Photo: Nina Bäcklund, 2009).

1.6.2 Chemical composition of morphological fractions

The nutritional value of whole crop forages such as maize depends on the DM contributions and intrinsic characteristics of its morphological fractions. The fractions that are most important for forage performance are the aboveground parts, i.e. stem, leaves, kernels and cobs. Stems, leaves and cobs have high concentrations of lignins, cellulose and hemicellulose (Boon *et al*., 2005; Masoero *et al*., 2006), while maize kernels are high in starch and low in fibre. Plant development and maturation affect the DM contributions of morphological fractions to the whole plant biomass yield (Phipps & Weller, 1979). At maturity, the fibrous fractions (e.g. stems, leaves, cobs) may account for about half of the total DM concentration (Hunt *et al*., 1992; Irlbeck *et al.,* 1993), and their digestibility may decrease as DM maturity increases (e.g. Verbič *et al.,* 1995; Fitzgerald & Murphy, 1998). However, their effects on the DM digestibility and nutritional value of whole plant forage maize are partially balanced by increasing kernel proportions (Bal *et al*., 1997).

1.6.3 Hybrid selection and performance

Choosing an appropriate maize hybrid is highly important for successful forage production (Struik, 1983; Darby & Lauer, 2002b), and the nutritive value of the crop since it is strongly related to the relative maturity of the hybrid (Argillier *et al.,* 2000). Important traits to consider when selecting forage maize hybrids are the DM yield, starch concentration and relative DM maturity in relation to the local climate (Jensen *et al.,* 2005; Nishida *et al.,* 2007). Breeders rank hybrids into maturity classes to facilitate choices, often using the FAO score/rating system, in which the maturity of the entire world's maize varieties are divided into nine classes (Zscheischler *et al.,* 1990). The earliest and latest maturing classes, which require the shortest and longest maturing periods are 100–200 and 900–990), respectively. The latter are suitable for regions like Mediterranean regions, where temperatures are relatively high for substantially longer periods of the year than in Sweden, where hybrids with FAO ratings between 180 and 250 are mainly cultivated (Thorell, 2005).

Forage maize has the potential to increase the competiveness of Swedish dairy and beef production. However, although more than 60 hybrids are included in official hybrid trials in Sweden (Gustafsson, 2008), Larson & Lindgren (2006) found that the farmers largely choose maize hybrids randomly. Consequently, their choices of hybrids are not always optimal and their cultivation may result in low quality forage that does not meet international standards of DM and nutritive value for maize. This highlights the need for rational and transparent hybrid ranking, and greater knowledge of the plants' development and forage quality under current Swedish growing conditions.

Hybrid performance trials are necessary for evaluating maize hybrids for silage as well as grain production, but both ranking and selecting them are complex issues, as numerous factors (e.g. quality of the silage, DM yields and digestibility) should all be considered. However, there are several international models for evaluating and comparing the performance of maize hybrids. For example, they can be described according to their agronomic performance or net energy for lactation (NEL) content in dairy cows at an intake level of 20 kg DM per day (NEL20, MJ/kg DM), according to the Scandinavian feed evaluation system NorFor (Volden, 2010). In Denmark, hybrids are evaluated according to their NEL20 GJ/ha and NEL20 MJ/kg DM contents, using a relative index (RI) based on comparisons with four reference maize hybrids (Table 2).

Table 2. Illustrative rankings of hybrids in Denmark, from "Landsforsøgene 2008"

		DM%					Norfor		
Hybrid	DM	Starch	WSC	NDF	iNDF	Harvest Ton DM/ha	NEL/kg	NEL/ha	RI
Revolver	38	33	3	41	207	13.8	6.29	87	97
Award	31	31	6	42	199	15.8	6.29	99	111
Arabica	28	28	6	43	216	14.1	6.04	85	95

DM=dry matter, WSC=water soluble carbohydrates, NDF=neutral detergent fibre, iNDF=indigestible NDF in g/kg NDF, NEL/kg=net energy for lactation at an intake of 20 kg DM, NELp20 MJ/kg DM, NEL/ha= NELp20 GJ/ha, RI= relative index.

The performance of maize hybrids can also be evaluated according to expected yields of milk from dairy cows fed on forage maize obtained using the spreadsheet model MILK 2006 (Shaver, 2006) developed in Wisconsin, USA. It balances the agronomic yield of hybrids against their feed value utilizing information obtained from chemical analysis and *in vitro* analyses (Schwab *et al.*, 2003), then calculates how much milk is expected to be produced when the specific hybrids are fed to cows (Lauer *et al.*, 2011). The milk output and feed intake are calculated utilizing the feed evaluation system NRC 2001 (NRC, 2001). Illustrative hybrid rankings obtained using the model are shown in Table 3.

Table 3.Results of maize hybrid evaluation trials in Arlington, Wisconsin, using the MILK 2006 model (Lauer *et al.*, 2011)

		DM%				MILK 2006	
Hybrid	DM%	Starch	NDF	NDFD	Harvest Ton DM/ha	Kg Milk/Mg DM	Kg Milk/ ha
Early	40	32	51	55	23.7	1471	31748

Late	42	34	49	55	24.2	1506	33095

NDF=neutral detergent fibre, NDFD=NDF digestibility in g/kg NDF

1.7 Evaluation of forage nutritional value

Evaluating forage nutritional value is very important for predicting the performance of animals that are fed it. A key parameter is digestibility, "the percentage of DM of an individual constituent that is digested as forage passes through the animal's digestive tract" (Dougherty & Collins, 2003). During passage through the tract, fermentation of carbohydrates occurs resulting in the production of volatile fatty acids, e.g. acetic, butyric and propionic acids, which are major sources of energy for the ruminant animal.

In vivo studies give the most relevant indications of digestibility, DM intake, milk production and live weight gain from animal feeds. However, animal trials are laborious and expensive, which limits their routine application. Therefore, several techniques based on *in vitro*, *in situ* and other methods like near infrared reflectance spectroscopy (NIRS) have been developed as alternatives to *in vivo* measurements. The *in situ* technique (Ørskov, 2000) has proven to provide robust predictions of forage *in vivo* organic matter digestibility (OMD) at commercial laboratories in the Nordic countries (Huhtanen *et al*., 2006; Krizsan *et al*., 2012). In this technique pre-weighed polyester bags containing feedstuff are incubated in the rumen of cannulated animals (Huhtanen *et al*., 1994) and the loss of feed from the bags is measured to estimate the amount that is degraded (Broderick *et al*., 1991; Krizsan *et al*., 2012).

The *in vitro* gas production technique measures the kinetics of fermentation of a given substrate with a given microbial population, thereby simulating fermentation in the rumen (Williams, 2000). It can provide estimates of rumen degradation rates of both specific chemical fractions (e.g. sugar, starch and fibre) of plants (Rinne & Nykänen, 2000). In the original protocols, a test substrate was incubated in a medium of rumen fluid and buffer solution warmed to 39° C, then gas produced from fermentation was collected in syringes and recorded either at regular intervals or at the end of fermentation (Williams, 2000). Since then, fully automated gas collection and monitoring systems have been developed (Cone *et al*. 1996), and the technique has been used to assess the nutritive value of both perennial forages (Huhtanen *et al*., 2000; Hetta *et al*., 2004) and maize (Boon *et al*., 2012).

Enzymatic methods, in which solutions containing specific substrate-digesting enzymes are used instead of rumen fluid are attractive techniques for evaluating forage quality since they avoid the problem of variations in rumen inocula. The most commonly used enzymes for assessing cell wall digestibility are cellulases obtained from *Trichoderma* species of fungi. Essentially, in enzymatic methods the test substrate is treated with acid pepsin or neutral detergent, to remove soluble constituents, then cellulase to degrade the cell walls (Jones & Theodorou 2000).

NIRS is a physical method, which depends on the measurement of light absorbed by the surface of samples using wavelengths in the infrared region of the spectrum (1100–2500 nm) (Beever & Mould, 2000). With the resulting absorption spectrum, it is possible to identify levels of chemical constituents, such as protein, fibre, starch etc in the samples. However it is first necessary to calibrate the apparatus against standard reference samples that have been analysed by more routine 'wet chemistry' methodologies (Beever & Mould, 2000). Its use for assessing forage quality was first reported by Norris *et al*. (1976), and its utility for evaluating forage maize has been reported in earlier studies (e.g. Volkers *et al*., 2003) and is now well accepted. The development of NIRS has opened possibilities to evaluate the chemical composition of forages with less time and resources than other techniques.

2 Objectives

The increasing interest in producing forage maize in Sweden and other Scandinavian countries has created needs for greater knowledge on the performance and rankings of maize hybrids at high latitudes under current conditions. Thus, major aims of the studies this thesis is based upon were to assess the performance of selected forage maize hybrids cultivated at several sites in the region, and the effects of key variables on the crops' development, agronomic performance and nutritional value. Further aims were to assess the utility of several techniques for evaluating the crops' nutritive value. More specific objectives were:

- To assess the effects of hybrid, maturity and site on the development, agronomic performance and nutritive characteristics of forage maize in Sweden.
- To evaluate the effects of hybrid and plant maturity on the chemical composition and nutritive characteristics of whole plants and morphological fractions of forage maize using an *in vitro* gas production technique.
- To explore the effects on the performance of forage maize of site, maturity, contribution and quality of morphological fractions using multivariate models.
- To evaluate the effects of hybrid, site and maturity on feed value predictions, acquired using different analytical techniques, for forage maize cultivated at high latitude sites.

3 Material and methods

The studies described in Papers I to IV were based on field experiments conducted during two years (2008 and 2009) at three locations in Sweden (Figure 4), spanning a latitudinal range of 470 km: Kristianstad (56°04'13"N, 14°19'11"E), Skara (58°23'15"N, 13°29'03"E) and Västerås (59°36'47"N, 16°38'07"E). Three maize hybrids with varying maturity ratings were used in the trials — Avenir (FAO 180), Isberi, (FAO, 190) and Burli (FAO, 210) — and the experiments were conducted according to a randomized complete block design.

Plants were harvested at increasing maturity during the season, dried and partitioned into morphological fractions: stem, leaf, cob and kernels. The fractions were milled through a 1 mm sieve. To represent the whole plants, reconstituted whole plant samples were assembled from the plant fractions from each plot based on their mean DM contributions to whole plants. Climatic conditions at the growing sites in both experimental years, 2008 and 2009, were within the ranges of weather conditions recorded in the previous decade (SMHI, 2011) and the plant material was representative for northern forage maize.

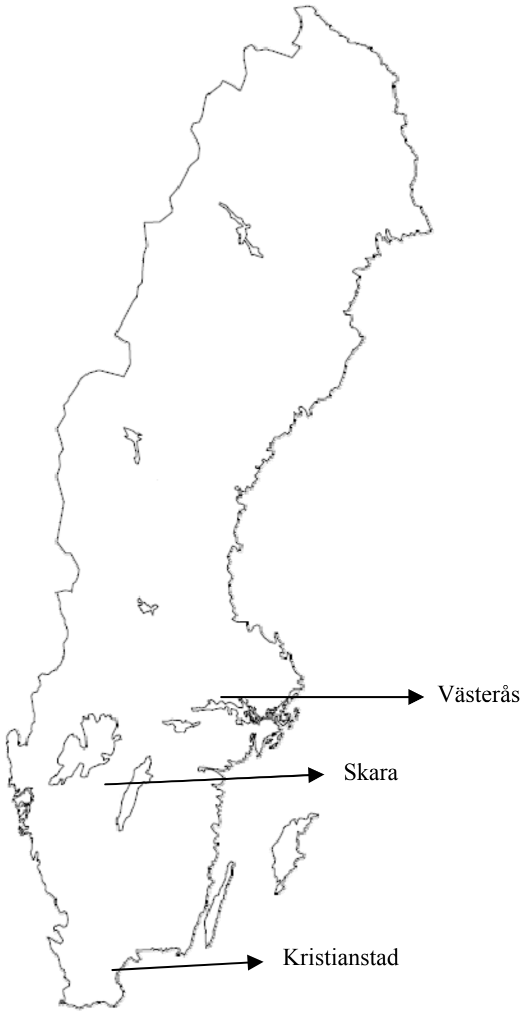

Figure 4. Locations of the field experiments in Sweden.

3.1 Paper I

Development of the maize hybrid plants was estimated in relation to accumulated CHU at all three sites: Kristianstad, Skara and Västerås. Accumulated CHU values for each site were calculated from May 1 (designated CHU– M1) and from day of sowing (designated CHU) until the last harvest, using the equation presented by Kwabiah *et al.* (2003). The silking, blister, milk, dough and dent stages (approx. DM concentration of 450 g/kg) were identified visually, using the Ontario guide for corn development (OMAFRA, 2009), and the times when the hybrids reached these stages were recorded as the dates when 50% of the plants in the plots had reached them. The chemical composition of whole plant samples was analyzed following the Nordic feed evaluation system, NorFor, procedures (Volden, 2011). The effects of hybrid, site, maturity and interactions between site and hybrid on the crops' chemical composition were evaluated by analysis of variance, using DM concentration as a proxy for maturity in the model.

3.2 Paper II

The rumen degradation characteristics of the plant material harvested from Kristianstad during 2008 were evaluated in the study described in Paper II using an *in vitro* gas production technique. Samples of whole plants and the morphological fractions (leaves, stems, kernels, cobs) were analyzed to assess effects of maturation (DM concentration) and hybrid on chemical composition and gas production parameters, acquired using automated equipment (Figure 5), and a general linear model (GLM). Briefly, samples of about 500 mg of organic matter (OM) were incubated for 72 h at 39 °C in 60 ml of buffered rumen fluid in 250 ml serum bottles, then true rumen digestibility of the OM and NDF was determined from the neutral detergent residues. The parameter values estimated from the GP profiles were used in the mechanistic two compartment rumen model developed by Huhtanen *et al.* (2008) to predict *in vivo* digestibility and estimate the first-order digestion rate of the digestible OM. The hybrids' nutritional values were evaluated by analysis of variance and the responses to maturation were evaluated using DM concentration as a covariate in the statistical model.

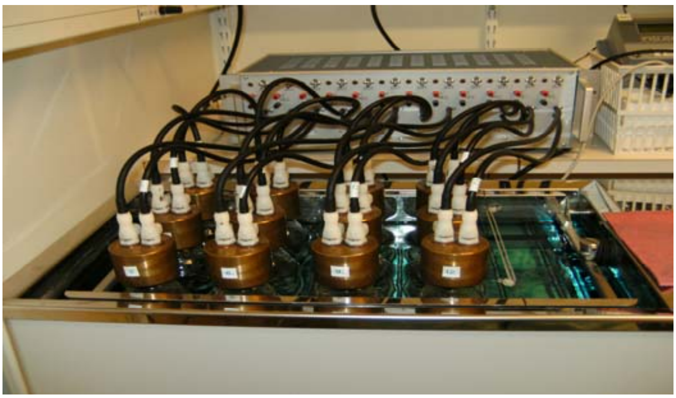

Figure 5. The apparatus for *in vitro* gas recordings from samples of organic matter in buffered rumen fluid incubated in bottles at SLU, Umeå (Photo:Anni Puranen).

3.3 Paper III

Chemical analyses of the samples representing whole crop maize plants and morphological fractions (Figure 6) from all three sites were conducted according to the procedures specified for the spreadsheet model MILK 2006 (Shaver *et al*., 2006). Principal Component Analysis (PCA) was then applied to explore the multivariate correlations among the morphological, agronomic and nutritional composition parameters. The nutritional and agronomic performance parameters were then entered in the MILK 2006 model spreadsheet to calculate the expected milk yield potential of the whole crop forage maize and its morphological fractions. In addition, two Partial Least Squares (PLS) regression (Martens & Naes, 1989) models with nutritive and agronomic characteristics as predictive parameters were developed, to predict estimated milk, kg milk/Mg DM and Mg milk/ha yields of the forage maize from the data set.

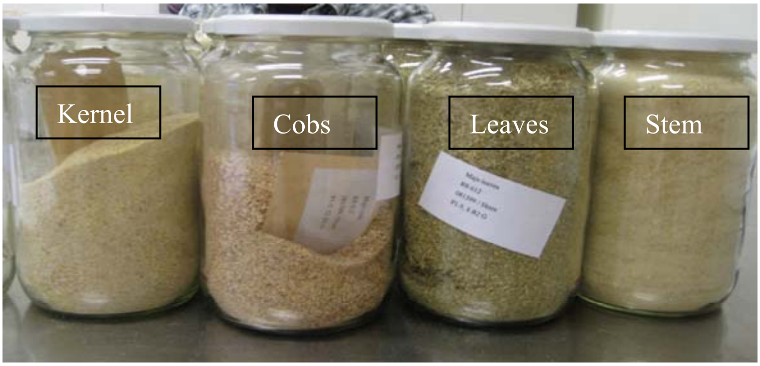

Figure 6. Milled samples of maize morphological fractions (Photo: Zohaib Mussadiq, 2010).

3.4 Paper IV

Whole plant samples collected from all three sites in 2009 were analyzed and evaluated using available analytical methods to estimate concentrations of indigestible NDF (iNDF) and OMD in forage maize. The tested methods included 288–h *in situ* incubation of samples in nylon bags (Figure 7) in the rumens of three lactating and cannulated Swedish Red cows, as described by Huhtanen *et al.* (1994). The iNDF was expressed as the NDF remaining in the bags after the incubations, excluding residual ash, following treatment with sodium sulphite. The digestibility of OM was calculated from the prolonged *in situ* incubation data according to the empirical equations published by Huhtanen *et al.* (2012). Further analyses of the material included assays using the enzyme digestible organic matter (EFOS) method (Weisbjerg & Hvelplund, 1993), and determination of rumen fluid digestible OM (VOS) by single 96–h incubations of forage samples in rumen fluid, as described by Lindgren (1979). In addition, the samples were analyzed by NIRS for predicting iNDF and OMD (Huhtanen *et al*., 2006) using two different calibration sets and instrumental systems coming from Finland and Sweden. The prediction equations were then evaluated by linear regression. Further, effects of hybrid, site and harvesting time on the predicted forage maize OMD and iNDF concentrations were evaluated by analysis of the residuals, as described by St-Pierre (2003).

Figure 7. Nylon bags for *in situ* incubation of samples in the rumen of dairy cows (Photo: Anni Puranen, 2011)

4 Results

4.1 Paper I

The results of the grading revealed differences between the hybrids in terms of the cumulative CHU required to reach given reproductive developmental stages, and between sites concerning the hybrids' developmental responses to cumulative CHU. The maize hybrids required the lowest cumulative CHU at Kristianstad and the highest at Västerås to reach given developmental stages. These differences affected the agronomic and nutritional performance of the maize hybrids at the growing sites. The late maturing hybrid gave the highest DM yields, and needed the highest accumulated CHU to reach the required maturity expressed as the DM concentration. However the early maturing hybrid reached the highest nutritive values, since its starch concentration at harvest (and hence OM digestibility) was highest.

4.2 Paper II

As maturity increased, the *in vitro* digestibility of NDF in all plant fractions declined. At the same time, the rate of rumen degradation of OM in the whole plants increased, according to the gas production analyses. Concerning the effect of hybrids, *in vitro* digestibility of OM and NDF of whole plants and fractions was observed to be highest in early maturing hybrids. Modelled first-order rates of rumen degradation followed the same pattern as the estimated *in vitro* parameters. Among the plant fractions, the DM contribution of stems showed the strongest negative correlation with modelled *in vivo* digestion.

4.3 Paper III

For all three maize hybrids included in the study, site had a major effect on the relative proportions of leaves, stems, kernels and cobs on a DM basis. The highest proportions of leaves and stems were observed at Västerås, while the proportion of kernels was greatest at Kristianstad followed by Skara. There were significant correlations between proportions of DM yield of the different plant fractions, chemical composition of the whole plants, net energy for lactation (NEL) and predicted milk yield indices. The contributions of individual morphological fractions to modelled milk yields estimated using the MILK 2006 spreadsheet model was highest for the kernels, followed by the leaves, stems and cobs. The major parameters affecting modelled milk yield kg/Mg DM according to the MILK 2006 model were the starch concentration, NDFD and proportion of stems. For milk yield/ha, forage DM yield per ha was the most important parameter.

4.4 Paper IV

Both the Swedish and Finnish NIRS calibrations of iNDF generated biased predictions, with low precision, of $iNDF_{IN\ SITU}$ (i.e. the iNDF estimated by the *in situ* technique). The residuals obtained from the two methods were related to effects of both hybrid and site. The root mean square error of prediction (RMSEP) was higher for $iNDF_{NIRS–F}$ residuals than for $iNDF_{NIRS–S}$ residuals (iNDF predicted by the Finnish and Swedish analyses, respectively). Residual analysis showed that all three methods used to predict OMD in forage maize systematically underestimated $OMD_{IN\ SITU}$, but the RMSEP was highest for residuals of OMD_{EFOS} and lowest for OMD_{VOS} (where $OMD_{IN\ SITU}$, OMD_{EFOS} and OMD_{VOS} refer to OMD values estimated using the *in situ*, EFOS and VOS methods, respectively). The OM digestibility was predicted with least precision when using values acquired by the EFOS method, the residuals of the predictions from OMD_{EFOS} were related to site only, while those of $OMD_{NIRS–F}$ were related to site, hybrid and harvest time. The residuals of the OMD_{VOS}-based predictions were not related to site, hybrid and harvest, but the prediction error indicated that it gave less precise results than the $OMD_{IN\ SITU}$ method.

5 Discussion

Historically, Swedish forage maize production levels have been relatively modest compared to those of countries such as Denmark, the Netherlands and Germany. However, maize cultivation has increased in Sweden recently, and may increase further if more knowledge concerning the cultivation, plant development and performance of maize hybrids at northern latitudes is obtained. Climatic data alone indicate that the growing conditions at the experimental sites were more suitable for maize cultivation than the production results indicated, as stated in Paper I. Thus, the aim of the studies underlying this thesis were to explore the effects of factors affecting forage maize hybrid productivity in Sweden, and evaluate methods for assessing its performance.

5.1 Performance of forage maize hybrids at high latitudes

5.1.1 Plant development in relation to crop heat units

For successful production of forage maize, it is essential that the selected hybrid, agronomic practices and environmental conditions allow maize to mature. In the study reported in Paper I the development of the forage maize at the high latitude trial sites, expressed as cumulative CHU was found to be affected by both hybrid and sites. The effect of hybrids was consistent with findings by Fairey (1980) and Millner *et al.* (2005), that hybrids differ in the accumulated thermal units required to reach particular stages of development, corresponding to their relative maturity ranking. The clearest manifestation of the effect of site on the plants' development was perhaps that grain-filling of all three hybrids reached the dent stage only at Kristianstad in both years (Paper I). Environmental effects on maize development have also been recorded in previous reported studies (Smith *et al.*, 1982; Cutforth & Shaykewich, 1990). All the hybrids reached the dent stage before the daily mean temperature dropped below 10 °C at Kristianstad, but not at Skara and

Västerås. This is a critical temperature, since it is reportedly the base temperatures for C4 grasses, e.g. maize (MacAdam & Nelson, 2003), and thus an important factor to consider when scheduling harvests of forage maize in regions with early onset of lower temperatures.

The weather data during the experimental years showed that all sites reached almost 2400 CHU between 1 May and the first frost, which is considered to be the minimum requirement for cultivating early maturing hybrids to reach the dent stage of development in Denmark (Mikkelsen, 2010). Accordingly, early maturing hybrids such as those used in this study should have been able to reach the dent stage (R5) under the prevailing climatic conditions. However, the growth stage assessments reported in Paper I showed that only the earliest maturing hybrid, Avenir, achieved complete grain filling (dent stage) in both years at Skara. At Västerås no hybrid developed beyond the milk stage (corresponding to 250 g/kg DM concentration) during any of the two years. That stage is below the recommended DM concentration at harvest for producing maize forage of acceptable quality (Jensen *et al*., 2005; Mikkelsen *et al*., 2008; Mikkelsen, 2010) and may cause losses of effluent to the environment during the ensiling process. The main reasons for the inability of the hybrids to develop and mature at the northern locations were the lower accumulation of thermal units in relation to the length of the season. The study presented in Paper I showed that a certain hybrid may not develop to the desired level (DM concentration) if it is cultivated in less favourable regions at higher latitudes. Hence, there is a need for regional testing of maize hybrids, in accordance with Stanton *et al*. (2007).

5.1.2 Agronomic and nutritive performance

The observed differences in development between the hybrids at the experimental sites (Paper I) contributed variations in the agronomic performance and nutritive characteristics of the plant material in all of the studies (Papers I, II, III & IV). These findings are in agreement with, but more pronounced than those of Cox *et al*. (1994) and Darby & Lauer (2002a), who previously reported effects of site on the yield and nutritive value of forage maize.

At Kristianstad, all hybrids reached the dent stage (Paper I), therefore their performance was evaluated at the recommended DM concentration for ensiling forage maize in bunker silos (Cox & Cherney, 2005), the most common method for conservation in Scandinavia. This indicated that all three tested hybrids (with FAO 180–210) were suitable for the most southern site. The performance of maize hybrids estimated at the same DM concentration maturity (Papers I & II) indicated differences in nutritional characteristics and thermal requirements between the hybrids. The earliest maturing hybrid showed higher OMD and consequently higher energy contents, than the later maturing hybrids, probably because of the higher kernel proportion and higher starch concentration in the hybrid Avenir (Paper II).

At Skara, the hybrids were evaluated at a DM concentration corresponding to the dough stage (Paper I), which is in the lower range of recommendations for harvest in the international literature (Fairey, 1983; Jensen *et al.,* 2005). The main reason for this is that few thermal units are added (Paper I) after the plants have reached a DM concentration of 250 g/kg. Most reports of maize silage cut as immature as that at Skara are from the British Isles (e.g. Givens & Deaville, 2001; Keady *et al.,* 2008). These constraints in growing conditions indicate that only early maturing hybrids like Avenir, with similar CHU requirements, should be used for forage production under conditions such as those in Skara. However achieving dough stage for harvest can result in acceptable forage maize (Johansson, 2010) under marginal conditions.

Both the yields and nutritional value, of all three maize hybrids, were lowest at Västerås compared to the other two sites. Results presented in Paper III also showed that the plant material from Västerås, which was the least mature and gave the lowest DM yields, performed less well according to the MILK 2006 model than maize from the other two sites. These findings agree with results presented by Darby & Lauer (2002a), who concluded that hybrids planted at sites in northern latitudes were often immature and had lower nutritive values than those cultivated at lower latitudes. These observations were attributed to the lower average temperatures and early frosts at the more northern sites. The findings presented in Paper I indicate that hybrids with a FAO score as low as 180 are unable to reach even the dough stage of development at Västerås, despite being early maturing, resulting in low nutritive value.

Consequently, there is a need for careful risk management in regions where cultivation of early maturing hybrids is common to reduce the risk of crop failure due to early frost. Adjustments of agronomic practices may also be required, e.g. planting the maize as early as May 1st to allow it to mature.

5.1.3 Nutritional composition of morphological fractions in relation to maturity

Some of the observed differences in nutritional characteristics of the whole plants reported in Paper I could be explained by variation in the relative DM proportions of the morphological fractions (Papers II & III). Hybrids grown in the most northern location, Västerås, had the highest leaf and stem proportions (Paper III), which may be attributed to the longer time to reach the number of thermal units required for development of kernels (Birch *et al.,* 1998) and hence prolonged period of vegetative development. The differences in proportions of plant morphological fractions and their chemical composition (Papers II & III) affected the modelled performance of the whole plant forage maize hybrids reported in Paper III, in agreement with previous studies on morphological quality (e.g. Hunt *et al.,* 1992; Verbič *et al.,* 1995). The results reported in Paper III suggest that a large part of milk production is likely to be derived from the kernels. However, the sum of the expected milk production derived from the other fractions shows that they make substantial contributions to the nutritional value of the whole plants.

The relative contributions of the different fractions may affect not only the energy concentration of the forage, but also its intake characteristics. For example, the OM and NDF digestibility of the leaf fraction declined with increasing maturity. This reduction was presumably mainly due to the transportation of available carbohydrates in the form of sugars to the ears for grain filling, which occurs during leaf senescence (Jones *et al.,* 1981). The stem fraction showed limited responses to increasing maturity in its chemical composition (Papers II and III), again in accordance with previous studies (Verbič *et al.,* 1995; Firdous & Gilani, 1999; Boon *et al.,* 2008). There were strong correlations between the NDF concentration of leaves, stems and cobs with the predicted milk yields (Paper III). This was probably due to the mobilization and translocation of non-structural carbohydrates from these fractions to developing ears with increasing maturation (Jones *et al.,* 1981; Coors *et al.,* 1997). Stems had lower NDFD than other fractions, which affects the forage quality by affecting DM intake (Shaver, 2006). This indicates that a higher proportion of stems in total DM reduces voluntary feed intake by cows, as suggested by the findings of Oba & Allen (1999). The negative effect of stem proportion on milk yields noted in Paper III may also be partly due to its interrelationship with kernel proportion, which reduces the energy available for milk production from forage, in agreement with the findings of Bal *et al.* (1997).

The starch concentration in the kernel fraction increased with increases in the DM concentration of the whole maize plants, in agreement with Cone *et al.* (2008), who related this to growth of ears and deposition of starch. The kernel fraction is important for increasing DM yields and feed values, according to Hunt *et al*. (1992) and Coors *et al.* (1997). The highest milk yields were obtained from the kernel fraction in our study (Paper III), probably related to a higher energy supply from starch, which can be readily used in milk production (Schwab *et al.,* 2003), followed by the leaf, stem and cob fractions. Results presented in Papers II and III showed that the cobs fraction is the smallest morphological fraction, and has both the lowest nutritional value and lowest

DM contribution to the plants' total DM. This is in agreement with studies by Verbič *et al.* (1995), and mainly due to the high concentration of NDF in the cobs and its limited degradation in the rumen (Paper II).

5.1.4 Estimation of rumen degradation and predicted digestion

Digestibility is the most important trait of forages in feed value determinations (Huhtanen *et al.*, 2006). The differences among hybrids in observed *in vitro* true OM digestibility of the whole maize plants reported in Paper II are likely related to the higher contributions of kernels to DM in the earlier maturing hybrids, and higher contributions of stems in the later maturing hybrids (Papers II & III). The NDFD was highest in Avenir (FAO 180), hence the DM intake and milk production should be highest for cows fed Avenir, based on the findings of Oba & Allen (1999) and Schwab *et al.* (2003). No response of *in vitro* true OM digestibility of hybrids to increased maturity was detected (Paper II), consistent with Jensen *et al.* (2005). However, quadratic responses of *in vitro* true OM digestibility to maturity, with a maximum digestibility at a maturity of 35% DM, have been reported by Johnson *et al.* (1999).

The *in vitro* true digestibility of OM and NDF in the leaf fraction declined with increasing maturity (Paper II). This can be mainly attributed to lignification and the transportation of available carbohydrates in the form of sugars to ears for grain filling, which occurs during leaf senescence (Jones *et al.,* 1981). In addition, increasing maturity is likely to reduce NDFD of the stem fraction through increases in cell wall thickness (Boon *et al.*, 2008) and lignification of the tissues (Jung & Casler, 2006). The reduction of *in vitro* true OM and NDF digestibility of the kernel fraction with increasing maturity is likely related to changes in the intrinsic characteristics of its starch and fibre components (Jensen *et al.,* 2005). Cobs represent the least digestible fraction, according to the *in vitro* analysis. The observed reduction of plant cell wall digestibility in forage maize with increasing maturity has been previously detected in both *in vitro* experiments (Deaville & Givens, 2001) and *in vivo* experiments (Jensen *et al.,* 2005).

As no *in vivo* studies were conducted in association with this experiment (Paper II) it is difficult to evaluate the validity of the total tract digestion values modelled, and the estimated first order digestion rates of OM from the observed plant samples. The validity of the *in vitro* technique applied, and the modelling procedure to interpret GP data is confirmed, given that the sum of the digestible fractions of the plant fractions, multiplied by their fractionation contributions on a DM basis, consistently showed only a small difference (20 g/kg OM) when compared with the estimated whole plant digestibility for all hybrids. The estimation of rumen degradation of the forage maize hybrids (Paper II) showed that a combination of *in vitro* recording and mathematical modelling has substantial potential utility for predicting the nutritional value of forage maize when routine *in vivo* analyses are impractical. Although dried, milled samples were used in the *in vitro* analysis, while cows eat the maize as silage, the technique appears to be a promising tool for evaluating the performance of ruminant feedstuffs.

5.2 MILK 2006 hybrid performance indices

In the study described in Paper III, the two output indices of the model MILK 2006 demonstrated that milk/ha was predicted well from a small number of variables, whereas milk/Mg DM was less well predicted, even when several variables were included. The dominant effect of DM yield in predicting the modelled milk yield/ha in our study, was supported by strong correlations between DM yield and milk/ha in accordance with results of cultivating hybrids at various sites in Wisconsin (Lauer *et al.,* 2011). Improving NDFD and increasing starch concentrations are important for maximizing hybrid performance as assessed by the milk/Mg DM indices provided by the MILK 2006 model. This has also been observed in animal trials, where a leafy hybrid with increased NDFD has been shown to increase the milk yield of dairy cows compared to a control hybrid with normal morphological proportions (Thomas *et al*., 2001). The strong effect of starch concentration on the MILK 2006 indices was probably a result of harvesting the plant material at a wide range of maturities, and consequently large variations in starch concentrations.

Findings from the study presented in Paper III may have implications for the selection of top-performing hybrids at high latitudes, because it fulfilled most of the criteria for ranking hybrids described by Lauer *et al*. (2011), including (*inter alia*) use of multi-location data and consistency of performance of the hybrids over years. It should be noted that due to sub-optimal growing conditions and the timing of harvests, in some cases the plant material used in the studies this thesis is based upon was harvested at immature stages with low DM concentration (Paper I & Paper III). Here the MILK 2006 model may not give valid information on the potential performance of maize hybrids. However, the model has been developed not only for ranking forage hybrid varieties, but also as an advisory tool to evaluate the outcomes of practical on-farm forage production (Shaver, 2006). It is therefore valuable to explore the applicability of the model to plants in a wider range of developmental stages, cultivated in a wider range of growing conditions, as in the study reported in Paper III. The finding that different variables influenced the output indicates that it may be difficult to identify a maize forage crop that has both high DM yield and high feed value, defined as calculated milk/Mg DM. It is therefore still debatable whether dairy farmers should use milk/ha or milk/Mg DM as a target for selecting suitable hybrid varieties and tuning crop management practices. Furthermore, it is concluded that even though the model evaluated in this study is widely used for hybrid selection and as a research tool, further studies based on production trials with dairy cows are needed to evaluate its true practical relevance.

5.3 Comparison of analytical techniques for estimating feed value of forage maize

The study reported in Paper IV highlighted the need to extend calibration sets for NIRS predictions of OMD and iNDF concentrations, as both the Swedish and Finnish NIRS analyses generated $iNDF_{IN\ SITU}$ predictions that had low precision and bias related to both hybrid and site. Thus, none of the tested methods could be used to generate unbiased predictions of forage feed value. Use of the *in situ* technique as a reference method is supported by a recent review by Huhtanen *et al.* (2012), who concluded that predicting $OMD_{IN\ SITU}$ from iNDF and NDF concentrations, as in this study, provides a good alternative to predicting $OMD_{IN\ VIVO}$ because it yields relatively small prediction errors compared to other methods. Accurate predictions of feed values are important for correctly ranking the performance of hybrids, as observed in Paper III. Biased predictions can lead to erroneous ranking of hybrids. The NIRS technique is widely used by plant breeders and large sums of money are spent on evaluating maize hybrids, but these sums may be wasted if the NIRS predictions are inadequate and biased. The plant material used in the study described in Paper IV differed from that used in other recent assessments (e.g. Gosselink *et al*., 2004; Jančík *et al*., 2011; Krizsan *et al*., 2012) of analytical techniques for evaluating feed value like that of De Boever *et al*. (1997) as it only included forage maize.

The variation in analytical precision of the iNDF procedure between laboratories was further highlighted in a recent ring test by Eriksson *et al.* (2012). The major problem complicating comparisons of the results obtained using different iNDF procedures in this study and others is the need for relevant *in vivo* data with which to compare the results, in order to identify which procedure gives the most relevant values. The NIRS technique provides an excellent high throughput analysis system for predictions of plant feed values. Various *in vivo*, *in situ* and *in vitro* methods have been applied in NIRS calibrations for routine determinations of parameters such as iNDF and OMD in forages (Huhtanen *et al*., 2006). In the study reported in Paper IV predictions based on the VOS technique were found to be the least sensitive to the effects of hybrid, site and maturity, in accordance with other studies

(Krizsan *et al.*, 2012). The EFOS method is the recommended procedure for whole crop maize in the Nordic feed evaluation system (NorFor) (Åkerlind *et al.*, 2011). Its predictions of $OMD_{IN\ VIVO}$ might be improved by developing forage-specific equations, as suggested by Jančík *et al.* (2011). Despite its poor predictive ability, the EFOS method has a major advantage over other methods, namely that access to rumen cannulated animals is not required. Thus, the technique should be easier to implement in laboratories and may be easier to standardize, like other enzymatic methods such as the pepsin cellulase technique for OMD predictions described by Jones & Theodorou (2000). Although the NIRS calibration set has performed well for a multitude of forage species (Huhtanen & Nousiainen, unpublished data), the reference plant material did not include a large number of forage maize samples, and this shortcoming needs to be considered when comparing the predictions of this technique with others. Taking these factors into consideration, the accuracy of NIRS predictions of $OMD_{IN\ VIVO}$ in forage maize cultivated at high latitudes would probably be substantially improved by extending the calibration set to include forage maize samples with a wide variation in maturity.

6 Conclusions

The studies presented in this thesis demonstrate that the DM concentration at harvest could be included in programs designed to assess and rank the potential agronomic and nutritive performance of maize hybrids. Although the later maturing hybrids gave the highest DM yields, the early maturing hybrid is likely to give the maximum DM intake and animal performance due to its higher fractional proportion of kernels and higher NDFD. The *in vitro* gas production technique showed promising potential for assessing the nutritive characteristics of forage maize in relation to maturity.

Multivariate correlation analyses showed that growing sites may have stronger effects on the morphological proportions and nutritive value of maize, than choice of hybrids. According to the model MILK 2006, one of the forage quality parameters of the hybrids, kg milk/Mg DM, was positively associated with starch concentrations and NDFD, while the other, Mg milk/ha, was most strongly associated with DM yield. It is therefore still debatable whether dairy farmers should use milk/ha or milk/Mg DM as a target for selecting hybrids.

Relevant and precise predictions of the feed value of forage maize are important for plant breeding programs and improving forage production, but none of the tested methods gave unbiased predictions of forage quality. Therefore, an extended calibration set must be developed for correct NIRS analysis and then validated independently against reference data obtained from *in vivo* measurements.

Using the tested hybrid for production of forage maize of acceptable maturity was possible in Kristianstad, possible in Skara if using the early maturing hybrid (*e.g.* FAO 180) but not possible in Västerås. Production at high latitudes is a risk due to late sowing, higher CHU accumulation for plant development, lesser DM maturity and nutritive value at harvest at the end of growing season. This implies that forage maize may be successfully grown in some warm years, but not in other years, at high latitudes.

7 Future perspectives

To improve forage maize production in northern locations, further research including trials with early maturing hybrids (low FAO scores) is needed. Breeding maize hybrids under Swedish conditions and may improve the agronomic performance. Another interesting perspective is to cultivate maize as a multifunctional crop, *e.g.* using the plant for biogas production (as in Germany) which could increase the profitability for using immature maize.

Future studies on forage maize hybrids at high latitudes should include more hybrids from each FAO maturity class and more sites to compare their agronomic and nutritional performance under Scandinavian conditions more thoroughly. This will provide valuable information to help farmers interested in growing forage maize to choose appropriate hybrids and apply suitable agronomic practices.

From a nutritional perspective, genetic modification of maize hybrids with higher NDFD, for example Brown Mid Rib maize, will be of interest for the producers. Combining maize with domestic protein-rich sources may be a good way to balance the diets for ruminant livestock. Furthermore, greater knowledge of the effects of agronomic factors, such as plant density and plant protection measures, on hybrid performance and forage quality at northern sites is required.

The development of accurate methods for predicting nutritive values of forage maize hybrids is another important area of future research. In the future, when calibrated using data from *in vivo* experiments, NIRS should be a valuable tool for this, and should also greatly facilitate maize breeding programs and hybrid evaluation.

8 Popular scientific abstract

Maize (*Zea mays* L.) is a warm season grass that is being increasingly used for diverse purposes globally, *e.g.* for human food, ethanol production, biogas production, feed for livestock and raw material for producing bio-plastics. The development of earlier maturing hybrids has markedly increased maize cultivation mostly as forage crop in Scandinavia. Forage maize has substantial advantages over perennial forages as it gives high dry matter (DM) yields and needs only one harvest per season. Increasing area for maize cultivation and development of newer hybrid raised needs for more knowledge on the performance of forage maize at higher latitudes, as in Sweden. Hence, the aims of the studies this thesis is based upon were to investigate the utility of available techniques for evaluating the agronomic performance and nutritive values for ruminants of maize hybrids. This involved field experiments at different sites, chemical analyses and laboratory studies.

The development of maize hybrid plants was examined in relation to accumulated crop heat units (CHU; which provide good temperature-based indications of the plant development period) at all sites. The timings of the maize reproductive stages: silking, blister, milk, dough and dent stages (corresponding to DM concentration of 450 g/kg) of the plants at all sites were monitored visually, and their development was found to be affected by the hybrid FAO maturity rating and site, with the longest growth periods being needed at Västerås (59 °N) to reach given developmental stages. These differences affected the agronomic and nutritional performance of the tested maize hybrids at the growing sites. At Kristianstad (56 °N) all of the tested hybrids reached acceptable DM maturity for forage cultivation, but at Skara (58 °N) only the early maturing hybrid Avenir, and none at Västerås. The hybrid Avenir reached the highest nutritive values, due to its higher concentrations of starch and consequently highest organic matter digestibility.

Assays to determine the effects of increasing maturation (DM concentration) on the hybrid rumen degradation characteristics of the plant material were conducted, based on gas production measurements in a laboratory. Samples of the whole plants and their morphological fractions (leaves, stems, kernels, cobs) were incubated in rumen fluid for 72 h at 39°C, and gas production was recorded with automated apparatus. As DM maturity increased, the degradability of fibre in all plant fractions declined, but that of organic matter in the whole plants increased. Marked effects of hybrid were that the degradability of the organic matter and fibre of whole plants, and their fractions, was highest in the early maturing hybrids.

In another study both whole plants and morphological fractions of all the maize hybrids cultivated at all sites were chemically analyzed to estimate the performance of the hybrids using the MILK 2006 model. Contributions of individual morphological fractions to milk yields estimated by the model were highest for the kernels followed by the leaves, stems and cobs. According to the MILK 2006 model, maize forage quality was positively associated with DM yield, starch concentration and fibre digestibility. It was also found that the growing site had major effects on the relative proportions of leaves, stems, kernels and cobs. The proportions of leaves and stems were highest at Västerås and the proportion of kernels highest at Kristianstad followed by Skara.

Whole plant samples were also analyzed, using available analytical methods, to estimate concentrations of non-degradable fibre and degradable organic matter (OM) in the forage maize. Tested methods included: an *in situ* method, where weighed samples are incubated in cows' rumens in nylon bags; enzyme-based and rumen fluid-based methods for determining digestible organic matter contents; and near infra red spectroscopy (NIRS) using two different calibration sets and instrumental systems. The results showed that all the methods generated biased predictions. Predictions of OM digestibility (estimated *in situ*) were least precise when based on data acquired using the EFOS method. Therefore, an extended calibration set must be developed for correct NIRS analysis and then validated independently against reference data obtained from *in vivo* measurements.

To avoid crop failure, use of early maturing hybrids and readiness of soils for planting no later than May 1 is recommended at high latitude sites. The results presented in this thesis show that the agronomic performance and nutritional value of forage maize hybrids at high latitudes will vary due to effects of site and hybrids on DM maturity at harvest.

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