# Effects of the Level, Type and Processing of Cereal Grains in Diets for Dairy Cows

Feed Intake and Performance

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# Effects of the Level, Type and Processing of Cereal Grains in Diets for Dairy Cows. Feed Intake and Performance


## Abstract

Milk yield in dairy cows is directly dependent on their feed or energy intake. Due to genetic improvements, dairy cows have increased their potential for milk yield. This implies that the animal's energy requirements are drastically increased. The presence of both physical and metabolic mechanisms for intake regulation and dynamic nature of lactating dairy cows has made ration formulation a challenging enterprise. Few strategies of ration formulation can be adapted to simultaneously address the issue of intake regulation and stimulate feed intake and milk yield in dairy cows. Strategies that may be useful in achieving these objectives include improving the grass silage quality and adjusting the rumen degradation of starch to match that of grass silage. Starch accounts for the bulk of the dry matter of cereal grains and has been shown to influence rumen functioning, fibre digestion and thereby animal performance. The overall aim of the studies presented in this thesis was to explore the scope for stimulating feed intake and milk yield in dairy cows through feeding management.

Three feeding experiments and one laboratory study were conducted. Paper I evaluated the effects of increasing the proportion of wheat in diets containing maize- and grass-silages. Paper II evaluated the effects of interactions between grass silage digestibility (high vs. medium) and concentrate starch type (barley vs. maize) in the diets. Paper III evaluated the effects of increasing the proportion of NaOH-treated wheat in grass-silage based diets, and Paper IV estimated the ruminal digestion kinetics of different cereal starch using the *in vitro* gas production technique and compared the gas production data to *in vivo* starch digestibility data.

The results obtained in Papers I-III indicated that the feed intake and milk yield were primarily affected by the level of concentrate and the digestibility of the grass silage in the diets. The results presented in Paper IV indicated that the differences between the digestion kinetics for starches of different origins are less than has been reported in literature, which is consistent with the results of feeding trials. Moreover, the predicted ruminal starch digestibility obtained using gas production data were in good agreement with the *in vivo* starch digestibility data.



*Keywords:* Concentrate supplementation, Grass silage digestibility, Intake regulation, Milk yield, Rumen escapable starch



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# Dedication

To my father, who is a great teacher, scholar and source of inspiration.

To all my teachers, for their encouragement and guidance.

# Contents

# List of Publications

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

I **M. Hetta, M. N. Tahir & C. Swensson. (2010)**. Responses in dairy cows to increased inclusion of wheat in maize and grass silage based diets. *Acta Agriculturae Scandinavica Section A-Animal Science* 60 (4), 219-229.

II **M. N. Tahir, M. Hetta & P. Lund.** The effects of and interactions between the maturity of grass silage and concentrate starch source when offered as total mixed rations on the performance of dairy cows and rumen degradation parameters. *Animal* (accepted)

III **M. Hetta, M. N. Tahir, S. J. Krizsan, A. Puranen & P. Huhtanen**. Effects of the inclusion of sodium hydroxide treated wheat on the voluntary feed intake and milk production in dairy cows (submitted).

IV **M. N. Tahir, M. Hetta, M. Larsen, P. Lund & P. Huhtanen.** *In vitro* estimations of the rate and extent of ruminal digestion of dried cereal feed fractions compared to *in vivo* data (submitted).

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

The contribution of Muhammad Naeem Tahir to the papers included in this thesis was as follows:

I Performed experiments in collaboration with co-authors, analyzed the data, and wrote the manuscript.

II Planning the research in collaboration with co-authors, performed the experiments, analyzed the data, and wrote the manuscript.

III Performed experiments in collaboration with co-authors, analyzed the data, and helped in writing the manuscript.

IV Planned the experiments in collaboration with co-authors, performed the experiments, analyzed the data, and wrote the manuscript.

# Abbreviations

| | |
|---|---|
| CP | Crude protein |
| DIM | Days in milk |
| DM | Dry matter |
| DMI | Dry matter intake |
| ECM | Energy corrected milk |
| ED | Effective degradability or digestibility |
| GP | Gas production |
| iNDF | Indigestible neutral detergent fibre |
| Kd | Fractional digestion rates |
| Kp | Fractional passage rate |
| ME | Metabolisable energy |
| MNE | Milk nitrogen efficiency |
| MY | Milk yield |
| NDF | Neutral detergent fibre |
| NDS | Neutral detergent solubles |
| OM | Organic matter |
| RES | Rumen-escapable starch |
| SR | Substitution rate |
| TMR | Total mixed ration |

# 1 Introduction

## 1.1 Dairy cattle and their feeding

Cattle are livestock animals that are kept by man all over the world. They consume diets containing coarse or fibrous feeds and convert them into valuable products such as meat and milk. The starting point of the digestive tract in cattle is in the buccal cavity, at the site of the parotid glands. Food then passes down a long pipe, the oesophagus, to a complex stomach consisting of four chambers known as the rumen, reticulum, omasum and abomasum. The stomach is then followed by the small intestine and the long spiral colon (Van Soest, 1994). The rumen and reticulum are usually treated as a single compartment, the reticulo-rumen which forms the largest part of the stomach. The reticulo-rumen is the most important chamber for the fermentation and absorption of fibrous feeds, and material can move freely between the two chambers. The digestive tract of cattle and other herbivores are home to a diverse microbial population that ferment fibrous feeds; a system that is absent in non-herbivores. The microbial population of the rumen includes protozoa, many species of bacteria, and some fungi (Van Soest, 1994).

Carbohydrates collectively comprise the largest components of cattle diets (NRC, 2001) and are the main source of energy in the diets of dairy cows. Carbohydrates provide energy for animal's growth and body activities, and for microbial activity. They are a source of carbon for microbial protein synthesis, and essential for healthy functioning of rumen (NRC, 2001). Volatile fatty acids (VFA) are produced by microbial carbohydrate fermentation and provide up to 70% of the energy requirements of the ruminants in the same way that glucose does for non-ruminants (Van Soest, 1994). Dietary carbohydrates can be provided as structural (mainly forages) and non-structural (cereal concentrates) carbohydrates (NRC, 2001). Silages made from perennial grasses and legumes, which are highly digestible, are the most common local forages

in the diets of dairy cattle in the northern regions of Europe and are supplemented with concentrates based on cereal grains. Starch accounts for the bulk (50-70% of dry matter; DM) of the most cereal grains and the concentration of starch varies across different cereals and also within individual feeds (Nocek & Tamminga, 1991; Mills *et al.*, 1999). Starch in the diets of dairy cows has been shown to influence the functioning of the rumen (Krause & Oetzel, 2006), fibre digestion, and animal performance (Mills *et al.*, 1999). A thorough understanding of the degradation characteristics of starch through gastro-intestinal tract is therefore needed in order to better describe the differences between cereals and their possible effects on feed intake and milk yield in dairy cows.

## 1.2 Voluntary feed intake in dairy cows

Milk yield responses in lactating dairy cows are more closely related to feed intake, which corresponds to total energy intake, than to diet digestibility (Crampton *et al.*, 1960; Mertens, 1987). Consequently high levels of feed intake are preferred for lactating dairy cows. Ideally, the cattle should be supplied with a ration whose energy content is balanced against their requirements so as to minimize potential physiological changes. Due to improvements in genetic potential, dairy cows have increased their potential for milk yield. This implies that the animal's energy requirements are drastically increased. High yielding dairy cows are unable to consume sufficient forages to meet their energy requirements for milk production (Allen, 2000). It is therefore required to increase the energy density of the diet in order to ensure sufficient total energy consumption. (Kesler & Spahr, 1964). However, when fed on such high-energy diets, feed intake among cattle becomes limited by the animals' energy requirements (Mertens, 1987). The lactating dairy cow has a dynamic nature: she is lactating, growing (or losing weight) and pregnant all at one time (Johnson, 1986). This dual mechanism of intake regulation and dynamic nature of the lactating cows makes it challenging to identify an optimal ration composition (Forbes, 1995). A range of ration formulation strategies have been developed with the aim of addressing intake regulation mechanisms and stimulating feed consumption (and thus milk yield) in lactating dairy cows. Most of these strategies focus on either improving the quality of the grass silage or adjusting the rumen degradation of starch to match that of grass silage. The rate and efficiency of starch degradation in the rumen may be adjusted through concentrate supplementation using the following:

- Increasing the proportion of cereal starch in the diets.

- Changing the cereal starch type so that it is more adapted to the rumen degradation of forage.
- Processing the cereal starch in order to achieve a useful degree of ruminal starch degradation.

### 1.2.1 Regulation of feed intake

It has been proposed that mature animals attempt to maintain equilibrium between their energy intake and expenditure via regulation of feed intake (Mertens, 1994). The feed intake is regulated on both a short- and long-term basis. The events within a day which affect frequency, size and pattern of feeding are considered to represent short-term regulation of feed intake, while long-term regulation affects average daily intakes over longer production periods during which the animal's maintenance requirements remain constant (Mertens, 1987; Forbes, 1995).

Understanding the regulation of feed intake requires a thorough understanding of both the animal and diet characteristics, and the interactions between them (Ingvartsen, 1994; Fisher, 2002; Huhtanen *et al.*, 2007). Important dietary factors that can potentially affect feed intake in dairy cows include the fill effect and the energy concentration of the diet, which are influenced by forage quality and concentrate supplementation (Ingvartsen, 1994; Allen, 2000). These factors in turn control overall dietary nutrient digestibility and animal's supply of metabolizable energy (ME). A number of factors affect feed intake within single lactation cycles in dairy cattle, including intake capacity and ME requirements (both of which depend on the animal's current position within the lactation cycle as well as the animal's production potential and health) (Ingvartsen, 1994).

### 1.2.2 Feedback signals

*Physical regulation*

In simple terms, short-term physical regulation of feed intake is a consequence of temporarily physical distension of the reticulo-rumen in particular or the gastro-intestinal tract in general. It can be caused by the properties of the diet, the characteristics of the animal, or both (Forbes, 1995; Allen, 2000). Physical regulation of intake is most important when dealing with forages, for which the effects of physical fill are more important than those of ME concentration or digestibility (Van Soest, 1994; Allen, 2000). The animal characteristic that has the greatest impact on the physical regulation of feed intake is the intake capacity when fill is a limiting factor. Although it is sensitive to many other

factors, physical regulation becomes primary factor when animal's energy requirements and the fill effects of its diet are both increasing (Allen, 2000).

*Metabolic regulation*

Metabolic regulation of feed occurs when the animal's diet is high in energy and low in fill and so its intake is limited by its ME requirements. The animal characteristic that have the greatest influence on metabolic regulation are its ME requirements and ability to digest and utilize the ingested feed. Dairy cows' energy requirements relate primarily to bodily maintenance, milk production, and reproduction. For non-pregnant lactating dairy cows with fixed maintenance requirements (*i.e.* animals that are neither gaining nor losing live weight), dietary energy demands are primarily driven by the animal's production potential. Dietary factors such as ME concentration and digestibility seem to strongly affect feed intake under metabolic regulation. (Conrad *et al.*, 1964).

### 1.2.3 Theories of intake regulation

*Mertens' bi-phasic theory*

Mertens (1994) proposed that the intake is a linear function of intake capacity of the animal and will increase as the animal's intake capacity increases. He described a simple relationship between intake ($I_f$) when fill is limiting, the filling effect of the forage or diet (F), and the animal's intake capacity (C). In this model, the C is given by the product of the F of the forage or diet and $I_F$ when fill is a limiting factor.

$$I_f \times F = C \qquad \text{Equation 1}$$

Conversely, $I_f$ is a reciprocal function of the F of the forage or diet and $I_f$ thus will decrease in a curvilinear manner as the magnitude of F increases. As discussed above, the animal's intake capacity is largely dictated by the physical volume of the reticulo-rumen (Forbes, 1995).

Similarly, feed intake is a linear function of animal's ME requirements and will increase with animal's increasing ME requirements. Mertens (1994) proposed a simple model to describe the relationship between dietary feed intake ($I_e$) under metabolic regulation, the ME concentration of the diet (E) and the animal's ME requirements (R).

$$I_e \times E = R \qquad \text{Equation 2}$$

According to equation 2, the magnitude of R is equal to the product of the $I_e$ when limited by energy demand and the E of the forage or diet. This implies that the $I_e$ is inversely proportional to the E of the forage or diet.

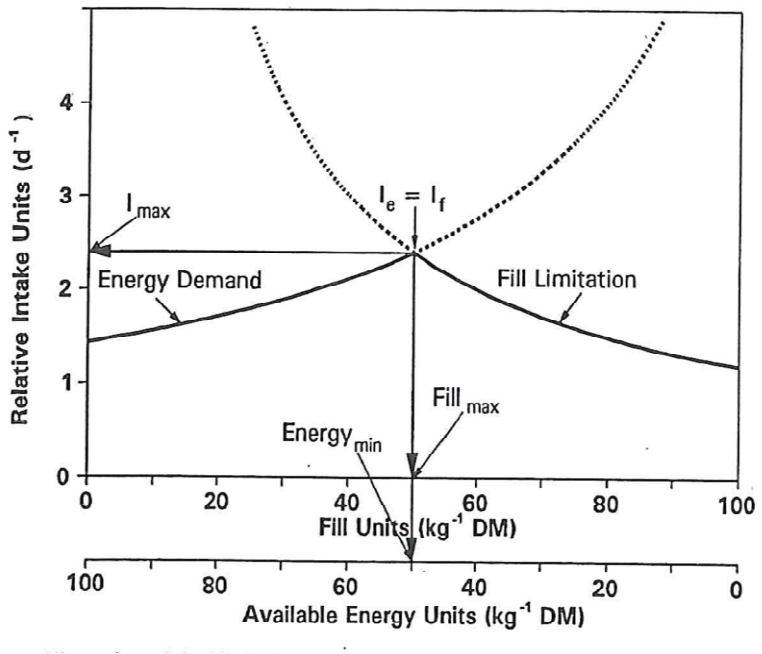


*Figure 1.* Illustration of the bi-phasic, discontinuous nature of intake regulation based on simple algebraic equations (Equation 1 and 2) describing expected intakes when limited by physiologic energy demand ($I_e$) or physical fill ($I_f$). Maximum intake ($I_{max}$) occurs at the intersection of the curves representing the two theories of intake regulation. The point of intersection defines the diet having maximum fill ($F_{max}$) and minimum energy concentration ($E_{min}$) which meets the animal's energy requirements and maximizes rumen fill (adopted from Mertens, 1994).

Figure 1 shows how the intake varies depending on the extent to which it is regulated by the fill of the diet and the animal's energy requirements.

*Interactions of feedback signals*

Regulation of feed intake is complex and is not governed by any single factor as has been reviewed by many authors (Mertens, 1994). Instead, multiple interactions of feedback mechanisms are responsible for the control of feed intake in dairy cows (Forbes, 1995). Fisher (2002) described that the distension or fill feedback mechanism should be viewed as a regulator of feeding behaviour of the animals, rather as a regulator of feed intake. The evidence that fill effect was not limiting intake has been provided by some experiments (Allen, 1996; Forbes, 1996). In these experiments, no reduction in feed intake

was observed and cows were able to increase their rumen capacity when distension or fill effect was increased (Fisher, 2002). This suggests that reticulo-rumen should be regarded as having some reserve capacity. However, in other experiments, set-point for feed intake took place to a degree of physical fill that is not physiological (Rinne *et al.*, 2002; Kuoppala *et al.*, 2009). The meta-analysis involving large data sets conducted by Huhtanen *et al.* (2007 & 2008a) give an indication of interactions of feedback mechanisms for the control of feed intake in dairy cows.

## 1.3 Feeding strategies to stimulate feed intake in dairy cows

### 1.3.1 Forage quality with respect to NDF concentration and digestibility

Forage consists of plant matter whose cell walls are primarily made up of polysaccharides, hydro-cinnamic acids, lignin, and proteins, and also called fibre (Van Soest & Wine, 1967). Fibre accounts for 30 to 80% of the DM of forage (Hatfield *et al.*, 1999). The most important structural carbohydrate in plant cell walls is cellulose, a polysaccharide, in which glucose monomers are linked together through a β 1-4 glucosidic linkage. The neutral detergent fibre (NDF) is a empirical determination of fibre, and could be defined as the portion of cell wall which is neutral detergent insoluble and consists of cellulose, hemicelluloses and lignin (Van Soest & Wine, 1967).

While the definition of forage quality is complex and depends on multiple factors, the quality of individual forages can be analyzed based on their consumption by ruminants, in terms of voluntary dry matter intake (DMI), digestibility and metabolism (Mertens, 1994). Van Soest *et al.* (1978) suggested that forage NDF is the most important chemical predictor of the forage quality. Mertens (1987) reported that forage NDF has been shown to correlate strongly with the volume or bulk density of the forage and thus with the fill effect of the forage or diet. If DMI is expressed as a percentage of the animal's live weight (LW), Mertens (1987) showed that the optimal daily NDF intake for dairy cows is 1.2% of LW when overall feed intake is limited by physical factors. This relationship was relatively constant across experiments. Mertens (1987) further argued that this relationship can provide reliable information on the intake capacity of dairy cows for optimal diets, that NDF alone can be used to predict forage intake (or total DMI if forage is the sole component of the diet). This information may be utilized in models to predict DMI of the cows (Mertens, 1987).

However, forages vary in terms of rumen digestibility (Huhtanen *et al.*, 2007; Kuoppala *et al.*, 2009) and there were reported reductions in the rumen NDF pool size with improving digestibility of grass silage (Rinne *et al.*, 2002;

Kuoppala *et al.*, 2009). Consequently, NDF data alone may not sufficient to fully describe the relationship between fill and the animal's intake capacity. It thus seemed that there was a need to define a term that would cover both the forage NDF concentration and digestibility. Therefore, the D-value was introduced in the 1980's (Mayne & Gordon, 1984), which is the digestible organic matter (OM) in the DM. Huhtanen *et al*. (2007) reported that the D-value was a more useful measure than NDF concentration or digestibility alone for estimating the intake potential of grass silage.

### 1.3.2 Concentrate supplementation

Supplementation of the diet with concentrates that are rich in either protein or energy is a common strategy to increase the density of nutrient supply to dairy cows through the ration. Feeding with mixed diets in which the basal components (usually forages) are supplemented with concentrates is different to feeding with diets based on forages alone. The introduction of cereal concentrates causes major changes in the nutritional status of the diet and the microbial population in the rumen (Elshazly *et al.*, 1961). Established concentrate supplementation strategies include: level, type and processing. The following sections discuss the ways in which concentrate supplementation can affect the total and forage DMI of dairy cows fed on grass silage based diets.

It should be noted that concentrate supplementation has been linked to reductions in forage intake in dairy cattle. This reduction is known as forage-concentrate substitution and the change in forage DMI per unit of additional concentrate is called the substitution rate (SR). The phenomenon of forage-concentrate substitution is practically important in both types of feeding systems e.g. feeding of concentrates separately (Laird *et al.*, 1981; Mayne & Gordon, 1984; Faverdin *et al.*, 1991) or feeding dietary treatments as total mixed rations (TMR) (Ferris *et al.*, 2001). Concentrates can replace forages by: 1) increasing the filling effect of the diet (i.e. the filling of the reticulo-rumen), 2) changing the fermentation pattern within the rumen, 3) providing an increased ME and nutrient supply, and 4) modifying eating behavior because they influence feed preferences and improve the nutritional status of the diet (Jarrige *et al.*, 1986). The mechanisms explaining the forage-concentrate substitution imply that multiple feedback mechanisms are involved.

Various factors can be accounted for the substitution of forages by concentrates which include: intake potential of forages, amount and type of concentrate supplementation and type of feeding system (*i.e.* whether the ingredients of the diet should be offered separately or as TMR) (Jarrige *et al.*, 1986). The SR value correlates positively with increases in the intake potential of the forage (Keady *et al.*, 2004; Huhtanen *et al.*, 2008a). Similarly, the SR

value has been shown to increase when the amount of concentrate supplementation is increased for any given forage type (Huhtanen *et al.*, 2008a). There are relatively little data on the relationship between the type of concentrate (with a high proportion of starch; starchy concentrates vs. easily digested plant cell walls; fibrous concentrates) and the SR value for dairy cattle. However, those data that are available clearly indicate that the SR values for cows fed with fibrous concentrates are lower than those for starchy concentrates (reviewed by Jarrige *et al.,* 1986).

*Cereal concentrate level*

Increased concentrate supplementation has reportedly affected feed and forage intake in dairy cows (Kesler & Spahr, 1964). The effects of increasing the amount of cereal concentrate supplementation in grass silage based diets have been evaluated in many feeding trials with dairy cows (Laird *et al.*, 1981; Faverdin *et al.*, 1991; Ferris *et al.*, 2001; Kuoppala *et al.*, 2004). While the intake and performance responses depended on the amount of concentrate supplementation applied, the following trends were consistently observed: increasing the amount of concentrate in the feed increased diet digestibility and raised the intake of total DM, digestible DM, and ME. Increased supplementation also enhanced milk yield (MY), milk protein and milk lactose yields but had a variable effect on milk fat concentration (Laird *et al.*, 1981; Mayne & Gordon, 1984). However, increasing the amount of concentrate supplementation had negative effects on fibre digestibility (and thus forage intake), feed utilization and feed efficiency (Stensig *et al.*, 1998; Kuoppala *et al.*, 2004).

*Cereal concentrate type*

Cereal concentrates differ substantially in their ruminal fermentation characteristics (Nocek & Tamminga, 1991; Mills *et al.*, 1999). This is primarily due to differences in the physical and chemical characteristics of cereal starches (Ørskov, 1986). Cereal starches are more easily degraded than tuber or root starches. The rate of digestion (kd) of concentrates is thought to influence the kd and digestibility of NDF (Stensig *et al.*, 1998) and thus the SR of the forage. The experiments conducted to compare the effects of starchy concentrates vs. fibrous concentrates did not show any significant differences between the two concentrate types (Castle *et al.*, 1981). Other report greater SR values for starchy concentrates (Faverdin *et al.*, 1991; Huhtanen *et al.*, 2008a) and still some other report lower SR values for starchy concentrates (Mayne & Gordon, 1984). There are limited data available on the effect of type of starchy concentrate on the SR of forages.

Starches from barley, oats and wheat are reported to be degraded rapidly and completely (*i.e.* more than 80% of starch intake is degraded) in the rumen of dairy cows (Nocek & Tamminga, 1991). These rapidly-degraded starches are associated with the rapid production and absorption of metabolites in the rumen and low ruminal pH values (Mills *et al.*, 1999; Silveira *et al.*, 2007). The rumen fermentation rate of maize starch is slower than that of barley, oats or wheat starch (Nocek & Tamminga, 1991; Mills *et al.*, 1999; Tothi *et al.*, 2003) which can reduce the rate of metabolite absorption and thus, maintain stable ruminal pH. Moreover, the degradation of maize starch may be so slow that it does not undergo complete fermentation in the rumen (McCarthy *et al.*, 1989). Previous reviews of starch digestion (Nocek & Tamminga, 1991; Mills *et al.*, 1999) have reported that the digestibility co-efficient for maize starch in the rumen is around 0.50 and 0.76, respectively, and that some of it is digested post-ruminally or excreted in the faeces. Digestion in the small intestine may increase its efficiency as a source of energy for MY (Reynolds, 2006).

*Cereal concentrate processing*

Cattle are not able to effectively utilize whole cereal grains so it is common for grains to be processed before being incorporated into dairy cattle rations. Processing of the cereal grains reduces their particle size and increases their potential exposure to bacterial and enzymatic action. Cereal grain processing is generally intended to improve the ruminal and total availability of starch (NRC, 2001) and can be achieved by physical or chemical means, or a combination of the two (Nocek & Tamminga, 1991). Physical processing methods often include grinding, cracking and rolling of the dry grains. The processing methods that involve heat and moisture in addition to physical processing are referred to as physiological processing methods, for example steam-flaking. The intensity and nature of grain processing depends on the nature of the material being processed and its intended purpose.

A second function of processing is to depress the rumen digestion of starch and to increase the amount of rumen escapable starch (RES). In fact, some chemical processing methods (*e.g.* treatment with formaldehyde or NaOH) are conducted to make cereal grains partly digested and absorbed post-ruminally (Nocek & Tamminga, 1991; Mills *et al.*, 1999). Chemical processing of cereals is intended to increase the amount of RES and has two advantages: 1) because it alters the availability of rumen degradable starch, it may change the pattern of rumen fermentation, resulting in less acidic conditions in the rumen and thereby stimulating forage intake as reviewed by Campling (1991), and 2) shifting starch degradation from the reticulo-rumen to the small intestine may improve the energy utilization of the diet, as suggested by Owens *et al.* (1986)

and Reynolds (2006) and as shown in Figure 2. However, chemical treatments used to reduce starch digestion in the rumen reduce energy supply to microbial protein synthesis and may therefore alter milk composition (McNiven *et al.*, 1995).

### 1.3.3 Forage-concentrate interactions

Interactions between dietary components can be used as an important tool to stimulate voluntary feed intake and milk production in lactating dairy cows. Forage-concentrate interactions are responsible for the different responses observed to a given concentrate when different forages are supplied (Dewhurst *et al.*, 2001). These interactions are due to the associative effects of dietary components. Positive associative effects, where grains increase voluntary intake and/or the digestion of forage, are usually due to the provision of a limiting nutrient in the grain which is deficient in the forage. Negative associative effects, in which grains decrease voluntary intake and/or digestion of forage, can cause low efficiency of utilization of grain (Dixon & Stockdale, 1999). Positive associative effects usually cause greater intake of ME than expected for example, protein or digestible fibre supplementation in the diets (Huhtanen, 1991).

When associative effects occur, the digestibility of one or more dietary components is affected which in turn affects feed intake (Dixon & Stockdale, 1999). Same or similar factors may be accounted for forage-concentrate interactions as those for forage-concentrate substitution. In a review of literature on associative effects of feeds, Huhtanen *et al.* (1991) suggested that the associative effects are most likely to occur at higher levels of feeding than those required for maintenance. It has been reported that the intake potential of forage is important for determining the strength of interactions because, a strong forage-concentrate interaction is reported for highly digestible grass silages (Keady *et al.*, 2004; Huhtanen *et al.*, 2008a).

## 1.4 Starch in dairy cow diets

### 1.4.1 Physical and chemical properties of starch

The non-structural carbohydrates consist of starch, sugars and organic acids (NRC, 2001). Unlike the latter two, starch is insoluble in cold water. Starch is stored as a reserve carbohydrates in various locations in different plants e. g. in seeds, fruits, tuber and roots (Mills *et al.*, 1999). Starch forms up 70 to 80% of total non-structural carbohydrates in cereal grains (Nocek & Tamminga, 1991). It is usually degraded rapidly and almost completely (the digestibility coefficients of most cereal starches are above 0.9) in the digestive tracts of

cattle (NRC, 2001). Starch is a polymer of glucose monomers and consists of two major molecules: amylose and amylopectin. Amylose contains only α 1-4 D glucosidic linkages between the glucose monomers but amylopectin also contains branches that are linked in straight chains by α 1-6 D glucosidic linkages. The structure of starch is highly organized and its two major constituents (e.g. amylose and amylopectin) are linked together by H-bonding. Starch granules are variable in shape: they may be spheres, ellipsoids, polygons, platelets or irregular tubules (Mills *et al.*, 1999). In addition, they have both organized crystalline areas, which resist water infiltration, and non-organized amorphous areas where water can move freely (Rooney & Pflugfelder, 1986). The crystalline areas are mainly composed of amylopectins and are of higher density than the amorphous areas, which are mainly made up of amyloses.

Swelling is a reversible process in which starch granules gradually take up water in the presence of heat and moisture. Gelatinization refers to the irreversible losses of the granular structure of the starch under moist conditions at temperatures above those required for swelling. Under such conditions, the intermolecular H-bonds in the crystalline areas of the starch granules are broken down (Zobel, 1988). This makes the granules more susceptible to enzymatic and bacterial attack in both the crystalline and the amorphous areas. The gelatinization temperature depends upon the proportion of amylose present and is therefore different for different starches. Retrogradation is the re-association of starch molecules after gelatinization. While H-bonds are re-established between amylose and amylopectin, the starch granules do not regain their native characteristics. Retrogradation produces forms of starch that are resistant to enzymatic attack (Nocek & Tamminga, 1991).

### 1.4.2 Starch digestion and utilization in dairy cows

*Rumen*

The first site of starch degradation is the reticulo-rumen (Figure 2) where starch is fermented by rumen microbes, principally amylolytic bacteria. Rumen degradation of starch is a direct competition between digestion and passage, and it seems that there is no upper limit for the extent of starch degradation in the rumen. However, the literature shows that an inefficient nitrogen supply to the rumen may limit microbial growth and the production of enzyme needed for starch digestion (Ortega Cerrilla & Mendoza Martinez, 2003). The rumen fermentation of starch is dependent on many factors involving the direct effects of variety, species and type and degree of processing of starch sources (Mills *et al.*, 1999) and frequency of feeding (Reynolds, 2006). The indirect effects of

the nutritional interactions such as type of forage fed to the dairy cows and the ruminal pH (Lykos *et al.*, 1997), the composition of the rumen microbial population, the dietary crude protein (CP) intake, and the availability of non protein nitrogen may affect rumen fermentation of starch.

Fifteen different amylolytic strains of bacteria have been identified by Kotarski *et al.* (1992), each of which produces between one and eight different amylolytic enzymes. The microbial α and β amylases catalyze extracelleular starch hydrolysis: the action of α amylase allows the degradation of amylose and the linear regions of amylopectin, while β amylase cleaves the starch chains at their end points and degrades both amylose and the peripheral regions of amylopectin. Thereafter, various other enzymes such as the maltases, maltose phosphorylases, and glucosidases cleave maltose and isomaltose to glucose or glucose 1-phosphate. Ruminal protozoa can reduce ruminal starch hydrolysis in at least two ways: 1) by ingesting bacteria in numbers sufficient to decrease ruminal fermentation rates, and 2) by ingesting starch granules and sugars, thus decreasing the accessibility of these substrates to fermentation by the fast-growing amylolytic bacteria as reviewed by Ortega Cerrilla & Mendoza Martinez (2003).

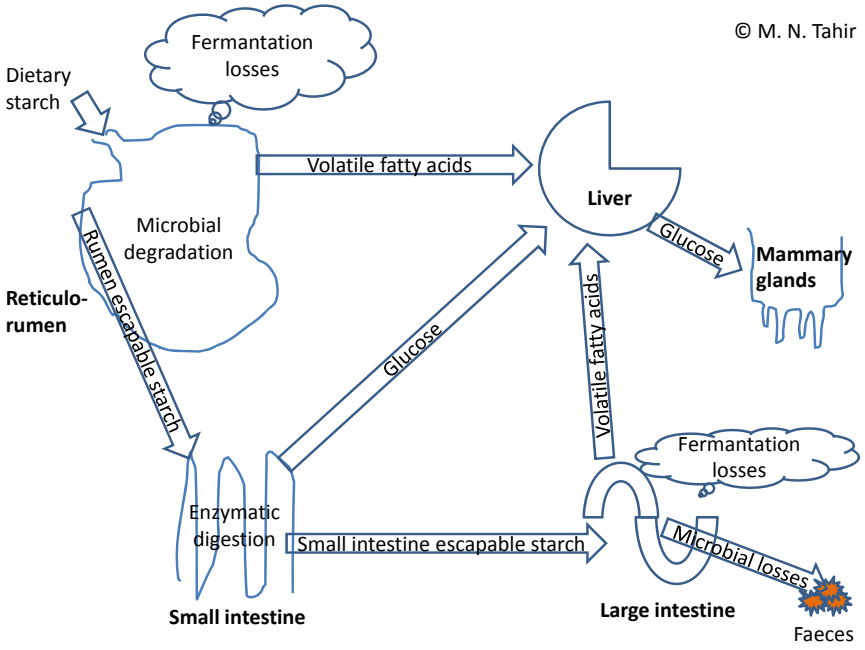


*Figure 2.* A schematic representation of starch digestion and utilization in dairy cows.

The intermediate product obtained from the hydrolysis of starch in the rumen is glucose, which is rarely detectable due to its rapid uptake and further metabolism. Glucose is very rapidly degraded to 3-carbon intermediates via glycolysis. Several possible chain reactions are then available for the conversion of these 3-carbon intermediates into VFA and are absorbed into the blood stream through the wall of the reticulo-rumen. The process of phosphorylation during VFA production provides energy for microbial growth and activity. The fermentation of high levels of starch in the rumen of cows offered high concentrate diets, gives rise to a high proportion of the glucogenic VFA, propionate (Mills *et al.*, 1999). In addition to VFA, starch fermentation in the rumen produces methane, carbon dioxide and heat.

*Small intestine*

Ruminal starch digestion differs from that in small intestine in two ways: 1) the quantity and composition of starch reaching the small intestine are different to those in the rumen due to the effects of ruminal pre-digestion, and 2) the enzymes produced by ruminal microbes digest starch in the rumen whereas in the small intestine, digestion is achieved by pancreatic enzymes and those secreted by the intestinal walls. The starch entering the small intestine also includes microbial polysaccharides, which make up a significant proportion of the microbial mass therein (Jouany & Thivend, 1972).

Enzymatic digestion of starch in the small intestine of ruminants is quite similar to that in non-ruminants. The pancreatic amylase, α-amylase, hydrolyses amylose and amylopectin to dextrins and linear oligo-saccharides of two to three glucose units. Further, hydrolysis is completed by the oligo-saccharidases secreted by the intestinal lining. The oligo-saccharidases such as maltases and isomaltases produce the glucose units which can then be absorbed. The two main routes for the transfer of glucose from the lumen of small intestine to the blood stream are active transport and diffusion in concert with the absorption of water (Huntington, 1997).

The suggestion that it may be possible for ruminants to use starch in a more energy-efficient manner by causing them to digest and absorb glucose rather than fermenting and absorbing organic acids (Owens *et al.*, 1986; Reynolds, 2006) promoted interest in increasing the amount of RES. It is therefore important to properly understand the starch digesting capabilities of the small intestine in ruminants. Huntington (1997) in a review of published studies on starch digestion reported that on average, 5 to 20% of the dietary starch intake is RES and is digested post-ruminally, principally in the small intestine or fermented in the hindgut. The data collected by Owens *et al.* (1986) showed a large variation in digestibility values of starch (from 0.45 to 0.88) in the small

intestine of growing cattle for two species of cereals; maize and sorghum. Owens *et al.* (1986) further reported that there seemed to be no ceiling for starch digestion in the small intestine and that there was a linear relationship between starch flow and digestibility in the small intestine ($R^2$=0.65).

*Efficiency of energy utilization with respect to site of starch digestion*

The site of starch digestion (Figure 2) determines the amount and type of energy available to dairy cows. The supply of ME is primarily via the absorption of VFA if starch is fermented in the rumen and via the absorption of glucose if it is digested in the small intestine. The differences in the energetic efficiency of starch utilization at these two sites can be explained by the differences in the corresponding metabolic pathways of digestion and absorption: starch fermented in the rumen must undergo many steps before it can be converted into glucose for the synthesis of milk. However, when digested in the small intestine, it can be directly absorbed through the lumen in the form of glucose as shown in Figure 2.

In a review of studies, Owens *et al.* (1986) reported that the amount of available energy from starch in growing cattle when digested in the small intestine is 42% greater than that obtained from digestion in the rumen. Similarly, Dixon & Stockdale (1999) reported that rumen fermentation of starch in dairy cows theoretically reduces the energy value of grain by 30-50%. Studies on the infusion of glucose or starch into the rumen or duodenum have shown that the efficiency of energy utilization was improved with duodenal infusions (Reynolds *et al.*, 2001; Boudon *et al.*, 2007). Reynolds (2006) concluded from a number of studies on the glucose infusions in dairy cows, that the post-rumen glucose infusions increased MY, decreased milk fat concentration and yield, and DMI when compared to infusions in the rumen. The decrease in milk fat concentration and yield with increasing MY indicate that total energy output through milk was not altered by the site of glucose infusions (Reynolds, 2006).

The production responses in dairy cows to the site of starch digestion have been unequivocal (Reynolds, 2006). Nocek & Tamminga (1991) concluded that there has been no clear evidence for the beneficial effects of the site of starch digestion on MY in most of the production studies reviewed. The exception to this conclusion was the study of McCarthy *et al.* (1989), where shifting the site of starch digestion by replacing barley with maize, in association with more DMI, increased MY and milk protein yield. In contrast, an advantage for the increased rumen digestion of starch in terms of MY and milk protein yield was concluded from a review of the published studies by Theurer *et al.* (1999). This suggests that increased starch fermentation in the

rumen increases energy supply for microbial protein synthesis, therefore, more metabolisable protein (MP) flowing to the small intestine. However, in these studies, also the total starch digestibility was improved by steam flaking of the grains.

### 1.4.3 Evaluation of starch

*Chemical analysis*

The starch concentration of ruminant feedstuffs has not been routinely measured in the past. Therefore, conventional methods of chemical analysis such as Weende method of proximate analysis, the crude fibre method (Van Soest & Wine, 1967) or the NDF method (Van Soest *et al.*, 1991) do not incorporate direct measurements of starch concentrations for different feeds. The starch concentrations of feedstuffs can however be measured by both the spectroscopic (Garnsworthy *et al.*, 2000) and enzyme digestion methods (Bach Knudsen, 1997). The enzymatic digestion method involves the complete degradation of starch to glucose using various enzymes, primarily glucoamylases (Bach Knudsen, 1997). The nature of these enzymes is such that they attack and break α 1-4 D, α 1-6 D and α 1-3 D glucosidic linkages. The starch must be gelatinized before it can undergo such enzymatic hydrolysis.

There is significant variation in the literature data on the starch concentrations of grains measured in different laboratories using different methods (Beever *et al.*, 1996). Information on starch concentrations is useful because it can be used to help determine the energy supply to the animals and is thus important when formulating rations. However, a weak but significant relationship between the starch concentration of various feedstuffs and the extent of ruminal starch degradation has been presented (Nocek & Tamminga, 1991).

The NRC (2001) fractionates non-structural carbohydrates into four fractions: 1) organic acids, 2) water-soluble carbohydrates, 3) starch and 4) neutral detergent soluble fibre. The Cornell Net Carbohydrate and Protein System (CNCPS) (Sniffen *et al.*, 1992) partitions non-structural carbohydrates into two main fractions: Fraction A is fast and consists of sugars and organic acids, and Fraction B1 is slow and consists of starch and pectins. The sizes of the different fractions in the CNCPS system are based on their rates of degradation determined using *in situ* degradation profiles. The fractional kd values for fraction B1 are feed-specific and highly variable among cereals.

*Digestibility techniques*

Digestibility is the most important of the factors that define the nutritive value of a feedstuff after it has been ingested by a ruminant (Crampton *et al.*, 1960). The digestibility determines the feed's available ME concentration. Several attempts have been made to develop a reliable method for the compartmental or total tract quantification of starch digestibility or degradability in dairy cows, including a range of *in vivo, in situ* or *in vitro* approaches. *In vivo* measurements are laborious, expensive, time consuming and require cannulated animals for both compartmental and total digestibility measurements (Owens & Hanson, 1992). However, they have a great advantage in that they provide information on responses in real animals. They should therefore always be used as a source of reference data when evaluating results obtained with other methods. It should be noted the values reported by different techniques are appropriate for comparative purposes within a study; however, they may not accurately represent real rates or extents or may not be comparable for a given feedstuff across studies and techniques. When evaluating digestibilities or degradabilities using different techniques, it is important to identify and evaluate the objective for the test procedure used.

*In vivo techniques*

The conventional method of measuring *in vivo* digestibility is total collection, where the digestibility of feedstuffs is determined by accurately measuring feed intake and faecal output (Van Soest, 1994). This technique may be stressful to the animals as they must be closely confined in digestion crates or fitted with harnesses and collection bags. An alternative procedure known as the marker method is to measure a substrate in the feed and faeces that is not absorbed or secreted by the animal. Markers are used routinely to estimate digesta flow and fecal output in ruminants (Owens & Hanson, 1992).

The *in vivo* measurements of ruminal digestibility are often achieved using flow measurements of digesta in the abomasum, proximal duodenum (Harmon & Richards, 1997; Titgemeyer, 1997) or omasal canal (Huhtanen *et al.*, 1997) using digestibility markers (Owens & Hanson, 1992). Huhtanen and Sveinbjörnsson (2006) discussed in detail the challenges and methodological problems associated with the measurement of ruminal starch digestibility *in vivo*. The primary source of variation in the estimates of ruminal starch digestibility is likely to be unrepresentative digesta sampling arising from either an inappropriately chosen sampling site or the nature or number of the digestibility marker used (Huhtanen & Sveinbjörnsson, 2006).

*Alternative techniques*

The *in situ* nylon bag technique, in which the measurements are conducted in the rumen of living animal, is the standard technique used to study the digestion kinetics of various feed stuffs (Ørskov & McDonald, 1979). The technique is based on the assumption that the disappearance of substrate from the bags represents actual rumen degradation by the rumen microbes and their enzymes (López, 2005). Since its development, the technique has been used extensively to study the ruminal digestion kinetics of a large range of feedstuffs and different feed parameters (Ørskov & McDonald, 1979; Offner *et al.*, 2003; Tothi *et al.*, 2003). However, this technique has been criticized for its drawbacks such as loss of undigested particulate matter from the bags due to mechanical degradation, contamination of the bag residues with matter that does not originate from the feed, and confined conditions within the bags, which may affect the activity of rumen microbes (Nocek & Tamminga, 1991; Noziere & Michalet-Doreau, 1996; López, 2005; Huhtanen & Sveinbjörnsson, 2006). The *in situ* kinetics of starch degradation and effective degradability (ED) are estimated by incubating the feeds in nylon bags in the rumen for a variable number and length of incubation periods. The data is usually fitted using the model proposed by Ørskov & McDonald (1979): disappearance = a + b $(1-e^{kdt})$, here the parameters a, b and kd represent the washable fraction, the non-washable potentially degradable fraction and the degradation rate of fraction b, respectively. The *in situ* ED can be calculated as: *in situ* ED = a + (b × kd)/(kd + kp). Here, the parameters a, b and kd have the same meaning as above, and kp represents the assumed rate of passage of particles.

*In vitro* techniques involve measuring digestion kinetics based on: 1) solubility, 2) the use of rumen fluids (Lanzas *et al.*, 2007; Stevnebø *et al.*, 2009), and 3) the use of enzymes (López, 2005). *In vitro* techniques that use rumen fluid provide data on digestion kinetics directly through gravimetric measurements of substrate disappearance (Sveinbjörnsson *et al.*, 2007) or indirectly through the measurement of gas production (GP) when feed is incubated in the presence of rumen fluid diluted with buffer solution (Menke & Steingass, 1988; Cone *et al.*, 1996; Lanzas *et al.*, 2007). The volume of gas produced using the GP technique is strongly correlated with *in vivo* digestibility, and various empirical equations have been developed to predict *in vivo* digestibilities based on chemical analysis and GP data for various feeds (Menke & Steingass, 1988). López (2005) listed important sources of variation for digestion kinetics measurements using *in vitro* techniques that involve the use of rumen fluid. These include the type of fermentation vessels used, the incubation conditions, the source and ratio of inoculum to substrate, and the size and preparation of substrate.

# 2 Objectives

The overall aim of the studies presented in this thesis was to explore the scope for stimulating feed intake and milk yield in dairy cows through feeding management. More specifically, the focus was on the effects of forage quality in terms of digestibility and concentrate supplementation. The specific objectives relating to these two aspects of feeding were as follows:

- To study the effects of increasing the amount of wheat in the diets of dairy cows fed on maize- and grass-silage based diets.
- To compare the effects of starches from maize and barley in the diets of dairy cows fed on grass silage of high or medium digestibility.
- To study the effects of processing wheat in the diets of dairy cows.
- To estimate the rate and extent of ruminal digestion of different starches using an *in vitro* GP technique and to compare the results obtained to the *in vivo* starch digestibility data.

# 3 Materials and methods

## 3.1 Paper I

The effects of increasing starch concentration in TMR of dairy cows were evaluated using a 4 × 4 Latin square dose response experiment with 28 multi parous Swedish red dairy cows. The animals were averaging 150 days in milk (DIM) at the start of the experiment, had an initial energy corrected milk (ECM) yield of 30 kg/day, and a LW of 625 kg. Four experimental diets comprising mixtures of maize and grass silage with wheat grain at four levels (8, 16, 24 and 32% of diet DM) were offered three times a day *ad libitum*. The experimental diets were formulated to contain 19, 22, 25 and 28% starch per kg diet DM. Forage maize (*Zea mays cv.* Baxxos) was harvested when the ears were at the dent stage, with an estimated DM content of 33%. A primary growth timothy grass (*Phleum pratense* L. *cv.* Grindstad) ley was harvested and chopped with a nominal chop length of 20 mm. Animals were milked twice daily. The digestibility of the DM and NDF was determined using indigestible NDF (iNDF) as an internal marker.

## 3.2 Paper II

Twenty-eight multi-parous Swedish Red dairy cows, 133 DIM, with an average MY of 30 kg/day and a LW of 624 kg were blocked by DIM and randomly assigned to seven replicated balanced 4 x 4 Latin squares with four 21-day experimental periods. The experimental diets consisted of four TMR mixtures consisting of two primary growth timothy grass (*Phleum pratense L. cv.* Grindstad) silages with different digestibilities (EGS and LGS), harvested with a 14-day difference in growth and supplemented with concentrates based either on barley or maize. All TMR contained identical proportions of forage (51%), concentrate (49%) and starch (21%) on a DM basis and were offered in a 2 × 2 factorial arrangement of experimental treatments three times a day *ad*

*libitum*. The CP, NDF and ME concentrations were balanced in diets based on the same types of silage. Total tract digestibility was measured using iNDF as an internal marker. The feeds' ruminal degradation parameters were determined using both *in situ* (nylon bag) and automated *in vitro* (gas production; Cone *et al.*, 1996) techniques. A first order kinetic model was fitted to the *in situ* degradation data (Ørskov & McDonald, 1979) and a two-pool Gompertz model to the *in vitro* GP profiles (Schofield *et al.*, 1994).

## 3.3 Paper III

An experiment was conducted to examine how increasing the proportion of NaOH-treated wheat in the diet of dairy cows affects DMI and MY, using diets based on rolled wheat and an oat/barley mixture for comparative purposes. Twenty-four multi-parous Swedish Red dairy cows, 147 DIM, with an average MY of 31 kg/day and a LW of 611 kg were blocked by DIM and randomly assigned to six replicated balanced 4 x 4 Latin squares with each experimental period spanning 21 days. The experimental diets consisted of four TMR mixtures consisting of grass silage (*Phleum pratense L. cv.* Grindstad) (52% on a DM basis) supplemented with concentrates (48%). Four diets were examined: an oat/barley mixture, 100% rolled wheat, 1:1 rolled wheat: NaOH-treated wheat, and 100% NaOH-treated wheat on a DM basis. Total tract digestibility was determined using acid-insoluble ash as an internal marker. The concentrate feeds' ruminal degradation parameters were also determined using an *in vitro* GP technique and a modeling procedure.

## 3.4 Paper IV

The rumen digestion characteristics of a range of commonly used cereal grains in ruminants' diets and their fractions were described using an automated *in vitro* GP technique (Cone *et al.*, 1996). The ruminal digestibility values predicted based on the GP data were compared to previously acquired *in vivo* data. Nine cereal feeds (WF) were dried and subjected to neutral detergent extraction according to the method of Van Soest *et al.* (1991) to isolate their NDF and neutral detergent soluble (NDS) fractions. The GP measurements were performed on duplicate samples in buffered rumen fluid (Menke & Steingass, 1988) at 39$^{o}$C on two occasions over a 72 h period. The fermentation residues were collected and analyzed for NDF concentration to estimate their true OM digestibility and *in vitro* NDF digestibility according to Hetta *et al*. (2004). The GP from the NDS fraction was calculated using a curve subtraction procedure (Schofield & Pell, 1995). A three-pool Gompertz

model (Schofield *et al.*, 1994) was fitted to the GP profiles and a two-compartment, dynamic and mechanistic rumen model developed by Huhtanen *et al.* (2008b) was used to predict the digestibility of the potentially digestible feed fraction and the effective kd according to the assumptions of distribution of GP volumes into different pools made by Stevnebø *et al.* (2009). The effective NDS kd values were further used to calculate ruminal NDS digestibility using the same two-compartment, dynamic and mechanistic rumen model and a suggested rumen retention time for concentrates in dairy cows (NRC, 2001).

# 4 Results

## 4.1 Paper I

The concentrations of estimated ME and supply of MP and protein balance in the rumen increased with the proportion of wheat grain in the diet. Increased proportion of wheat grain in the diet resulted in linear increases ($P<0.01$) in the daily DMI and ECM yield of the dairy cows. Dry matter digestibility, concentrate intake, and milk protein concentration and yield increased ($P<0.01$) with the wheat proportion, while the intake of forage, milk fat concentration, total NDF digestibility, feed efficiency and milk nitrogen efficiency (MNE) decreased ($P<0.01$). The increased inclusion of wheat in the diets did not affect the intakes of NDF or iNDF. There was a significant ($P<0.01$) linear increase in faecal concentrations of nitrogen and linear decreases in the concentrations of NDF and iNDF. The linear regression coefficients (obtained from the estimated responses to changes in the wheat proportion of the diet on a DM basis) for the relationship between dietary wheat concentration and DMI, MY and ECM production were 0.24, 0.10 and 0.08, respectively.

## 4.2 Paper II

Compared to the EGS silage, the LGS silage had greater concentrations of DM, NDF, iNDF and $NH_3N$ in total nitrogen and lower concentrations of CP and lactic acid. All diets based on the same silage types had similar DM, CP, NDF and starch concentrations, and similar calculated ME values. Cows offered EGS-diets had greater ($P<0.01$) DMI, ECM yields, milk component yields and greater total DM and OM digestibility, but these were not affected by the concentrate starch source ($P>0.05$). The intake of NDF, the composition of the milk and the total starch digestibility were not affected ($P>0.05$) by the

experimental diets. No interaction between the maturity of the grass silage and the starch source was observed (P>0.05) for DMI, diet digestibility or ECM yield. Both grass silages and concentrates had similar rates of ruminal degradation of NDF when measured *in situ*. The *in situ* DM and starch degradation rates for barley concentrate were greater (P<0.01) than those for maize concentrate. The *in vitro* OM gas production rates and extents were similar for both concentrates.

## 4.3 Paper III

Increasing the proportion of NaOH-treated wheat in the diet did not affect DM intakes or ECM yields (P>0.05) but reduced (P<0.01) milk protein concentrations, MNE (P<0.05), milk urea nitrogen, and urinary nitrogen (P<0.01), as well as tending (P=0.10) to decrease milk protein yields. There were no differences between the oat/barley mixture and rolled wheat diets in terms of DMI, MY, or milk composition. Increasing the proportion of NaOH-treated wheat in the diet caused linear increases (P<0.05) in both faecal nitrogen and urinary volumes.

## 4.4 Paper IV

The true OM and *in vitro* NDF digestibility determined for the WF ranged from 0.804 to 1.011 and from 0.362 to 1.107, respectively. The *in vitro* NDF digestibility determined for the aNDF fraction ranged from 0.410 to 0.985. The effective kd values estimated using the GP data varied from 0.118 to 0.282/h for the WF and from 0.123 to 0.301/h for the NDS fraction. They were also less (P<0.05) for maize than for small grains (SG) but did not differ between barley and wheat (P>0.05). The effective kd values for the aNDF fraction ranged from 0.039 to 0.082/h and did not differ (P>0.05) either between maize and SG or between barley and wheat. The relationship between the observed starch digestibility and predicted ruminal NDS digestibility was described by the following equation: Observed ruminal starch digestibility = 0.85 × predicted ruminal NDS digestibility + 0.14. The predicted ruminal NDS digestibility determined using GP data closely matched the *in vivo* data on starch digestion ($R^2$=0.81). The effective kd values for the WF correlated strongly ($R^2$=0.94) with those for the NDS fractions.

# 5 Discussion

## 5.1 Regulation of feed intake

A summary of the average values of the response parameters as affected by the concentrate supplementation (Papers I-III) is presented in Table 1. The mean intake of NDF, when expressed as the percentage of LW was 1.12%, which is close to the value presented by Mertens (1987), indicating that intake may have been limited by physical factors. This observation is supported by the suggestion of Allen (2000) that intake in high producing dairy cows is regulated by physical means. In addition, the observation of increased DMI with increasing concentrate supplementation (Paper I) satisfies the theory that feed intake in dairy cows increases with decreasing fill effect of the diet (Mertens, 1994). However, the later observation does not appear to agree with the results presented by Huhtanen *et al.,* (2008a) who found curvilinear responses in DMI with increasing concentrate supplementation.

Although improving digestibility of the grass silage improved forage DMI (Paper II) which satisfies that decreasing the NDF will improve feed intake (Mertens, 1994), the intake of NDF was marginally reduced. Huhtanen *et al.* (2007) found quadratic responses in the intake of NDF with improving digestibility of grass silage. Further, rumen evacuation studies (Rinne *et al.*, 2002; Kuoppala *et al.*, 2009) indicate that rumen NDF pool size was reduced with highly digestible grass silage in high yielding dairy cows. This indicates that the cows were unable to use their full reticulo-rumen capacity, suggesting some metabolic factors controlling feed intake. These observations support the theory of interaction of multiple mechanisms involved in the regulation of feed intake (Fisher, 2002; Huhtanen *et al.*, 2007).

## 5.2 Feeding strategies to stimulate feed intake in dairy cows

In dairy cow diets, voluntary silage intake has regularly increased with increasing silage digestibility (Rinne *et al.*, 1999; Huhtanen *et al.*, 2007; Kuoppala *et al.*, 2008). Among the parameters that define the intake potential of the grass silages, Huhtanen *et al.* (2007) identified the D-value (which is influenced by the maturity of the grass used to make the silage) as the most important factor. Huhtanen *et al.* (2007) reported that the intake of digestible OM was strongly and linearly associated with silage D-value. The DMI and MY responses of the studied cows to improved forage digestibility in Paper II were similar to the results reported in these studies. A 100 g/kg difference in

Table 1. *A summary of the average values of the response parameters for diets with different concentrate supplementation as tested in Papers I through III (no. of treatment means = 12).*

| Parameters | | | | | Concentrate supplementation[a] | | |
|---|---|---|---|---|---|---|---|
| | Mean | SE | Minimum | Maximum | Level | Type | Processing |
| Days in milk | 143 | 2.2 | 133 | 150 | | | |
| *Intake (kg/day)* | | | | | | | |
| DM | 20.5 | 0.57 | 17.6 | 23.8 | + | ‖ | ‖ |
| NDF | 6.9 | 0.20 | 6.3 | 8.4 | ‖ | ‖ | ‖ |
| Starch | 4.5 | 0.26 | 3.3 | 6.7 | + | ‖ | ‖ |
| *Milk production (kg/day)* | | | | | | | |
| Milk | 25.6 | 0.27 | 24.2 | 27.3 | + | ‖ | ‖ |
| ECM | 28.3 | 0.34 | 26.2 | 30.2 | + | ‖ | ‖ |
| *Milk composition (g/kg)* | | | | | | | |
| Fat | 46.6 | 0.49 | 44.2 | 49.5 | − | ‖ | ‖ |
| Protein | 37.5 | 0.21 | 36.5 | 39.0 | + | ‖ | − |
| Lactose | 47.0 | 0.24 | 45.8 | 48.2 | ‖ | ‖ | ‖ |
| MUN | 4.68 | 0.246 | 3.83 | 5.81 | ‖ | ‖ | − |
| *Ratios* | | | | | | | |
| ECM/DM intake | 1.40 | 0.026 | 1.28 | 1.58 | − | ‖ | ‖ |
| CP milk/CP intake | 0.27 | 0.013 | 0.22 | 0.36 | − | ‖ | − |
| ME intake/ECM | 8.8 | 0.22 | 7.5 | 10.0 | − | ‖ | ‖ |
| NDF intake/LW | 1.12 | 0.036 | 1.02 | 1.37 | ‖ | ‖ | ‖ |

[a] Linear effects of the concentrate supplementation described in terms of concentrate level (Paper I), concentrate type (Paper II) and concentrate processing (Paper III). The signs +, − and ‖ denote positive, negative and null treatment effect.

DM, dry matter; NDF, neutral detergent fibre; ECM, energy corrected milk yield; MUN, milk urea nitrogen (m mol/l); CP, crude protein; ME, metabolisable energy; LW, live weight.

total OM digestibility of the two grass silages was related to a difference of 3 kg in DMI, 2.1 kg in MY and 2.3 kg in ECM.

The high digestibility grass silage diets were associated with increased yields of milk constituents (Rinne *et al.*, 1999; Kuoppala *et al.*, 2008) resulting from increased milk yields. The increase in milk protein concentration and yield observed with highly digestible silages was probably a reflection of the improved energy status and increased MP supply to the cows (Lykos *et al.*, 1997). Previous studies have demonstrated that feeding with highly digestible grass silage results in poor MNE (Rinne *et al.*, 1999; Kuoppala *et al.*, 2008). Our MNE data are consistent with these previous findings. The MNE was more closely related to the dietary concentration than the intake of CP (Huhtanen & Hristov, 2009). The low dietary CP concentration of the mature grass silage, which resulted in a reduced CP supply per unit DMI, may explain the improved MNE for the mature grass silage diets.

Among concentrate supplementation evaluated in the various feeding experiments (Papers I through III) presented in this thesis, only the level of concentrate supplementation appeared to influence DMI and MY in dairy cows (Table 1). The overall ratios of ME to ECM yield observed in this work were greater than those reported in previous experiments with dairy cows (Rinne *et al.*, 1999; Ferris *et al.*, 2001; Kuoppala *et al.*, 2004). Although changes in the LW of dairy cows were not monitored, this is still an indication of partitioning of marginal incremental ME towards the body tissues rather than the mammary glands. Kuoppala *et al.* (2004) indicated that the partitioning of marginal incremental ME between body tissues and mammary glands generally changes in favour of body tissues. The improper partitioning of energy towards body tissues was further enhanced by the late stage of lactation of the cows, since dairy cows tend to increase their body reserves as their number of DIM increases (Britt *et al.*, 2003).

### 5.2.1 Concentrate supplementation: effects on forage and total dry matter intake

*Cereal concentrate level*

Compared to the forages alone, introducing more concentrate in the diet increased the dietary starch concentration and the cow's starch intake but also increased the digestible portion of the diet, which resulted in greater kd as suggested by Stensig *et al.* (1998). The kp of the diet also rose with the amount of concentrate supplementation, which is similar to the observations of Robinson *et al.* (1987). The fast clearance of the rumen with highly digestible diets favors a greater DMI in dairy cows (Faverdin *et al.*, 1991).

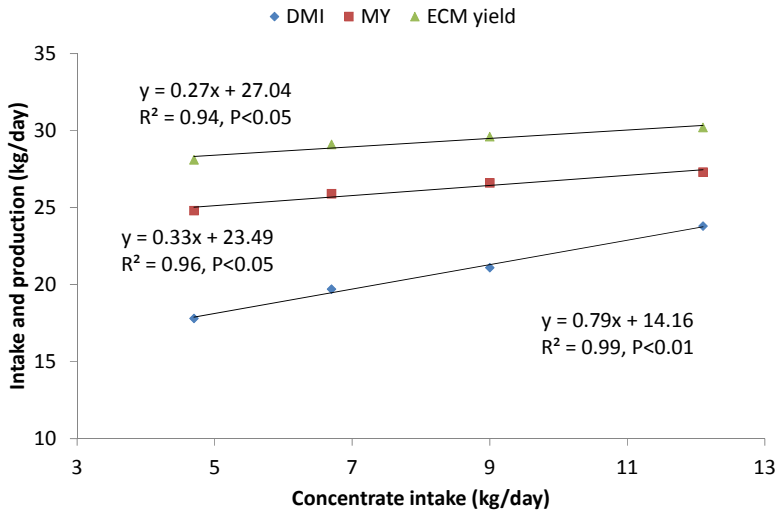


*Figure 3.* A graphical representation of the effect of increasing the proportion of concentrate in the diet on total dry matter intake (DMI), milk yield (MY) and energy corrected milk (ECM) yield as reported in Paper I.

The increasing amount of starchy concentrate supplementation can be interpreted as an increasing amount of starch since there was a strong correlation ($R^2$=0.98, P<0.01) between the starch concentration of the diet and starch intake. Figure 3 illustrates the effects of increasing concentrate intake on the total DMI and subsequent MY and ECM yield of the cows as reported in Paper I. The responses in DMI to increasing proportions of concentrate/starch in the diet are often described by a linear relationship (Ferris *et al.*, 2001). The linearity of this relationship is maintained up to a certain level of supplementation. However, at very high supplementation levels , there has been a drop in total DMI which might affect the response function (Laird *et al.*, 1981).

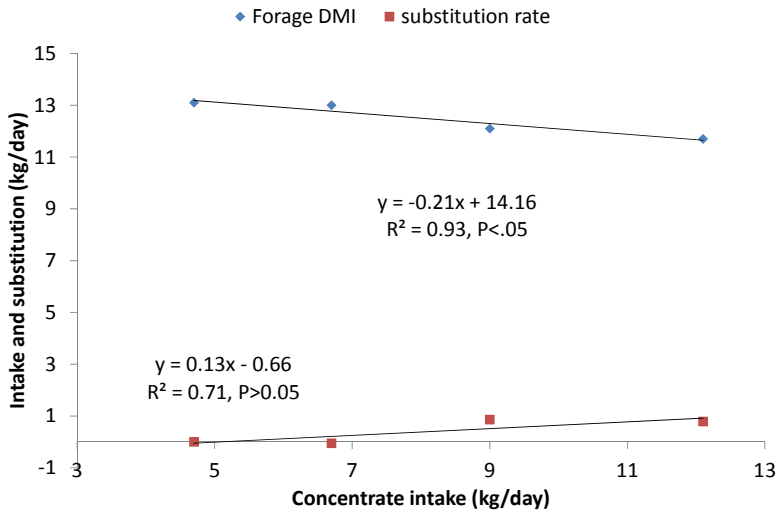


*Figure 4.* A graphical representation of the effect of increasing the proportion of concentrate in the diet on forage dry matter intake (DMI) and forage substitution rate as reported in Paper I.

High starch diets may reduce fibre digestion in the rumen (Stensig *et al.*, 1998). Nousiainen *et al.* (2009) found that increasing the proportion of concentrates in the diet reduces digestion due to a decrease in the utilisation of NDF. Similar observations were made by Stensig *et al.* (1998) who found that the kd of NDF decreased at high starch or sugar levels. Although kd values for ruminal digestion of NDF were not determined in this work, the intake and digestion data for grass silage clearly indicated the mechanism underpinning these observations (Paper I). The reduction ($P<0.05$) in forage intake as concentrate intake rises is clearly shown in Figure 4. The calculated mean SR was low but the SR value rose with the proportion of concentrate in the diet, which has also been found by Huhtanen *et al.* (2008a).

*Cereal Concentrate type*

Despite the clear differences in the calculated ME and MP concentrations of the rolled wheat compared to the oat/barley mixture (Paper III) and in the MP concentrations of the barley compared to the maize (Paper II), no significant differences in DMI or MY were observed. Our hypothesis that cereal concentrates based on maize would increase DMI compared to those based on barley in high digestibility grass-silage based diets did not prove to be true; this

finding is in line with that of Khalili *et al.* (2001) but not with those of others (McCarthy *et al.*, 1989; Overton *et al.*, 1995; Silveira *et al.*, 2007). There are two plausible reasons for the absence of a relationship between the concentrate starch type and the DMI of the dairy cows. First, the expected differences in the kd values of the cereal starches may not be that large as expected. This could be taken to imply that the two types of concentrate starch should have similar digestibilities (Paper II and III). This suggestion appears to contradict previous findings of greater kd values and ruminal digestibility for barley compared to maize (Nocek & Tamminga, 1991; Yang *et al.*, 1997; Mills *et al.*, 1999). While no *in vivo* data on ruminal digestibility for these studies are available, this speculation was supported by the *in vitro* data shown in Table 2. These observations suggest that the pattern of rumen fermentation may have not been affected by the kd of the starch from rapidly degrading cereals and thus would have had no effects on fibre digestion of grass silage as suggested by Stensig *et al.* (1998).

The comparable ruminal digestibility data for different starch types may suggest equal amount of estimated RES flowing to the cow's lower tracts. Many studies on dairy cows have indicated similar results of intakes and lactation performance for different cereal concentrate types (Yang *et al.*, 1997; Khalili *et al.*, 2001). To the author's knowledge, the studies in which maize-based concentrates increased DMI in dairy cows, forage maize constituted a substantial proportion of the forage (McCarthy *et al.*, 1989; Overton *et al.*, 1995). The inclusion of maize both as a concentrate and as part of the forage may have increased the amount of RES as indicated by McCarthy *et al.* (1989) and thus the efficiency of grain utilization as suggested by Dixon & Stockdale (1999). Second, the applied feeding system may have influenced the results. TMR feeding, unlike separate feeding of concentrates is known to reduce diurnal variation in rumen pH (Robinson, 1989), thus diluting the effects of differences in starch kd arising from different starch types. However, Khalili & Huhtanen (1991) found no difference in NDF digestibility (or enzyme activity) when sucrose was fed twice daily or continuously infused into the rumen.

Lastly, the processing of the starch may also have been partly responsible for the comparable animal performance results obtained with different starch types, since the processing of grains could have altered the nutritive characteristics of their starch (Mills *et al.*, 1999). The reasons presented herein are purely speculative since exact factors that govern concentrate availability in the rumen are unknown.

Based on the information provided by the *in vitro* digestion kinetics data, one may conclude that NaOH-treated wheat in the diet (Paper III) significantly increased the estimated amount of RES and partially shifted the site of starch digestion from the rumen to the small intestine and hindgut. This was confirmed by increased faecal nitrogen concentrations and faecal nitrogen output. However, the findings that all dietary treatments caused comparable DMI in dairy cows does not support the suggestion made by Dewhurst *et al.* (2001) and Reynolds (2006) that silage intake might increase if starch digestion were shifted from the rumen to the small intestine. The results obtained in this work also failed to confirm the suggestion of McNiven *et al.* (1995) that the less acidic rumen conditions induced by reduced starch digestion might increase the overall rate and efficiency of digestion. Instead, our findings are consistent with those of Phipps *et al.* (2001), who reported that feeding with NaOH-treated grains did not improve fibre digestion relative to that observed with cracked grains in cows fed maize-silage based diets.

The results of Paper III suggest that the potential benefits of using NaOH-treated wheat to increase intestinal starch digestion are at least partly lost due to increased starch fermentation in the hindgut. The hindgut fermentation of starch reduces the energy available to rumen microbes and increases the output of microbial matter in the faeces (Figure 2). This may be because of the limited capacity of the small intestine for starch digestion (Kreikemeier *et al.*, 1991) in lactating dairy cows consuming high concentrate diets. Ortega Cerrilla & Mendoza Martinez (2003) noted that the inefficiency of ruminants' small intestine at digesting large amounts of starch is at least partly due to the low levels and activity of the secreted pancreatic amylase and partly because of the low glucose absorption capacity of the small intestine. Further, they proposed that the presence of glucose or starch hydrolysates might de-regulate pancreatic amylase secretion, while an increased dietary intake of CP could enhance the production and secretion of pancreatic amylase and thus starch digestion.

The results of increased faecal nitrogen concentration, faecal nitrogen output and the observed reductions in urinary nitrogen concentrations indicate a shift in nitrogen excretion from the urine to the faeces. These responses are similar to those reported for abomasal infusions of pectin (Gressley & Armentano, 2005). These data suggest that the decreased urinary nitrogen concentrations observed with NaOH-treated wheat may decrease ammonia volatilization, but, at the same time, increased urinary volumes increase the costs of manure management.

### 5.2.2 Concentrate supplementation: effects on milk production and composition

The responses in MY are highly correlated with the amount of concentrate supplementation (Ferris *et al.*, 2001). The increases in MY caused by increasing the proportion of concentrate in the diet were relatively small compared to those observed for DMI (Figure 3). This may be partly due to the fact that the ME supply relative to cow's production potential was high even for diets with lower levels of starch, and partly because the cows were late in lactation, as has been suggested in other studies (Britt *et al.*, 2003). This second conclusion is also supported by a comparison with the results of Ferris *et al.* (2001), who observed stronger MY response following an increase in DMI for cows that were 26 DIM at the start of the experiment. The concentrate starch type (Paper II) did not significantly affect the MY, probably because there were no differences in DMI and thus probably no differences in ME intake when consuming the different concentrate starch types. In some cases, maize supplements have been found to increase MY relative to that observed with barley (McCarthy *et al.*, 1989; Overton *et al.*, 1995; Khalili *et al.*, 2001) but this effect is not always observed (Yang *et al.*, 1997). In most cases, MY increases have been related to increased feed intakes (McCarthy *et al.*, 1989; Overton *et al.*, 1995). However, Khalili *et al.* (2001) observed no difference in feed intake between barley and maize concentrates. Although the use of NaOH-treated wheat in Paper III increased the flow of nutrients into the small intestine and lower tract, no differences in MY were observed. This observation seems inconsistent with the suggestion of Boudon *et al.* (2007) that the glucose supply to the mammary glands is a strong limiting factor for milk synthesis.

The effects of increasing the concentrate proportions in the diets on milk fat concentration and yield were variable. In Paper I, increasing the proportion of starch in the diets clearly decreased the milk fat concentration but had no effect on milk fat yield. A reduction in forage concentration in the diets and a reduced forage to concentrate ratio might have changed the relationship between the production of acetate and propionate in the rumen. These factors may alter fat concentration in the milk (Lechartier & Peyraud, 2010). The quadratic effects of including NaOH-treated grain on fat composition and yield may suggest that a 1:1 combination of rolled wheat/NaOH-treated wheat was the best of the tested wheat treatments in terms of milk fat output (Paper III).

Increased milk protein for diets with high concentrate proportions (Paper I) suggests that increasing the microbial protein flow to the small intestine, together with a higher supply of ME, has a positive effect on milk protein concentration as suggested by Lykos *et al.* (1997). The reported effects of

increasing the concentrate proportions in the diets on milk protein concentration are very consistent throughout the published studies (Kuoppala *et al.*, 2004). The concentrate starch type did not appear to affect milk composition in Paper II despite the expected differences in the MP supply for the two grains; this finding is consistent with the results of other researchers (McCarthy *et al.*, 1989; Yang *et al.*, 1997; Silveira *et al.*, 2007). The increased MP supply has been suggested to increase the flow of amino acids to the small intestine and milk protein yield (McCarthy *et al.*, 1989). This suggestion was supported by the observations of Khalili *et al*. (2001). Milk protein concentrations were observed to decrease linearly and there was also a trend for decreased milk protein yield as the proportion of NaOH-treated grain in the diet was increased (Paper III); this may be a result of decreased microbial protein synthesis in the rumen due to reduced ruminal starch digestion. This finding agrees with the observation of McNiven *et al.* (1995) for a reduced duodenal non-ammonia nitrogen flow in cows fed on NaOH-treated barley compared to those fed dried rolled barley.

## 5.3 Quantification of ruminal starch digestibility

### 5.3.1 Alternative techniques

Although much effort has been devoted to finding a reliable, accurate and precise method for studying the digestion kinetics of starch and subsequent ED, every known method has both characteristic merits and draw-backs. The two techniques used in this work (i.e. *in situ* and *in vitro*) gave different ED estimates (Table 2). Previous studies using the *in situ* approach yielded kd values for maize ranging from 0.05 to 0.09/h and from 0.35 to 0.63/h for barley (Offner *et al.*, 2003; Tothi *et al.*, 2003). The kd values for maize and barley determined using the *in situ* method and presented in Table 2 were consistent with those reported from previous studies. The kd values for maize and SG obtained using the *in vitro* technique were in the middle of these ranges.

The *in situ* method suggested the presence of greater differences in the ruminal digestion kinetics of starch than the *in vitro* method; the *in situ* method tends to overestimate starch degradation rates (and consequently ED values) for rapidly-degraded feedstuffs, and underestimates the rate of starch degradation for feedstuffs that are degraded slowly as reported in previous studies (Offner & Sauvant, 2004). The equation for predicting the starch digestibility in the rumen from *in situ* ED data of Offner *et al.* (2003), developed by Offner & Sauvant (2004) does not agree well with those based on *in vivo* experiments and has a high slope bias. The poor accuracy of these estimates is probably due to particulate losses (Huhtanen & Sveinbjörnsson,

2006) and the low microbial activity inside the bags relative to the rumen (Noziere & Michalet-Doreau, 1996).

The *in vitro* GP technique showed some promising results and a strong correlation ($R^2$=0.81) (Paper IV) when compared to the *in vivo* starch digestibility data (Nocek & Tamminga, 1991; Larsen *et al.*, 2009). The GP technique has some advantages over the *in situ* nylon bag technique: 1) it does not assume that everything that disappears is degraded, 2) it can be applied to different feed fractions as well as the whole feeds (Schofield *et al.*, 1994; Schofield & Pell, 1995), and 3) fully automated systems generate and record a large number of data points, making it possible to use more sophisticated models when estimating kinetic parameters (Cone *et al.*, 1996).

Table 2. *Rate and extent of ruminal digestion of dry matter (DM) or organic matter (OM) for various feedstuffs determined by in situ and in vitro methods.*

| Item | | kd[a] (1/h) | | ED[b,c] | | References[d,e] |
|---|---|---|---|---|---|---|
| | | *In situ* | *In vitro* | *In situ* | *In vitro* | |
| Barley pelleted | | 0.536 | 0.134 | 0.890 | 0.728 | Paper II |
| Maize pelleted | | 0.059 | 0.116 | 0.705 | 0.699 | Paper II |
| Oat-barley mix | | --- | 0.161 | --- | 0.763 | Paper III |
| Wheat rolled | | --- | 0.161 | --- | 0.763 | Paper III |
| Wheat NaOH-treated | | --- | 0.112 | --- | 0.691 | Paper III |
| Maize | | --- | 0.133 | --- | 0.759 | Paper IV |
| Small grains | Barley | --- | 0.245 | --- | 0.875 | Paper IV |
| | Oats | --- | 0.210 | --- | 0.836 | Paper IV |
| | Wheat | --- | 0.244 | --- | 0.872 | Paper IV |

[a] Fractional degradation rates of DM (*in situ*) or OM (*in vitro*).
[b] ED=Effective ruminal degradability was calculated assuming the rumen as a single-compartment chamber and for a fractional rate of passage of 0.05/h in Paper II and III.
[c] ED was calculated assuming the rumen as a two-compartment chamber. The rumen retention time was divided (0.20: 0.80) into a rate of release and rate of passage, respectively in Paper IV.
[d] A single-pool kinetic model was fitted to the *in situ* data (Paper II) and a two-pool Gompertz model to the *in vitro* data (Paper II and III).
[e] A three-pool Gompertz model was fitted to the *in vitro* data and the resulting predicted kinetic parameters were used in a modeling approach (Paper IV).

When the *in vitro* fractional starch kd and kp values are known, it is possible to predict *in vitro* ED values using the equation: *in vitro* ED = kd/(kd + kp). Lanzas *et al.* (2007) reported starch kd values for maize, barley and wheat based on their GP system that were very similar to our results presented in Paper IV. However, their predicted ED values calculated using a kp of 0.06/h, were lower than the previously reported *in vivo* starch digestibility (Nocek & Tamminga, 1991; Larsen *et al.*, 2009). The observations of GP profiles for the

grain NDS fraction (Paper IV) indicated that the digestion kinetics of grains cannot be described using a first order kinetic model. Because the passage of grain particles does not appear to follow first order kinetics as found by Tothi *et al.* (2003), we therefore used a two compartment rumen model to describe the selective retention of feed particles (Huhtanen *et al.*, 2006).

The starch kd values determined using the GP technique are confounded by the degradation of microbial cells when the fermentable substrate is depleted. This can substantially reduce the estimated starch kd (Tahir *et al.*, 2011) but can be corrected for by fitting multiple-pool models such as the Gompertz model described by Schofield and co-workers (1994) to the gas data and excluding the pool(s) with the least amount of GP, the slowest GP rates and long lag periods (GP from microbial cell degradation and slow degradable fibre) as was done in Paper IV or as described by Stevnebø *et al.* (2009). Another advantage of using multiple-pool models is that it makes it possible to determine digestion rates of the NDS fraction by incubating grain (or other high starch) samples without NDF extraction in the GP system.

# 6 Conclusions

The studies that this thesis is based upon partially support the idea that factors controlling feed intake function in an interactive manner. The results demonstrate that the feed intake and animal performance in terms of milk yield were mainly affected by the level of concentrate and the improved digestibility of the grass silage in the diets. However, the small marginal responses in milk yield and reduced feed utilization do not justify the increased use of grains in diets containing high digestibility grass silage, which may have negative economic effects on farmers. The concentrate starch type did not affect dairy cow performance, suggesting that true differences between concentrate starch types in terms of rumen availability might be smaller than was previously reported in the literature. No interaction between silage grass maturity and concentrate starch type was identified. It is concluded that NaOH-treatment of grain makes it possible to increase the amount of rumen escapable starch and partially shift the site of starch digestion from the rumen to the small intestine and hindgut. However, this does not significantly affect the cows' production, suggesting that the site of starch digestion is not of the utmost importance. Moreover, the negative effects of the NaOH-treatment on milk composition and the increased loss of microbial matter in the faeces indicate a less efficient utilisation of available energy. The predicted ruminal effective degradability of starch differed depending on the method of analysis used and how the results were interpreted. The results presented indicate that, when measured using the *in vitro* gas production technique, the differences between the digestion kinetics of starches of different origins are less pronounced than those obtained using the *in situ* technique, which is consistent with the results of the feeding trials. Moreover, the predicted ruminal effective degradability of starch obtained using gas production data and modeling approach were in good agreement with the *in vivo* starch digestibility data.

## 6.1 Implications

Cows prefer eating concentrates in their diets and this causes corresponding increases in milk yields. However, due to the reductions in returns per kg feed offered and the environmental issues involved in the transportation and utilization of such feeds, high level feeding of concentrates is not recommended. The results presented in this thesis imply that making silage from grasses harvested at an early stage of growth improves feed intake and milk production in dairy cows, lower DM yields and increased nitrogen losses are associated with such a type of grasses. NaOH treatment of cereals is not recommended because it increases the cost of processing and manure management without having any positive influence on the performance of dairy cows. Lastly, since the cereals grains used in the studies presented in this thesis had comparable feeding values, any one of them could be used to supplement a dairy diet with equal effect, depending on their availability and market prices.

# 7 Future perspectives

The feed intake and production data reported in feeding experiments presented in this thesis create the opportunities for farmers and stakeholders to update their knowledge concerning the performance of dairy cows when fed on high digestibility grass silage diets. Further, these data and the data on laboratory studies as well as on chemical analysis encourage researchers and scientists to incorporate them into their feed evaluation systems. The feed intake data presented may be useful for understanding how regulatory mechanisms function in dairy cows consuming high digestibility grass silage diets. However, these data partially indicate the presence of multiple feedback mechanisms and their interactions. This implies that data on control mechanisms related to animal behaviour in combination with feed intake data may improve the understanding of feed intake regulation in dairy cows.

Despite differences in nutritive values reported by alternative feed evaluation methods presented in this thesis and the literature, different grains had comparable feeding values for dairy cows. These findings leave questions for future research on the factors which govern rumen fermentation in order to better understand the differences between different grains in terms of their availability in the rumen and post-ruminally. It would also be interesting to investigate the capacity of ruminant small intestine to digest and utilize large amounts of starch.

The efforts to find out a reliable method for describing digestion kinetics of grains/starch will continue in the future. The *in vitro* GP technique showed promising results regarding ruminal digestion kinetics of grains when compared to the *in vivo* starch digestibility data. This technique can further be utilized to determine the rate of digestion of a large range of grains and other feeds in order to generate parameter values that can be used to improve the present feed evaluation systems.

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