An overview of silage research in Finland: from ensiling innovation to advances in dairy cow feeding

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Because of the climatic conditions, the Finnish milk production research has focused to improve the utilisation of grassland, mainly as conserved forages. The main research areas have been ensiling, evaluation of the forage feeding value, predicting nutrient supply from grass silage-based diet and the effects of forage quality and concentrate supplementation on milk production responses. Due to changes in ensiling technologies and variety of forage crops new silage additives have been adopted. A centralized system for the analysis of forage energy value is based on NIRS calibration. It was calibrated against in vitro pepsin-cellulase solubility method that was validated against in vivo digestibility. The concentration of indigestible neutral detergent fibre was found to be a useful parameter both in empirical models predicting forage digestibility and mechanistic rumen models predicting the amounts of absorbed nutrients. Models predicting relative intake potential of forages and total diet were developed, and an intake model combining animal and diet effects independently of each other was developed. Using meta-analysis approaches a nutrient response model was developed for dairy cows for milk, energy corrected milk and protein yield. Feed evaluation, intake and nutrient response models form now the basis of practical Finnish ration formulation system that can optimize diets according to maximum income over feed cost in addition to minimum feed cost.

Key words: grass silage, ensiling, feed evaluation, nutrient intake, milk production

Introduction

Milk production systems in different climatic zones have developed to utilize local feed resources. Due to the short grazing period (100–120 days) in Finland grazed grass cannot contribute more than 20–25% of total feed energy intake for dairy cows. This has increased the importance of conserved forages in dairy cow rations. Relative competitiveness of grass in Finland is high, since in the main milk production regions grass dry matter (DM) yields are more than two-fold compared with cereal grains (Kangas et al. 2010). Grasses can utilize efficiently the long days in early summer, and daily DM growths exceeding 200 kg are common (e.g. Kuoppala et al. 2008). The nutritive value of forages in terms of digestibility is high due to the relatively cool climate and long day length which delay the lignification of cell walls (Van Soest et al. 1978, Deinum et. al. 1981). Earlier high concentrate costs and a shortage of protein supplements favoured forage-based feeding systems, but since Finland joined the EU in 1995 subsidised grain and protein prices have reduced the competitiveness of grassland production.

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Ensiling

The control of major preservative factors of silage (e.g. pH, water activity, epiphytic flora), and their interactions, is the basis for biologically and economically efficient silage production. Virtanen (1933) was first to show systematically the importance of low pH and inhibition of plant and microbial enzymes in silage preservation. By using hydrochloric and sulphuric acids he introduced the A.I.V.-method and established the principle of rapid achievement of pH 4 to suppress respiration of plant cells, to prevent degradation of proteins and vitamins and to avoid clostridial fermentation. He also showed that different crops, e.g. leguminous plants vs. grasses, require different amounts of acids to achieve target pH.

Ensilability of silage crops

Ever since the innovation of A.I. Virtanen, the control of silage fermentation by silage additives has been the core of ensiling in Finland. In the late 1960's, combinations of inorganic acids and organic acids, mainly formic acid (FA), and additives containing formaldehyde were in the focus of research (Ettala et al. 1975). The corrosive nature of inorganic acids and other hazardous effects of formaldehyde were reasons to abandon these products later.

The research done in Norway (Saue and Breirem 1969) demonstrated the effectiveness of FA which became the most commonly used silage additive also in Finland. Direct acidification using a relatively high application rate of FA (approximately 4 L t^{-1}, expressed as 100% w/w) has facilitated that relatively wet and low sugar crops, predominantly timothy, meadow fescue and some legumes, can be ensiled successfully. The climatic conditions in Finland exclude the more easily ensiled crops like perennial ryegrass and fodder maize. This highlights the importance of adjusting harvesting and ensiling management according to crop characteristics and local conditions (Lampila et al. 1988). The most important ensilability factors of crops were clearly presented by Weissbach et al. (1974) in an equation predicting anaerobic stability and clostridial development from crop dry matter (DM), buffering capacity (BC) and water soluble carbohydrates (WSC).

Increasing size of Finnish farms and demand for high labour efficiency in the ensiling systems have been the major reasons for the technological development, like pre-wilting and harvesting techniques related to it. Although some of the techniques, e.g. chopping with harvesters and additive applicators, have had some important positive effects on silage quality the biological efficiency has not necessarily increased. Gordon (1989) concluded in Northern Ireland that a harvesting system based on wilting decreased the output of animal product per hectare by 13% as compared to a direct-cut system. The increasing popularity of wilting, a concomitant decrease of application rate of FA and a shift to using biological additives have all changed the challenges of ensiling. Effluent losses and the risk of clostridial fermentation decreases with increasing DM content but at the same time wilting may increase nutrient losses during drying, impair the microbiological quality of crop and expose the silage to aerobic deterioration.

Wilting grass to DM content of 300 g kg^{-1} did not alone prevent clostridia (Ettala et al. 1982) but in favourable harvesting conditions it supports achievement of good fermentation quality and feeding value without additives (Heikkilä et al. 2010). However, an ensiling system based on baling of high DM grass without additive is more susceptible to unfavourable harvesting conditions and to lower feeding value of silage as compared to ensiling in bunker silo with lower DM content and FA-based additive (Jaakkola et al. 2008). In spite of the low butyric acid and ammonia N content of untreated bale silage having relatively high DM content (380 g DM kg^{-1}), the use of inoculants or FA improved milk production and sensory quality of milk (Heikkilä et al. 1997). This demonstrates that fermentation parameters of high DM silage insufficiently describe the value of silage in animal production. The unpredictability of weather conditions and variation in crop DM and WSC concentration and epiphytic flora are important factors to be considered in the risk management of ensiling and when making decision on the use of additives. Currently 50–60% of the Finnish farm samples analysed in the laboratory of Valio Ltd are from silages treated with acid based additives, 25–30% from silages treated with biological additives and 10–15% from untreated silages (J. Nousiainen, personal communication).

A risk of undesirable fermentation is higher when forage and grain legumes with high BC are ensiled as compared to grass species. Slight wilting of lucerne, galega, red clover and lotus to 250 g DM kg^{-1} alone was not sufficient to avoid poor fermentation in research made in Germany, Sweden and Finland (Pahlow et al. 2002). Wilting to 400 g DM kg^{-1} prevented the production of butyric acid, but silage quality was further improved by the use of additives. The challenging ensiling characteristics of forage legumes are alleviated in a mixture with grass species having lower BC. Similarly, when whole-crop field beans and field peas were ensiled without an additive, inclusion of 0.25 to 0.50 of wheat ensured a good fermentation (Pursiainen and Tuori 2008). However, common vetch with a high BC and a low WSC concentration was best ensiled using FA to prevent extensive protein degradation.

Preservation of small grain cereal crops has been successful in our conditions when harvested at the dough stage (300–400 g DM kg^{-1}) and when ensiling is based on a low pH generated by fermentation and/or acid based additives (Vanhatalo et al. 1999b, Jaakkola et al. 2009). Ensiling of cereal crops either untreated or treated with urea resulted in clostridial fermentation (Alaspää 1986). The low DM content of whole crop cereals even at a late maturity in our conditions does not support alkaline preservation. Extensive research in 1970's in Finland demonstrated that ensiling of high moisture grain is an efficient storage method as an alternative to grain drying. Early harvest, crimping and treatment with an additive diminishes the challenges of short growing season, increases the grain yield and reduces the use of fossil fuels. In the later studies the use of dry barley and ensiled barley resulted in the same animal performance in growing cattle (Huhtanen 1984) and dairy cows (Jaakkola et al. 2005).

Restriction of fermentation

The variation in crop characteristics and application rate of FA, and probably the variation in the evenness of FA application, in different experiments explains the inconsistent results obtained in the fermentation quality of FA-treated silage and consequently in animal responses (Harrison et al. 2003, Kung et al. 2003). A high application rate of FA restricts fermentation resulting in lower content of total acids (TA; lactic acid plus volatile fatty acids [VFA]) and ammonia N, and higher content of residual WSC in silage as compared with extensively fermented untreated or inoculated silage (Chamberlain et al. 1992, Heikkilä et al. 1998, Shingfield et al. 2002a). With lower FA application rates the differences in fermentation profiles are smaller. The low ammonia N content in silage reveals that FA treatment inhibits the conversion of herbage protein to non-protein-nitrogen (NPN) and increases the proportion of peptide N in silage NPN as compared with untreated silage (Nagel and Broderick 1992, Nsereko and Rooke 1999). The extent of silage fermentation thus dictates the amount and type of nutrients available for animals. Consequently, the nutritive value of restrictively fermented silages can be equal to that of respective barn dried forages (Jaakkola and Huhtanen 1993).

The effects of increasing level of FA on silage fermentation pattern have been linear (Jaakkola et al. 2006a) or curvilinear (Chamberlain and Quig 1987, Jaakkola et al. 2006b). This indicates that the balance and survival of desirable and undesirable microorganisms may differ with the characteristics of ensiled material and the additive. Due to the corrosive nature and handling problems of pure FA the commercial additives generally contain salts of FA like ammonium and sodium formate. Ammonium tetraformate maintains good silage quality if applied according to the molar concentration of acid (Randby 2000). Replacing FA (5.1 kg t^{-1}) with increasing proportion of ammonium formate up to 45% delayed the drop of pH in unwilted (210 g DM kg^{-1}) and wilted (406 g DM kg^{-1}) grass silage while the quality of silage was not compromised (Saarisalo and Jaakkola 2005).

Even a low application rate of FA disrupts cell membranes and releases soluble cell contents (Kennedy 1990, Jaakkola et al. 2006a). As a result, in wet material increased effluent losses partly offset the advantages of reduced fermentation losses. As a positive effect, cell wall degradation leads to efficient consolidation and may increase storage density as compared with untreated silage. This could partly explain why the use of a high rate of FA may result in good aerobic stability and low yeast count despite restricted fermentation and high residual WSC content in silage (Saarisalo et al. 2006) which often have been considered risk factors for aerobic stability.

Formic acid has a selective bactericidal effect but it is not specifically effective against yeasts (McDonald et al. 1991). More antifungal alternatives applied in a combination with FA have sometimes improved (Heikkilä et al. 2010) but sometimes not (Lorenzo and O'Kiely 2008) the aerobic stability as compared to untreated silage. The increased risk of aerobic deterioration concerns mainly wilted FA silages since low-DM or minimum wilted FA-treated grass silages have been shown to be more stable than untreated and inoculated silages (Pessi and Nousiainen 1999). As underlined already in the studies of Ettala et al. (1982) the feeding rate and good silo management are the key issues in preventing aerobic deterioration. However, even a small amount of oxygen may start the growth of yeasts and moulds responsible for aerobic deterioration. The use of combinations of hexamethylene-tetraamine, sodium nitrite, sodium benzoate and sodium propionate has improved the quality and storage stability of silage made from wilted grass (Lingvall and Lättemäe 1999, Knicky and Spörndly 2009).

Stimulation of fermentation

The interest on enzymes and inoculants as silage additives increased in Finland in the late 1970's (Vaisto et al. 1978, Poutiainen and Ojala 1982). Compared to early products, the improvements in inoculants and better understanding of the conditions in which inoculants are effective have generally improved the results (Kung et al. 2003). Inoculants alone are unable to produce enough lactic acid to lower the pH to an acceptable level if the WSC

content of the original crop is a limiting factor (Seale et al. 1986). A content of 25–30 g kg^{-1} in fresh material has been suggested to ensure sufficient production of fermentation acids in untreated silage (Wilkinson et al. 1983, Pettersson 1988). Accordingly, a high WSC content of grass (32 g kg^{-1}) resulted in minor differences in the fermentation of untreated silage and silages treated with inoculants or enzymes (Rauramaa et al. 1987). The amount of fermentable substrate can be increased by using efficient enzymes as an additive or the ensilability can be increased by wilting which increases the content of WSC on a fresh basis. The decreased rate of N fertilization has also enhanced ensilability by increasing WSC content of grass. However, the concomitant lower nitrate content may have an opposite effect since nitrite and nitric oxide, the reduction products of nitrate, effectively inhibit clostridia (Spoelstra 1985, McDonald et al. 1991).

Another purpose of using cell-wall degrading enzymes as an additive was to increase the rate and/or extent of digestion of cell wall carbohydrates in the rumen. The degradation of fibre in the silo was shown to increase with increasing cellulase level (Vaisto et al. 1978, Huhtanen et al. 1985). However, enzyme treatment had no consistent effect on organic matter digestibility but it decreased fibre digestibility in cattle (Jaakkola and Huhtanen 1990, Jaakkola et al. 1990) and in sheep (Jaakkola 1990; Table 1). Enzymes clearly affected the most easily degradable fraction of fibre which is also completely degraded in the rumen. On the other hand, with a successful combination of cell-wall degrading enzymes even a high-moisture (172 g kg^{-1}) and low-WSC (16 g kg^{-1}) grass was well preserved (Jaakkola et al. 1991). Generally the ensiling results with enzymes have been inconsistent. Kung et al. (2003) suggested that e.g. the lack of synergistic activities of enzyme complexes or environmental factors (pH, temperature) may be the potential reasons for failures in improving silage fermentation with enzymes.

Table 1. The effect of enzyme treatment on silage NDF or crude fibre concentration and digestibility, and on organic matter digestibility.

Reference	Animal	Silage treatment [1]	NDF or crude fibre, g kg^{-1} DM [2]	NDF or crude fibre digestibility [2]	Organic matter digestibility
Jaakkola (1990)	Sheep	Untreated	666	0.707	0.678
		Formic acid	619	0.695	0.683
		Cellulase 200 ml t^{-1}	609	0.672	0.677
		Cellulase 400 ml t^{-1}	596	0.666	0.675
		Cellulase 800 ml t^{-1}	579	0.619	0.644
Jaakkola and Huhtanen (1990)	Cattle	Formic acid	301	0.655	0.739
		Cellulase 400 ml t^{-1}	272	0.583	0.724
Jaakkola et al. (1990)	Cattle	Formic acid	303	0.600	0.701
		Cellulase 150 ml t^{-1}	284	0.540	0.688

[1] Formic acid application rate 4 L t^{-1} (as 100%), enzyme treatments: glucose oxidase 50 000 IU t^{-1}+ cellulase produced by *Trichoderma reesei*, activity 25 000 nanokatal HEC (hydroxyethyl cellulose) ml^{-1}.
[2] NDF (Jaakkola 1990), crude fibre (Jaakkola and Huhtanen 1990, Jaakkola et al. 1990)

Selection of effective bacteria strains for the use as inoculants is crucial for successful ensiling. A screening method using grass extract proved to be useful in strain selection (Saarisalo et al. 2007). *Lactobacillus plantarum* strain (VTT E-78076) having a broad-spectrum antimicrobial activity against gram positive and gram negative bacteria, and Fusarium moulds, was originally isolated from beer (Niku-Paavola et al. 1999, Laitila et al. 2002) but was shown to be also efficient in producing lactic acid, lowering pH rapidly and especially decreasing the ammonia-N production in grass silage (Saarisalo et al. 2006, Saarisalo et al. 2007). However, the antimicrobial properties were not efficient enough to improve aerobic stability (Saarisalo et al. 2006).

One possibility to overcome the inability of lactic acid to prevent yeast and mould growth is to use chemical additives in combination with the inoculants (Weissbach et al. 1991). Skyttä et al. (2002) showed that a combination of a selected inoculant, potassium sorbate and sodium benzoate inhibited *in vitro* the growth of four spoilage yeast strains isolated from grass silage. In two ensiling trials the combination of lactic acid bacteria and sodium benzoate (0.3 g kg^{-1}) had variable effects on the aerobic stability of wilted grass silage showing that the minimum effective application rate of sodium benzoate varies (Saarisalo et al. 2006). As shown in the meta-analysis of Kleinschmit and Kung (2006) improved aerobic stability has been observed in different types of forages when acetic and propionic acid production in silage fermentation is increased with *L. buchneri* inoculation. In our experiment, buffered propionic acid and a combination of *L. plantarum* and sodium benzoate were more efficient than a combination of *L. plantarum* and *L. buchneri* to prevent heating of high DM silage (Jaakkola et al. 2010).

Feed evaluation

Silage fermentation quality

Practical on-farm silages show wide variation in the fermentation quality due to e.g. crop characteristics, additives used and ensiling technologies. In-silo fermentation can influence the profile of absorbed nutrients and especially intake potential compared with fresh herbage (Huhtanen et al. 2007). Silage quality assessment with traditional wet chemistry for on-farm feeds is too expensive. For the analysis of farm samples Moisio and Heikonen (1989) developed a rapid electrometric titration method (ET). From the titration curve the concentrations of lactic acid, VFA, WSC, amino acid carboxyl groups and the protein degradation products (ammonia, amines) can be predicted (Moisio and Heikonen 1989). Later work revealed that ET over-predicted WSC, especially for extensively fermented or very dry samples. The system has been used for on-farm silage assessment for more than 20 years, with the exception that WSC are currently determined with the NIRS from dried samples. A comparable ET system has been also studied in UK (Porter et al. 1995) as an alternative or an additional silage measurement to either wet or dry NIRS (Park et al. 1998). However, direct comparisons between dry or wet NIRS and ET have shown that ET can be more accurate especially for VFA and ammonia-N (M. Hellämäki, personal communication).

Silage composition with reference to nutrient availability

The first step in a successful feed chemistry system is to divide forage DM into (1) cell contents that can be digested by mammalian enzymes and (2) a cell wall fraction that can only be digested by anaerobic microbial fermentation. The proximate feed analysis (Weende system) has been available for over 100 years, and it divides feed OM into crude protein (CP; 6.25 × N), crude fat (EE), crude fibre (CF) and nitrogen free extracts (NFE). Within the system, CF should represent the least available and NFE readily available feed components with a high true digestibility. The primary problems associated with NFE and CF fractions (Van Soest 1994, Huhtanen et al. 2006b) were realised by Paloheimo (1953), who initiated research to develop improved analytical methods for plant cell wall. In the pioneering work, Paloheimo and co-workers (Paloheimo and Paloheimo 1949, Paloheimo and Vainio 1965) used a weak hydrochloric acid and a two-stage ethanol extraction to remove cellular contents to describe vegetable fibre. Despite the correct criticism against fractionating feed carbohydrates into CF and NFE, these methods were too laborious, not applicable to faecal samples and the fibre residue was contaminated with protein. Based on these ideas, Van Soest (Van Soest 1967, Van Soest and Wine 1967) introduced the neutral detergent (ND) fractionation, which mainly resolved these drawbacks. The evaluation based on a wide dataset of silages (Huhtanen et al. 2006b) clearly demonstrated the biological weaknesses of the proximate feed analysis.

Neutral detergent (ND) fractionation (Van Soest 1967) divides forage DM into neutral detergent fibre (NDF) and neutral detergent solubles (NDS). Originally NDS was calculated as DM – NDF, but because ash does not provide energy, expressing NDS as organic matter (OM – NDF) may be preferable. True digestibility of the NDS fraction is close to unity (Van Soest 1994, Weisbjerg et al. 2004) when estimated by the Lucas test. The Lucas test allows estimation of ideal nutritional entities that have a uniform digestibility across a wide range of feedstuffs by plotting the digestible nutrient concentration in DM against the nutrient concentration in DM. The slope of regression provides an estimate of the true digestibility and the intercept is an estimate of the metabolic and endogenous faecal matter (M). Huhtanen et al. (2006b) reported a value of 0.963 for true NDS digestibility for different forages. Regrowth silages had a lower true NDS digestibility (0.925), the reasons for which are not known. Based on the Lucas principles the concentration of digestible OM (DOM; g kg^{-1} DM) can be expressed as:

$$DOM\ (g\ kg^{-1}\ DM) = NDS + dNDF - M \quad [1]$$

Given that digestible NDF (dNDF) = NDF × NDF digestibility coefficient (NDFD), NDS = OM - NDF, M = 100 and digestibility of NDS = 1.00, the equation [1] can be written as:

$$DOM\ (g\ kg^{-1}\ DM) = 1.00 \times (OM - NDF) + NDF \times NDFD - 100 \quad [2]$$

The equation [2] indicates that variation in DOM and OMD (OM digestibility) of forages is primarily a function of the concentration and digestibility of NDF, implying that the main emphasis in the evaluation of forage feeding value should be focused to the NDF.

A fraction of NDF in forages is completely indigestible even if it is subjected to digestion for an infinite time. This fraction can be defined as indigestible NDF (iNDF), and it can be determined e.g. by extended incubations *in situ* (Huhtanen et al. 1994) or *in vitro* (Van Soest et al. 2005). We have used a 12-d *in situ* incubation using bags with a small pore size (6 – 17 μm) to avoid particle losses. Potentially digestible NDF (pdNDF) is then calculated as:

$$\text{pdNDF (g kg}^{-1}\text{ DM)} = \text{NDF - iNDF} \quad [3]$$

Since iNDF is by definition a uniform nutritional entity with constant zero digestibility, equation [2] can be rewritten as:

$$\text{DOM (g kg}^{-1}\text{ DM)} = (\text{OM - NDF}) + \text{pdNDF} \times \text{pdNDFD - 100} \quad [4]$$

where pdNDFD is pdNDF digestibility. This equation indicates that variation in DOM is a function of iNDF concentration and pdNDFD. The smaller coefficient of variation (4.1 vs. 11.4%) and range (0.79–0.94 vs. 0.48–0.87) in pdNDF digestibility compared with total NDF digestibility for the wide range of silages (Huhtanen et al. 2006b) indicates that pdNDF is a more ideal nutritional entity than total NDF. Digestibility of pdNDF was on average 0.85 with a mean faecal pdNDF output of 60 (sd 23; range 13–105) g kg^{-1} DM intake (Huhtanen et al. 2006b). Faecal pdNDF can be defined as updNDF (= faecal NDF - iNDF) that represents the loss of potentially digestible OM in addition to obligatory losses of M.

Prediction of silage digestibility

Digestibility measured in sheep fed at maintenance still forms the basis of many feed evaluation systems. However, this method is not applicable for on-farm silages, and even not often for research samples. Hence, much research has been conducted to develop OMD prediction systems that are suitable for extension purposes i.e. that are rapid, accurate, precise and inexpensive. For this purpose, empirical models based on silage composition, *in vitro* methods using either rumen fluid or commercial fibrolytic enzymes and several *in situ* incubation procedures have been studied. In Finland, a database (n = 86) including grass and legume silages harvested at different maturity with detailed chemical analysis and *in vivo* digestibility in sheep has been collected (see Huhtanen et al. 2006b) to standardize *in vitro* or *in situ* OMD prediction models. In carefully conducted *in vivo* trials measurements of OMD are associated with a SD of 0.02 units (Van Soest 1994). For studies conducted according to Latin square designs the residual SD (RSD) was 0.014 units (Nousiainen 2004); i.e. determination of forage *in vivo* OMD in 4 × 4 Latin squares would be associated with a minimum inherent error of 0.007 units. However, the development of any prediction model for silage OMD should take in account inter- and intra-laboratory variation in both *in vivo* and *in vitro* OMD measurements and laboratory analyses. To tackle this problem in Finland, we adopted a strategy that *in vivo* and *in vitro* determinations as well as laboratory analyses and NIRS calibration are conducted only in one or two forage laboratories with standardized methods. Supporting this strategy, Hall and Mertens (2012) reported relatively high 95% probability limits for within-lab repeatability and between-lab reproducibility (0.102 and 0.134, respectively) for *in vitro* forage NDFD as determined according to the method by Goering and Van Soest (1970).

Many attempts have been made in developing regression equations that relate various chemical components to forage OMD, but without success owing to large interspecies and environmental variation (Van Soest 1994). In the Finnish silage dataset statistically significant relationships between chemical components and OMD were identified, but the prediction error using CP, NDF and ADF as independent variables was not markedly lower than the SD for *in vivo* OMD (Huhtanen et al. 2006b). Lignin was the best single predictor of OMD, but this entity could only account for proportionately 0.43 of observed variation, whilst the prediction error (0.042) is too high for practical feed evaluation. Van Soest et al. (2005) suggested a universal and constant relationship between lignin and iNDF over several types of forages (iNDF = 2.4 × Lignin). However, evidence from the Finnish forage dataset does not support this, suggesting that biological methods are required for predicting forage iNDF and OMD (Huhtanen et al. 2006b).

Several *in vitro* laboratory methods have been used for estimating forage OMD. The two-stage rumen fluid *in vitro* technique by Tilley and Terry (1963) and Goering and Van Soest (1970) are the most widely used methods. Tilley and Terry (1963) demonstrated a close correlation between DMD determined *in vivo* and *in vitro* and reported that the values determined *in vitro* were almost the same as those determined in sheep. However, even with a good lab practice it is important to calibrate any *in vitro* method using *in vivo* data to derive reliable prediction equations (Weiss 1994, Nousiainen 2004).

Due to several practical difficulties in conducting the rumen fluid *in vitro* method enzymatic *in vitro* procedures for the determination of forage digestibility have been studied (Jones and Theodorou 2000, Nousiainen et al. 2003a and 2003b). In principle, the methods include removing cell solubles with HCl-pepsin or ND followed by incubation in a buffered enzyme solution. This procedure results OM solubility (OMS) that differs from *in vivo* OMD in at least two key respects; no metabolic and endogenous matter is produced and the capacity of commercial enzymes to degrade NDF is substantially less than that of rumen microbes (McQueen and Van Soest 1975, Nousiainen 2004a). In predicting *in vivo* OMD from OMS the coefficient of determination (R^2) was 0.804 and RSD 0.025 digestibility units (n = 86, Huhtanen et al. 2006b). Because the relationship was highly dependent on forage type, using a forage specific correction equation increased R^2 to 0.925 and decreased RSD to 0.015. With a mixed model regression analysis, RSD was further decreased to 0.010 units, indicating that OMS predicted OMD within a study very accurately. The reduction in RSD can be attributed to differences between sheep used in digestibility trials and/or the contribution of between-year variation in the relationship between OMS and OMD. Using the general OMS correction underestimated the OMD of primary growth grass silages but overestimated OMD in regrowth grass and whole-crop cereal silages (Huhtanen et al. 2006b). The OMS method was also successfully used in predicting OMD for herbage samples taken before ensiling, provided that silages are well-preserved (Huhtanen et al. 2005). Owing to the problems in standardizing OMS method in different laboratories (Nousiainen 2004a), it is recommended that each laboratory should develop their own forage specific correction equations. In conclusion, the OMS method provides a reliable basis for OMD prediction, but caution should be directed to forage specificity. A recent comparison (Jančík et al. 2011) of different laboratory methods in predicting OMD revealed that OMS gave substantially higher OMD estimates than empirical iNDF equation or mechanistic model using gas *in vitro* production kinetics, especially for *Lolium perenne*. This suggests that specific OMS correction equations may be needed even for different grass species.

Equation [4] suggests that iNDF should correlate closely to forage OMD. Indeed, the evaluation of the Finnish dataset showed that iNDF correlated with in vivo OMD for silages made from 1st cut and regrowth grass (Nousiainen et al. 2003b), and over a wider range of silage types (Huhtanen et al. 2006b). The relationship between iNDF and *in vivo* OMD was more uniform compared with OMD equation based on OMS. Mean square prediction error of OMD was 0.010 for a mixed regression model (within study) and 0.019 for a fixed regression model. A reliable prediction of OMD can be attributed to a more consistent digestibility of pdNDF compared with total NDF and the inverse relationship between iNDF content and the rate of pdNDF digestion. However, iNDF seems to underestimate the digestibility of legume silages, particularly lucerne, probably because of their higher rate of pdNDF digestion relative to iNDF concentration (Rinne et al. 2006). Precision of OMD estimates was slightly improved when the concentrations (g kg^{-1} DM) of iNDF and NDF were used:

$$OMD = 0.882 - 0.00121 \times iNDF - 0.00011 \times NDF \quad [5]$$

Prediction error of this model was 0.0174 and 0.0090 for the fixed and mixed model regression, respectively, and the respective parameter estimates were biologically sound. The more recent work with a wider range of forage types (Krizsan et al. 2012) confirmed that empirical OMD equation based on forage iNDF forms a relatively universal basis for NIRS, especially for a more heterogeneous sample population. Under-prediction of OMD for lucerne silages by iNDF (Rinne et al. 2006, Krizsan et al. 2012) suggests that this assumption is not always true. An additional advantage of iNDF in forage evaluation is that it can be predicted with a relatively good accuracy by NIRS either on scans from dried feed (Nousiainen et al. 2004a) or faeces (Nyholm et al. 2009). In our digestibility dataset, *in vivo* OMD could be predicted almost as accurately from iNDF determined by NIRS as with iNDF determined by 12-d *in situ* incubation. However, it must be highlighted that both feed and faecal iNDF calibrations are based on reference values obtained from two laboratories that have standardized *in situ* procedure with no substantial inter-lab bias in the iNDF values and scans from only one NIRS lab. Evidence from the iNDF ring-test (Lund et al. 2004) suggests that a reliable reference database for NIRS cannot be established by simply compiling data from several labs.

NIRS applications in forage evaluation

Since Norris et al. (1976) first introduced NIRS equations for predicting forage quality, considerable progress has been made to implement NIRS applications for silage analysis. The development of computers, optical devices and calibration soft wares has facilitated this process (Deaville and Flinn 2000). Although individual wavelengths in the NIR spectrum lack specificity to important feed parameters, especially being non-specific for functional properties of feeds (e.g. NDF, digestibility, intake potential), quantitative analysis of forage quality by NIRS is possible by

calibrating the reflectance spectrum against biologically sound reference methods (Deaville and Flinn 2000, Nousiainen 2004). NIRS applications for forage evaluation include quantitative analysis of both cell wall (NDF, iNDF) and cell content (CP, WSC, silage fermentation products) characteristics (Deaville and Flinn 2000, Nousiainen 2004). The scans may be obtained from dried and finely ground or coarse wet samples, although the latter may be less accurate. Interpretation of published NIRS equations reveal that OM digestion and cell wall lignin bonding of forages is associated to spectral regions near to 1650–1670 and 2260–2280 nm (Deaville and Flinn 2000). In agreement with this, Nousiainen et al. (2004a) demonstrated that the absorbance in these regions was negatively correlated with the iNDF content of grass silages.

The precision and repeatability of NIRS are known to be much better than any feed chemistry method (Deaville and Flinn 2000). Consequently, within a single lab NIRS calibration statistics often suggests very accurate prediction of any feed trait. When several chemical, *in vitro* and *in situ* reference methods in calibrating silage OMD were compared (Nousiainen 2004), the calibration statistics for all of them showed high R^2 and a low standard error of calibration (SEC) and cross validation (SECV). However, the total error of prediction (*in vivo* vs. NIRS) was highly dependent on the biological validity of the reference method used. Therefore caution should be used in the choice of calibration method for NIRS. A relatively high correlation (R^2 0.23) between the residuals of OMD estimates based on iNDF or OMS in the Finnish dataset (Huhtanen et al. 2006b) suggests that *in vivo* reference values include some random error. Therefore it is likely that with NIRS the true errors may be smaller than apparently estimated. For commercial laboratories OMS method may be the most practical choice to calibrate the NIRS for the prediction of OMD (Nousiainen 2004a, Huhtanen et al. 2006b).

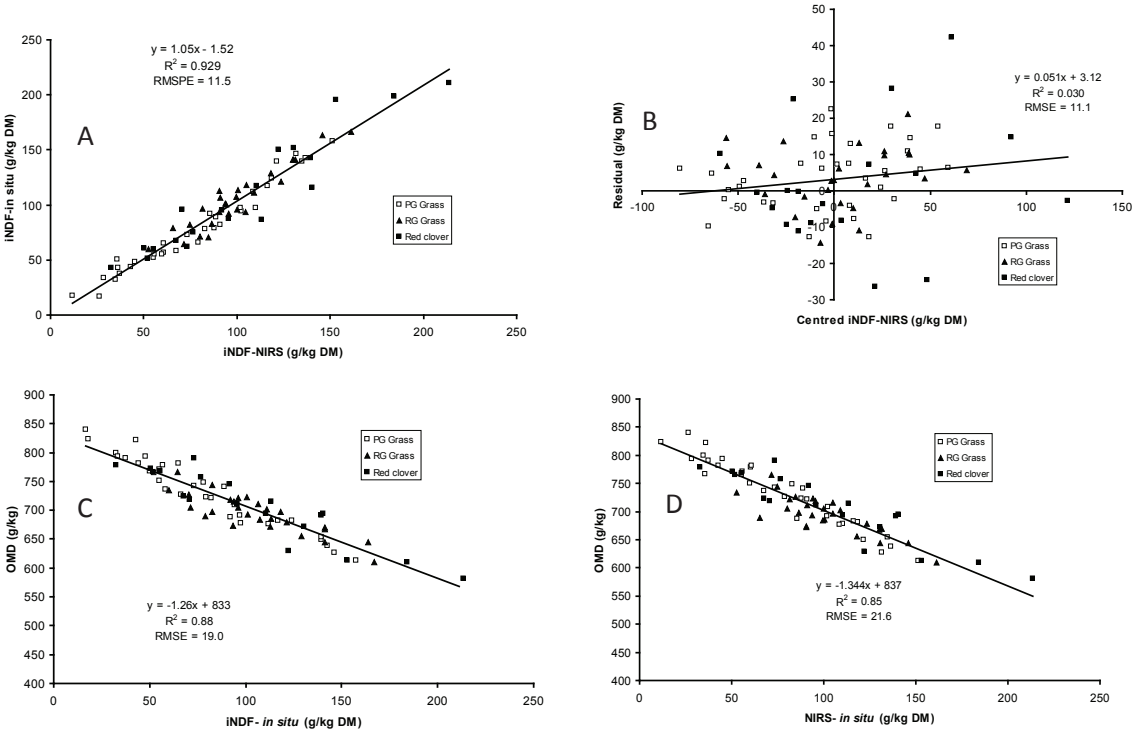

Fig. 1. The relationship between NIRS predicted and *in situ* determined iNDF (A) with residual analysis (B) and predictions of *in vivo* OMD from iNDF determined in situ (C) or by NIRS (D). In residual analysis each NIRS predicted value is centred by subtracting of mean predicted from each predicted value (n = 80). (Data from Huhtanen et. al. 2006b).

By using forage specific corrections for OMS and a sufficiently diverse range of reference samples, total prediction performance can be considered satisfactory. The standard error of prediction (SEP) for D-value using OMS based calibrations was circa 17–20 g kg^{-1} DM (Huhtanen et al. 2006b), consistent with a RSD of 14 g kg^{-1} DM for measurements of OMD in digestion trials (Nousiainen 2004). Alternatively iNDF can be used for OMD or D-value calibration for NIRS in one of two ways; (1) predict digestibility with a direct regression equation (Nousiainen 2004) or (2) use a summative method of uniform feed fractions (Huhtanen et al. 2006b). In the future, NIRS may be used to predict forage traits for use in dynamic digestion models. The digestion rate of pdNDF can be calculated from OMD, NDF and iNDF using the Lucas principle for the NDS fraction and constant passage kinetic parameters at maintenance intake (Huhtanen et al. 2006a). Incubation of isolated NDF in automated *in vitro* gas production system resulted in similar digestion rate of pdNDF as estimated from the *in vivo* data (Huhtanen et al. 2008c).

Digestibility at production intake

Digestibility determined in sheep fed at maintenance describes the intrinsic digestibility of the diet, i.e. *in vivo* digestibility under optimal conditions (Mertens 1993). Feed values for cattle diets are traditionally computed using these digestibility coefficients by summing up individual dietary components. In general, the digestibility coefficients for a given feed are similar in sheep and cattle (Yan et al. 2002). Because diet digestibility decreases with increased feed intake, energy values are adjusted for the level of feeding in many feed evaluation systems. In a recent meta-analysis based on the evaluation of 497 diets in lactating cows, OMD was on average 0.038 units lower in dairy cows fed at production levels of intake compared with OMD estimated at maintenance intake (Huhtanen et al. 2009). Digestibility in cows was shown to decrease with DM intake, the extent of depression being greater for highly digestible diets (Huhtanen et al. 2009). Dietary CP concentration had a positive effect on OM and NDF digestibility, while OMD decreased in a quadratic manner with increases in the proportion of whole-crop silage in the diet and linearly with concentrate fat intake (Table 2). The RSD of a multivariate mixed regression model was 0.007 indicating that the differences in OMD between the diets of lactating cows could be predicted accurately from digestibility at maintenance, feed intake and diet composition (Huhtanen et al. 2009). Interestingly, there was no difference in the accuracy of OMD prediction in cows when OMD at maintenance were determined either *in vivo* with sheep or based on predictions from various *in vitro* measurements. The variation in OMD in dairy cows was almost completely related to the concentration and digestibility of NDF (Huhtanen et al. 2009). This indicates that the negative associative effects of feeding level and diet composition on OMD at the production level of intake are mainly associated with decreased NDF digestibility. It is therefore important to distinguish between iNDF and uNDF. Indigestible NDF is not digested by ruminants, whereas uNDF represents faecal output of pdNDF per kg DM intake. Total faecal NDF also includes a proportion of pdNDF (i.e. uNDF) that is not digested because the retention time in the fermentation compartments is not long enough for complete pdNDF digestion. In dairy cows fed at production level of intake pdNDFD was substantially lower than in sheep fed at maintenance (0.75 vs. 0.85) resulting in a greater loss of potentially digestible NDF in faeces.

Table 2. The best-fit equation for multiple regression of OM or NDF digestibility (OMD or NDFD, respectively) in lactating dairy cows[1]; adjusted RMSE for OMD 7.1 g kg^{-1} (n= 497) and for NDFD 12.4 g kg^{-1} (n = 394) (Huhtanen et al. 2009).

Effect	Unit	Estimate (g kg^{-1})	
		OMD	NDFD
Intercept		18.4	-285
OMD_m[2]	g kg^{-1} DM	0.651	
pdNDF[3]/NDF	g kg^{-1} NDF		0.647
DMI	kg d^{-1}	-2.72	-4.85
Ln CP[4]	g kg^{-1} DM	53.7	101
Wcrop[5]		22.2	-28
WCrop × WCrop		-61.4	-70
(NFC[6]/NDF) × (NFC/NDF)			-55
Cfat[7]	g kg^{-1} DM	-17.7	-33

[1)] All values are adjusted for the random study effect. [2)] OM digestibility determined at maintenance level of feeding in sheep or with a corresponding *in vitro* method. [3)] pdNDF = potentially digestible NDF. [4)] Natural logarithm of crude protein concentration. [5)] Proportion of whole crop cereal silage in forage (kg kg^{-1}). [6)] Concentration of non-fibre carbohydrates in the diet (g kg^{-1} DM). [7)] Concentrate fat

Nutrient supply

Feed intake

Accurate prediction of DM intake (DMI) is a prerequisite for the formulation of economical dairy cow diets. Despite intensive research, no generally accepted intake model has been developed. Limited success is at least partly due to complicated interactions between the animal and feed factors, and difficulties in distinguishing and quantifying these factors. Many intake models include observed milk yield as a predictor of intake. However, these models are primarily useful in predicting intake required to sustain a given level of milk production, as stated by Keady et al. (2004a). It should also be remembered that the yield can only be known retrospectively after the diet has been fed (Ingvartsen 1994). Several attempts have been made to develop prediction equations for practical ration formulation using multiple regression equations for individual animal data. However, these models usually

have large residual errors, and consequently the effects of e.g. silage fermentation characteristic were non-significant.This is probably due to large between animal variations in intake within a diet and study, and large between study variations both in the intake and composition of diets. Mixed model regression analysis with random study effects allows estimation of quantitative relationship between dietary variables and DMI and the relative intake potential of diets.

The first relative silage DMI index (SDMI-index) model included D-value (g digestible OM in DM), a quadratic negative effect of total acid (TA) concentration and a logarithmic of ammonia N (Huhtanen et al. 2002a). Volatile fatty acids, especially propionic acid, had a stronger negative effect on intake than lactic acid. Digestibility was a much better predictor of SDMI than CP and NDF. The effects of D-value and fermentation quality were combined into a single index by defining standard silage (SDMI-index = 100) and that 0.10 kg DM is one index point. Root mean squared prediction error (RMSE) adjusted for the random study effect was 0.41 kg d^{-1}, i.e. the model predicted precisely the differences in the intake potential of silages within studies. The model was revised to include other variables that significantly influence SDMI (Huhtanen et al. 2007). In addition to D-value and fermentation characteristics, the revised model includes the concentrations of silage DM and NDF, harvest of grass silage (primary vs. regrowth) and forage type (grass, legume and whole-crop). Silage DM concentration influenced quadratically SDMI with maximum intake with a DM concentration of 350–400 g kg^{-1}. Intake of regrowth silages was 0.4 kg DM d^{-1} less than that of primary growth silages when the differences in other variables were taken into account. Both legume and whole-crop silages displayed positive associative effects on SDMI, i.e. the intake of silage mixtures was greater than the mean of the two silages when fed alone. Maximum NDF intake was observed at a D-value of 640 g kg^{-1} DM suggesting that the cows do not use the full rumen capacity when fed high D silages. Indeed, the rumen NDF pool has reduced with increased silage digestibility (Bosch et al. 1992, Rinne et al. 2002) despite increased SDMI. These observations do not support the bi-phasic intake regulation theory (e.g. Mertens 1994); it rather suggests that DMI is regulated by interplay between physical and metabolic factors. In the revised model fermentation variables were simplified to the linear negative effect of TA concentration. However, with silages displaying secondary fermentation the intake predictions can be improved by including acetic acid or VFA in the model (Eisner et al. 2006). The adjusted RMSE of the revised model was 0.34 kg d^{-1} and it explained 0.85 of the variation in SDMI within a study. D-value, fermentation quality and DM concentration were the three most important variables.

It is well-known that both the amount and composition of the concentrate supplements influence SDMI. Therefore the next step in developing the intake prediction model was to include concentrate factors in the model (Huhtanen et al. 2008a). Total DMI increases with increased concentrate DMI (CDMI) but the increases diminished at high levels of supplementation; i.e. substitution rate increased. Substitution rate also increased with increased intake potential (SDMI-index) of silages. Interestingly, SDMI explained the variation in substitution rate better than any single component of it. The interaction between forage intake potential and concentrate supplementation is also included in the Feed into Milk model, presented by Keady et al. (2004b). In their model silage intake potential is determined by NIRS calibrated against standardized intake data by cattle. In addition to CDMI, the model of Huhtanen et al. (2008a) includes the quadratic effect of supplementary protein intake, negative linear effect of fat and positive linear effect of concentrate NDF. The adjusted RSME of the CDMI model in studies in which different concentrate treatments were used with the same silage was 0.27 kg. The two indexes were combined to a single total DMI index (TDMI-index) that describes quantitative differences in DMI within a study by assuming the effects are additive. In the model evaluation the observed DMI response at 0.095 kg/index point was close to default value of 0.100 and the adjusted RMSE of the TDMI-index model was 0.37 kg DM d^{-1}.

Evaluation of the TDMI-index model indicated that quantitative differences in the intake potential of the diets can be estimated accurately. The modelling was based on an assumption that within a study the animal factors (e.g. yield, live weight [LW]) are similar for all diets. However, in practical ration formulation in addition to relative intake potential related to diet characteristics, accurate predictions of actual intake including animal factors is required. Most intake prediction models use milk yield and live weight as animal variables. Because milk yield is a function of both cow's genetic potential and diet characteristics, it is important that animal and diet variables are modelled independently of each other to avoid double-counting. It is important to note that cow's genetic intake potential does not increase when she is fed a better diet; the intake response is entirely due to the diet effect. To avoid this double-counting and to have unbiased estimates of diet effects in the model, we used standardised energy corrected milk (sECM) rather than observed yield to describe the production potential of the cow (Huhtanen et al. 2011b). Observed ECM was adjusted for days in milk, TDMI-index and dietary metabolizable protein (MP) concentration, i.e. to predict how much the cow would produce at a given stage of lactation when fed a standard diet. An advantage of this approach is that all data is available at the time of prediction, in contrast to observed

ECM yield. The final model comprised sECM, LW, days in milk as animal factors and TDMI-index to describe the dietary intake potential. The regression coefficient of TDMI-index (0.088) remained close to the default value suggesting that the true animal and diet effects were separated properly.

Rumen fermentation

Typically the molar proportion of propionate is low in cattle fed diets based on restrictively fermented grass silages with moderate levels of concentrate supplementation; for example in the review of 34 diets fed to growing or lactating cattle the molar proportion of propionate was only 165 mmol mol^{-1} (Huhtanen 1998). Water soluble carbohydrates are fermented to lactic acid and VFA during ensilage with the extent and type depending on ensiling characteristics of forages and additives used. These changes have a strong influence on ruminal fermentation pattern. Increased concentration of silage lactic acid increases propionate in rumen VFA. Intraruminal infusions of lactic acid demonstrated that propionate is the main end-product of lactate fermentation (Chamberlain et al. 1983, Jaakkola and Huhtanen 1992). Jaakkola and Huhtanen (1992) calculated that propionate comprised about 50% of the end-product of lactate fermentation in the rumen. Consistently, increased lactic acid concentration in silage has consistently increased propionate in rumen VFA (van Vuuren et al. 1995, Harrison et al. 2003). In contrast to lactic acid, the effects of silage WSC on rumen fermentation pattern have been inconsistent: sometimes butyrate (Jaakkola et al. 1991, 2006a) and sometimes acetate (Cushanan et al. 1995, Huhtanen et al. 1997) has increased.

Rumen fermentation pattern in cattle fed grass silage-based diets appears to be rather resistant to increased concentrate supplementation. The effect of dietary starch concentration on the proportion of propionate in rumen VFA was not significant in multiple regression models derived from the Nordic dataset (107 diets in 29 studies) (Sveinbjörnsson et al. 2006). In this dataset, dietary lactic acid concentration had the strongest effect on rumen propionate suggesting that silage lactic acid is a more important factor influencing rumen fermentation pattern than starch. Mixed model analysis of an unpublished Finnish dataset (106 diets) indicated that dietary starch concentration influenced rumen propionate in a quadratic manner with a minimum at 200 g kg^{-1} DM. In the same dataset, molar proportion of acetate decreased quadratically and that of butyrate increased linearly with increased starch concentration. The results suggest that at low levels of concentrate (starch) supplementation silage lactate dominates the rumen fermentation pattern, whereas at moderate levels of dietary starch concentrations the role of rumen protozoa becomes more important. The number of rumen protozoa increases with increased starch supplementation (Rooke et al. 1992, Jaakkola and Huhtanen 1993) and this may explain the changes in rumen fermentation pattern with increased concentrate supplementation in cattle fed grass silage-based diets.

As for increased starch supplementation, the effects of fat supplementation on rumen fermentation pattern are also rather small in cattle fed grass silage-based diets. In the analysis of the Finnish dataset there was a quadratic positive response in rumen propionate to increased dietary concentration of concentrate fat. The model predicts 10 – 15 mmol mol^{-1} increases in rumen propionate for dairy cows fed 500 g d^{-1} of supplementary fat as plant oils. Only at high inclusion rates of plant oils quantitatively important changes in rumen fermentation pattern can be expected in animals fed grass silage-based diets (Tesfa 1993, Shingfield et al. 2008).

Protein supply

Microbial protein synthesised in the rumen comprise the major part of the supply of amino acids (AA) absorbed from the small intestine. Regression coefficients of bivariate regression model predicting milk protein yield were five times greater for bacterial metabolizable protein (MP) compared with feed MP both in North American and North European dairy cow trials covering a wide range of dietary ingredients (in total >1 700 diets) emphasizing the importance of microbial protein (Huhtanen and Hristov 2009). It has generally been believed that the efficiency of microbial protein synthesis (MPS) is lower in animals fed grass silage-based diets than in those fed dried or fresh forages, but there is little experimental evidence to support this. Three reasons have been suggested for the lower efficiency of MPS: silage fermentation products provide less ATP for microbial growth than WSC (Chamberlain 1987), the nature of N constituents (more ammonia and NPN) and asynchronous energy and N release from the silage (Thomas and Thomas 1985). Microbial protein production in the rumen increased when silage fermentation was restricted using formic acid based additives (e.g. Jaakkola et al. 1991, 2006a, Huhtanen et al. 1997). In addition to increases in measured MPS, increased plasma concentrations of AA, particularly branched-chain AA, (Nagel and Broderick 1992, Huhtanen et al. 1997) indicated a greater amount of absorbed AA in response to restricting in-silo fermentation. There were no differences in the total or microbial protein flow at the duodenum between diets based on dried hay or restrictively fermented silage harvested simultaneously from the same sward (Jaakkola and Huhtanen 1993, Table 3). All these results suggest that the preservation method *per se* does

not influence MPS and that the extent, and possibly type, of the in-silo fermentation are more important factors influencing the protein value of forages than preservation method.

The asynchrony between energy and N supply, often assumed to be a main reason for the low efficiency of MPS, has attempted to be minimized by feeding soluble carbohydrates. Feeding sugar supplements has decreased rumen ammonia N concentration (Syrjälä 1972, Chamberlain et al. 1985). However, the marginal increases in MPS with sugar supplements have not been greater than those predicted from the increased supply of fermentable energy (Chamberlain and Choung 1995), i.e. there were no extra benefits from a better synchrony. In line with this, Khalili and Huhtanen (1991) reported significant increases in microbial protein flow with different sucrose supplements in cattle fed a grass silage-based diet. However, the continuous infusion of sucrose decreased rumen ammonia N and increased microbial N flow numerically more than feeding sucrose twice daily, despite a better synchrony of energy and N release with the latter. Similar conclusions can be drawn from the studies of Henning et al. (1993) and Kim et al. (1999); continuous supply of energy stimulated MPS more than attempts to catch high post-prandial ammonia concentrations by pulse doses of rapidly fermentable carbohydrates.

Table 3. The effect of forage preservation method (silage vs dried hay) and the application rate of formic acid in silage on the flow of nitrogen at the duodenum (g day^{-1}).

Reference	Treatment	Ammonia-N	Non-ammonia N	Microbial N	Feed N
Jaakkola and Huhtanen (1993)	Silage (formic acid 4 L t^{-1})	5.0	148.2	83.4	52.8
	Dried hay	4.8	142.8	73.1	57.8
Jaakkola et al. (2006a)	Untreated	3.9	114.5	49.0	53.4
	Formic acid 2 L t^{-1}	4.2	126.1	57.3	56.7
	Formic acid 4 L t^{-1}	4.5	128.4	58.4	57.9
	Formic acid 6 L t^{-1}	5.1	136.9	65.4	59.4

Formic acid application rate expressed as 100% solution

Despite their rather small contribution to the total MP supply, forage factors influencing the supply of rumen undegraded protein (RUP) have been investigated more intensively than factors influencing MPS. Studies conducted with the *in situ* method have suggested large differences in ruminal degradability of forage protein, but seldom these differences have been realized as production responses. Two reasons can be suggested for this discrepancy: the differences in RUP supply are overestimated by the current methods and/or that the value of forage RUP is low. In the analysis of omasal flow data the slope between the predicted (NRC 2001) and measured feed N flow was 0.76 (Broderick et al. 2010) suggesting that the differences in ruminal degradability of dietary CP are smaller than the model predictions based on the tabulated *in situ* data. The models computing ruminal degradability from the kinetic data assume that the immediately disappearing fraction (buffer/water soluble N) is degraded at infinite rate. However, there is a plenty of evidence that soluble non-ammonia N (SNAN) fractions can escape from the rumen in the liquid phase (e.g. Choi et al. 2002, Reynal et al. 2007). Ahvenjärvi et al. (2007) reported using ^{15}N labeled silage buffer soluble N that approximately 15% of SNAN fraction escaped ruminal degradation in dairy cows. Consistent with these results, a meta-analysis based on 253 diets showed no negative influence of the proportion of SNAN in silage on milk protein yield when a silage MP values were calculated using a constant CP degradability irrespective of the proportion of soluble N (Huhtanen et al. 2008b). The meta-analysis of milk production data (Huhtanen et al. 2010) silage D-value and especially intake potential were more important determinants of milk protein yield than silage CP or ammonia concentrations.

Production responses

Silage digestibility

The effects of silage quality on feed intake and production responses can be attributed to intrinsic nutritive value of grass at the time of harvest and changes in the composition of grass during ensilage. In the northern latitudes the digestibility of primary growth grasses decreases very rapidly (0.65 %-units d^{-1}; in the dataset of Huhtanen et al. 2006b) with concomitant rapid increases in grass DM yield. Therefore the timing of the harvest of primary growth of grass is one of the most important management decisions in a dairy farm. Improved silage digestibility, expressed as D-value, clearly increases intake and ECM yield (Fig. 2). The average increases in silage DMI and ECM yield were

0.027 and 0.045 kg per one g kg^{-1} in D-value. In the studies of Kuoppala et al. (2008) and Randby et al. (2012) intake of grass (mixtures of timothy and meadow fescue) silage was 17 kg DM d^{-1} when fed with 8 kg d^{-1} of concentrates. These results indicate a high intake potential of restrictively fermented grass silages harvested at early stages of maturity and wilted to DM concentration of approximately 300 g kg^{-1}. The effects of silage digestibility on milk fat concentration have been variable and usually small, whereas milk protein concentration has increased with improved digestibility (Rinne et al. 1999a, Kuoppala et al. 2008), probably reflecting an increased energy supply. In all studies (Fig. 2) the silages were supplemented with different levels of concentrate allowing calculation of concentrate sparing effects of improved silage digestibility. The average ECM yield response was 0.48 (SE = 0.04) kg ECM per kg increase in concentrate DMI. The average "concentrate sparing effect" was 0.81 (SE = 0.12) kg DM per 10 g kg^{-1} DM increase in silage D-value. Assuming that silage D-value decreases 5 g kg^{-1} DM per day, one day delay in harvest corresponds to 0.22 kg decrease in ECM yield or 0.45 kg DM greater concentrate requirement to maintain ECM yield.

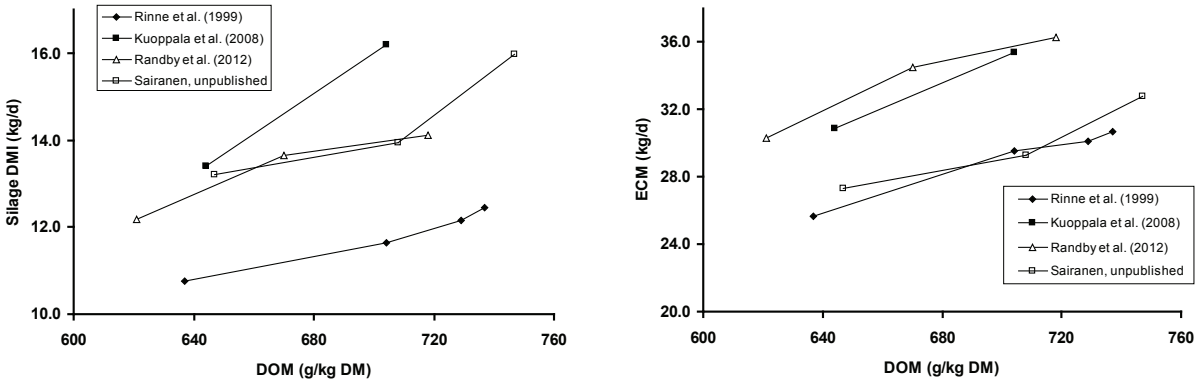

Fig. 2. The effects of the concentration of digestible organic matter (DOM) on silage DM intake and ECM yield. The values are means over 2 or 3 concentrate levels in each study.

Silage fermentation

In a meta-analysis of data from silage fermentation studies (47 studies, 234 diets), both the extent and type of in-silo fermentation influenced milk production variables (Huhtanen et al. 2003). In this dataset the silages were harvested at the same stage of maturity and ensiled with different additive treatments. The yields of milk, ECM and milk components decreased with increased concentrations of lactic acid and VFA in silage. Numerically the effects of VFA were stronger than those of lactic acid. Proportional decreases in the yield of milk components with increasing extent of in-silo fermentation were the smallest for lactose and the highest for milk fat. When silage DMI was included in the prediction models, the effect of TA concentration on milk yield was not significant. However, increased silage TA concentration reduced the ECM yield even when silage DMI was included in the model, but the regression coefficient was much smaller (-5.8 vs. -18.6 g per 1 g TA kg^{-1} DM). It can be concluded that the effects of in-silo fermentation on the production of milk and milk components are mainly derived from the changes in feed intake.

Milk fat and protein concentrations decreased with increased in-silo fermentation (Huhtanen et al. 2003). Reduced milk protein concentration can be attributed to decreased feed intake and the lower efficiency of MPS, whereas the lower fat concentration is most likely related to the reduced proportion of lipogenic VFA in the rumen. The effects of silage TA concentration remained negative even at fixed DMI indicating that the changes in the composition of absorbed nutrients influenced milk composition beyond the responses related to DMI. Decreases in milk protein yield with increased in-silo fermentation were not greater than those predicted from reduced intake, even though negative effects of high TA concentration in silage on the efficiency of MPS are well-documented (Harrison et al. 2003). It is possible that increased propionate production from silage lactate increases hepatic gluconeogenenis thereby sparing AA from being used for glucose production. Higher plasma glucose concentration in cows fed extensively fermented silages compared with those fed restrictively fermented silages (Heikkilä et al. 1998, Shingfield et al. 2002b) support this hypothesis. However, the lack of responses to dietary supplementation of propylene glycol of cows given restrictively fermented silages (Shingfield et al. 2002a, Jaakkola et al. 2006b) do not support the hypothesis that the diets based on restrictively fermented silages are specifically limited by the glucose supply.

Improved silage fermentation can be realized as increased yield or as "concentrate sparing effect". Compared with formic acid-treated silage the cows given untreated silage required an additional 2.9 kg concentrate per cow per day to produce the same amount of milk fat plus protein (Shingfield et al. 2002a). The "concentrate sparing effect" of formic acid treatment was greater than reported by Mayne (1992) and Keady and Murphy (1996). The greater value in the study of Shingfield et al. (2002a) may be related to the higher levels of concentrate feeding, and therefore smaller marginal responses to supplements attained.

Concentrate supplementation

It is well-known that concentrate supplementation decreases silage DMI but increases total DMI. Silage DMI decreased by 0.45 kg and total DMI increased by 0.55 kg per 1 kg increase in concentrate DMI in our data-set from milk production trials (233 treatment means from concentrate supplementation studies, Huhtanen et al. 2008a). The effects of concentrate DMI on total DMI were strongly curvilinear with decreasing responses at high levels of supplementation. When the data was divided into two groups according to the relative silage DMI index (<100 [mean 91] and >100 [mean 107]) the total DMI increased less (0.51 vs. 0.61 kg per kg increase in concentrate DMI) for silage of high compared with low intake potential, respectively. As a result of interactions between the forage quality and the level of concentrate supplementation substitution rates can be high, even close to 1.0, in cows fed high quality grass silages with moderate to high amounts of concentrates (Kuoppala et al. 2008, Randby et al. 2012).

The mean linear ECM yield response to increased concentrate allocation was 0.71 kg kg^{-1} concentrate DM, but it decreased with the increasing supplementation level (Huhtanen et al. 2008a). With high quality silages, marginal production responses to increased concentrate allocation were small (Kuoppala et al. 2008) or even negative (Randby et al. 2012). Small production responses are related to the high substitution rate, negative associative effects in digestion and possibly repartitioning nutrient towards body tissues with high concentrate levels. Although the digestibility of concentrates at maintenance level is greater than that of forages, diet digestibility in dairy cows at production level was not related to the concentrate intake (Nousiainen et al. 2009). Interestingly, when the data-set from concentrate supplementation studies were divided according to mean milk yield (<27 kg d^{-1} and >27 kg d^{-1}) the linear ECM responses were greater (0.76 vs. 0.63 kg kg^{-1} concentrate DM) at low (mean 23 kg d^{-1}) compared with high (31 kg d^{-1}) production level. This is mainly because total DMI responses were greater (0.65 vs. 0.47 kg per kg concentrate DM) at low production level. In the analysis of a larger dataset ECM yield responses to increased ME intake did not depend on the production level of the cows (Huhtanen and Nousiainen 2012).

Protein supplementation

Proper determination of animal protein requirements is critically important for maximizing production and minimizing N input in dairy production systems. Efficiency of N utilization in milk production is relatively low at 25–28% (Huhtanen and Hristov 2009). Although increasing N input usually increases milk protein yield, conversion of dietary N to milk N will decrease. Earlier, when the feed protein evaluation was based on digestible CP the strategy in Finland was to increase CP concentration in grass silage by high levels of N fertilization and early harvest (Hiltunen 1979). As discussed before, maturity stage at harvest has a strong influence on intake and milk production. However, when CP concentration in grass silage was increased from 120 to 150 g kg^{-1} DM by greater application rate of N fertilizer feed intake or output of milk and milk protein were not influenced, while provision of additional N in concentrate supplements improved all of these parameters (Shingfield et al. 2001).

Inclusion of protein supplements such as soybean and rapeseed meals in grass silage-based diets increased milk protein yield, but at the same time reduced the efficiency of N utilization (Huhtanen and Hristov 2009). The increases in milk protein yield ranged from 98 (soybean meal) to 136 g kg^{-1} increase in CP intake (untreated rapeseed meal) in recent meta-analysis by Huhtanen et al. (2011a). Similar differences were reported in a single study by Shingfield et al. (2003), who compared soybean mean and rapeseed expeller at four graded isonitrogenous levels (Table 4).

Plasma AA profiles suggested that rapeseed increased the supply of histidine and branched-chain AA compared with soybean meal (Shingfield et al. 2003). Positive production responses to supplementary protein in cows fed grass silage-based diets are partly associated with increased ME intake resulting from a greater silage DMI (Huhtanen et al. 2008a) and improved diet digestibility (Nousiainen et al. 2009). Marginal responses to incremental ME (0.16–0.18 kg ECM MJ^{-1} ME) in protein studies (Huhtanen et al. 2011a) were greater than usually obtained with increased inclusions of concentrate feeds (about 0.10). This may indicate that a greater AA/ME ratio in absorbed nutrients can improve the efficiency of ME utilization for milk production. Data from a whole lactation study (Law et al. 2010) indicated that calculated ME balance was greater for cows fed low vs. medium and high protein diets,

but the differences in blood metabolites, body condition score or live weight change did not indicate any true differences in energy balance.

Table 4. The effects of graded levels of rapeseed expeller (R) and soybean meal (S) supplementation on milk production and plasma amino acids (AA) in cows fed grass silage based diets (Shingfield et al. 2003). The number refers to crude protein concentrations in concentrates.

	Control	R150	R180	R210	S150	S180	S210
Intake (kg DM day^{-1})							
Silage	11.6	11.9	12.3	12.4	12.0	12.2	12.5
Total	20.0	20.6	20.9	20.8	20.3	20.4	20.5
Production							
Milk (kg day^{-1})	26.2	28.2	29.3	30.1	26.9	27.0	28.5
ECM (kg day^{-1})	29.8	31.2	31.6	32.8	30.3	30.4	32.1
Protein (g kg^{-1})	33.5	33.9	33.7	33.4	34.3	33.6	33.8
Protein (g day^{-1})	859	930	967	993	902	889	954
Milk N / N intake (g kg^{-1})	307	295	280	271	290	262	262
Plasma AA (µmol L^{-1})							
Lysine	76	80	92	93	73	88	90
Methionine	20	21	23	25	19	22	20
Histidine	18	28	41	51	24	29	34

Two main strategies, reducing ruminal CP degradability of supplementary protein and balancing profile by absorbed AA by using AA supplements or balancing dietary ingredients, to improve milk N efficiency have widely been investigated. In the meta-analysis (Huhtanen et al. 2011a) untreated and heat-treated rapeseed meal elicited similar milk protein yield responses. This is consistent with the meta-analysis by Ipharraguerre and Clark (2005), who did not find any differences in milk production between soybean meal and different RUP sources. According to the meta-analysis of Huhtanen and Hristov (2009), ruminal CP degradability had a significant effect on milk protein yield, but calculated marginal responses to MP derived from reduced degradability was only 6–8%. It has been suggested that the protein supplements treated to reduce ruminal protein degradability have not increased milk yield as the untreated supplements already met the cow's MP requirements. To test this hypothesis Rinne et al. (1999b) fed untreated and heat-treated rapeseed meal at four different levels. Both supplements increased milk and protein yields linearly, but no differences between untreated and treated rapeseed feeds were observed.

Methionine and lysine are often considered as limiting and/or co-limiting AA in dairy cows, but there is no evidence that these AA limit milk protein production in cows fed grass silage-based diets (Choung and Chamberlain 1992 and 1995, Varvikko et al. 1999). Vanhatalo et al. (1999a) infused post-ruminally histidine alone or in combinations with methionine, lysine or both (Table 5). Histidine increased significantly milk protein yield, whereas lysine, methionine or lysine + methionine did not produce any further response. Later studies (Huhtanen et al. 2002b, Korhonen et al. 2000) confirmed that histidine was the first limiting AA in cow fed low CP grass silage based diets. Attempts to identify methionine, lysine and branched-chain AA as the second limiting AA were not successful (Vanhatalo et al. 1999a, Huhtanen et al. 2002b, Korhonen et al. 2002). A recent study by Lee et al. (2012) suggested that histidine could also be a limiting AA in cows fed low CP diets based on maize silage. It is possible that after the first-limiting AA the differences between the next limiting AAs are small in cows fed grass silage–based diets, and that the ranking of these AA can vary between experiments.

Analysis of data from milk production trials clearly indicated that dietary CP concentration was the best single variable predicting milk N efficiency (Huhtanen and Hristov 2009). Intake of N has often been used as a predictor of milk N efficiency (e.g. Castillo et al. 2000), but the adverse effect of increased N intake is much stronger when derived from increased dietary CP concentration rather than from increased DMI. As could be expected from its relatively small effects on milk production, ruminal protein degradability had a relatively small influence on milk N efficiency (Huhtanen and Hristov 2009). Milk urea concentration is closely related to dietary CP concentration and it predicted the differences between diets in milk N efficiency and calculated urinary N output accurately (Nousiainen et al. 2004b) suggesting that it can be used as a farm diagnostic tool.

Table 5. Effects of postruminal infusions of amino acids (AA) on milk production and plasma AA concentrations. The amounts of amino acids and glucose (Gluc) infused (g day^{-1}) are given in brackets.

	DMI	Milk	Protein	Protein	Plasma AA (μmmol L^{-1})		
	(kg day^{-1})	(kg day^{-1})	(g kg^{-1})	(g day^{-1})	Lys	Met	His
Vanhatalo et al. (1999)							
Control	16.1	22.9	30.4	695	82	21	18
His (6)	16.3	23.6	30.6	721	77	17	53
His + Met (6.5)	16.3	23.7	31.0	728	90	33	57
His + Lys (19)	16.2	24.2	29.8	717	120	18	47
His + Met + Lys	16.4	23.7	31.1	729	115	30	38
Korhonen et al. (2000)							
Control	17.8	27	31.9	861	72	21	23
His (2)	18.2	28.1	31.3	877	82	23	28
His (4)	17.9	28.1	32.2	907	86	21	51
His (6)	17.9	28.8	32	919	91	23	64
Huhtanen et al. (2002b)							
Control	16.6	23.6	29.4	691	79	18	21
His (6.5)	16.7	24.4	29.7	715	73	17	52
Gluc (250)	16.6	24.2	29.3	706	69	19	17
His + Gluc	17.1	25.0	30.2	748	69	18	52
His + Leu (12)	17.0	24.7	29.9	736	76	19	52
His + Gluc + Leu	17.0	24.9	30.2	751	72	19	49

Conclusions

Silage research in Finland during the latest 30 years has systematically focused on the production and ensiling of grass and legume silages with special reference to the utilization and supplementation of silages in cattle production. This work has facilitated the development of ration formulation systems based on meta-analyses of large and comprehensive datasets that has been compiled mainly from Finnish and North European studies. Successful economical dairy cattle ration optimization requires (1**)** a well-performing feed evaluation system, (2) accurate and cheap feed analyses for on-farm produced silages, (3) DM intake prediction models integrating independently dietary and animal constraints and (4) equations to estimate true nutrient supply and marginal production responses to changes in nutrient intake. Based on these principles Huhtanen and Nousiainen (2012) presented milk production response models that are currently used in practical feed ration planning in Finland.

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