Rumen fermentation profile and intestinal digestibility of maize and grass silages

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Thesis

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Abstract

Maize and grass silages are commonly used as major feed materials for dairy cows in Europe and are becoming common parts of dairy cow rations in other parts of the world. The nutritive value of maize and grass silages varies greatly due to variation in chemical composition. A combination of different factors such as the use of various cultivars, fertilization practices, growing conditions, harvesting technology, maturity at harvest and ensiling conditions cause this variation in chemical composition. The first aim of this thesis was to investigate relationships between the chemical composition and the *in situ* rumen degradation characteristics and *in situ* mobile nylon bag digestibility of dietary nutrients of maize and grass silages. Maize and grass silages with a broad range in chemical composition and quality parameters were selected from different Dutch commercial farms. The broad range in the chemical composition of the maize and grass silages resulted in a large variation in rumen degradable fractions of dry matter (DM), organic matter (OM), crude protein (CP), neutral detergent fibre (NDF) and starch. The intestinal digestibility of CP, NDF and/or starch was affected by the concentration of these components in the maize and grass silages, by the rumen incubation time and the rumen escape content. Regression equations were developed describing relationships between the chemical composition and the *in situ* ruminal and postruminal degradation characteristics of dietary nutrients of maize and grass silages. A number of the developed regression equations presented in this thesis can be used for accurate and rapid estimation of the ruminal and postruminal degradation characteristics of dietary nutrients of maize and grass silages, without conducting time consuming and expensive *in situ* experiments. The second aim of this thesis was to determine whether three cows are sufficient to cover the variation between individual cows in *in situ* rumen degradation characteristics of dietary nutrients of maize and grass silages. Significant differences ($P<0.05$) were found between individual cows for a number of parameters of DM, OM and CP of maize silages, indicating that four or more cows should be used for nylon bag incubations of maize silages. For grass silages, no significant differences ($P>0.05$) between individual cows were found for all the parameters of DM, OM, CP and NDF. The results suggest that using three cows are sufficient for nylon bag incubations of grass silages and pooling of rumen incubated residues is allowed to obtain a representative sample. The third aim of this thesis was to compare two fractionation methods; the washing machine method and a modified method, for nitrogen (N) and starch fractions of maize silages and N fractions of grass silages. The N and/or starch fractions of maize and grass silages determined, using the washing machine method (washing with water for 40 min) and the modified method (shaking with buffer solution for 60 min) were compared. The different methodological approaches of both methods resulted in different values for the washout (W), the soluble (S) and the non-washout (D+U) fractions of N of maize and grass silages and for the W, the insoluble washout (W-S) and the D+U fractions of starch of maize silages. The loss of insoluble small particles of starch was less during shaking of nylon bags in buffer solution, compared to washing nylon bags in the washing machine. Therefore, large differences were found between the D+U fractions of starch determined by both methods compared to the D+U fractions of N of maize silages. The developed regression equations for W, S and D+U fractions of N in grass silages and for D+U fractions of starch in maize silages determined by both methods can be used for rapid estimation of these fractions from chemical characteristics of maize and grass silages. The information on nutrient bioavailability of maize and grass silages presented in this thesis can be used to more accurately formulate dairy ration in terms of maintenance, health and production of dairy cows.

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CHAPTER 1

General introduction

Milk production, meat production, growth and performance of ruminants depend on the animal's potential (genetics) as well as on the feed intake and the nutritive value of the consumed feeds. Forage intake and nutritive value are important for animal health and welfare, which correlate strongly to overall animal performance. The nutritive value of forages is largely determined by the energy and nutrient concentrations and their bioavailability, but also factors such as palatability, contaminations and physical aspects. Forage composition can be highly variable due to amongst others, forage genotype, cultivars, maturity stage, fertilisation level, soil type, climatic conditions, storage and preservation methods (Hoffman *et al*., 1993; Vanhatalo *et al*., 1996; Elizalde *et al*., 1999). Major factors affecting forage quality and ultimately animal performance are shown in Figure 1.

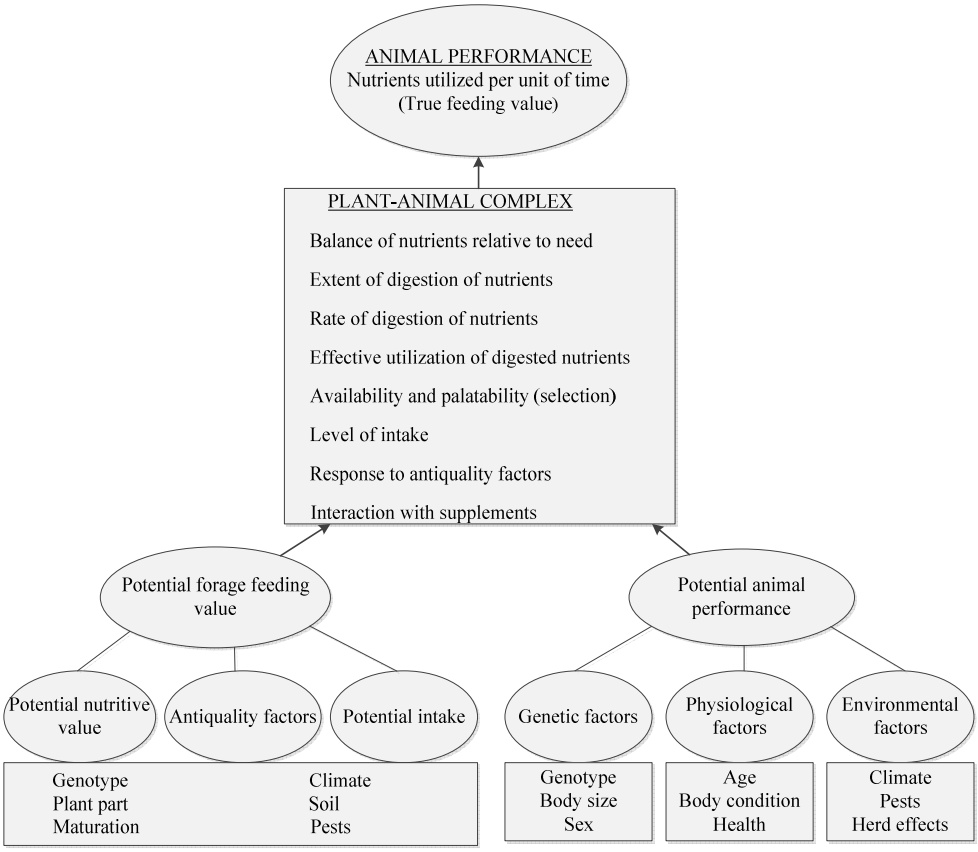

Figure 1 Major forage and animal factors affecting animal performance (Marten *et al*., 1988).

Maize and grass silages are commonly used as major feed materials for dairy cows in Europe and North America (Dawson *et al*., 2002, Ettle and Schwarz, 2003) and are becoming common parts of dairy cow rations in other parts of the world. Grass silage protein is more degradable in the rumen than that of grass hay and fresh grass, due to the fermentation process during ensiling (De Boever *et al*., 2004). Maize silage, with its high energy and low protein content, can replace up to 66 % of grass silage in the diet of dairy cows without a decline in milk yield (O'Mara *et al*., 1998). Keady *et al*. (2008) also reported positive effects on feed intake of dairy cows when grass silage was partly or totally replaced by maize silage. Maize silage is a low cost energy source (starch and fiber) and can replace ration concentrates to a certain extent which ultimately reduces feed cost. The chemical composition of maize and grass silages varies between different cultivars, ensiling conditions, growing conditions, fertilisation, harvesting practices, and maturity at harvest (Cone *et al*., 1999; Fernandez *et al*., 2004; González *et al*., 2010). A broad range in chemical composition of maize and grass silages is used in practice. The research described in this thesis focusses on effects of the broad range in the concentration of chemical components on the feeding values of maize and grass silages for dairy cows, determined by the *in situ* nylon bag and mobile nylon bag (MNB) techniques.

Modern ruminant feed evaluation systems aim to estimate the bioavailability of energy and nutrients of feedstuffs. This information on nutrient bioavailability is important to optimize ration formulation for ruminants in terms of performance, nutrient losses, animal well-being and economical profitability. Several *in situ*, *in vitro* and *in vivo* techniques have been extensively used to measure nutrient digestion of concentrates, feed ingredients and forages fed to ruminants. *In vivo* techniques, although by definition involving the measurement on the species of interest thereby being appropriate, are often expensive, labour intensive, time consuming and subject to errors associated with markers and inherent animal variation (Stern *et al*., 1997). A number of *in situ* and *in vitro* laboratory techniques have been developed and used as alternatives to *in vivo* measurements to predict the rumen degradation and intestinal digestion of dietary nutrients of different feed ingredients (Stern *et al*., 1997). *In vitro* techniques are less laborious, less time consuming but are more suitable for large scale evaluation of ruminant feeds (Stern *et al*., 1997). Among *in vitro* techniques, the gas production technique has been widely used in the last two decades (Cone *et al*., 1996) for accurate determination of the level and rate of degradation of feedstuffs by obtaining large numbers of data points, more than other *in vitro* and *in situ* techniques (Huhtanen *et al*., 2008). However, using the gas production technique, the dilution of ruminal inoculum, type of

buffer, particle size and the diet of the donor animals affect the results (Cone *et al*., 1989; Stern *et al*., 1997). The *in situ* nylon bag technique is another method to estimate the ruminal rate and degree of degradability and intestinal degree of digestibility of different chemical components like dry matter (DM), organic matter (OM), crude protein (CP), neutral detergent fibre (NDF), starch and minerals (Harazim *et al*., 2002; Homolka *et al*., 2008; Jančík *et al*., 2008). The advantage of the *in situ* nylon bag technique is that the digestive process takes place in the rumen (nylon bag technique) and intestine (MNB technique) of live animals (Stern *et al*., 1997).

***In situ* nylon bag technique**

The *in situ* nylon bag methodology is considered a reference methodology in many feed evaluation systems (e.g. Dutch DVE/OEB_{2010} system, Nordic NorFor system, Feed into Milk (FiM) system and French PDI system) to determine the rumen degradation characteristics of the dietary nutrients of different feedstuffs (De Boever *et al*., 2002). Several factors affect the estimates of the *in situ* rumen degradation of nutrients, including bag and sample size, bag material and pore size, sample processing, animal diet, animal effect, feeding level and feeding frequency, bag insertion and removal procedures, rinsing procedures, mathematical models, correction for microbial contamination, incubation time period and number of replicate animals and bags required to obtain repeatable estimates of ruminal degradation (Michalet-Doreau *et al*., 1992; Vanzant *et al*., 1998). Several suggestions are discussed in different studies to standardize the *in situ* procedure but the lack of a standard rinsing procedure and correction for microbial contamination of rumen incubated residues are the major factors which cause variation in *in situ* results (Lindberg, 1985; Vazant *et al*., 1998). The *in situ* methods used to estimate the feeding value of ruminant feeds are based on the characterization of nutrient fractions, the rumen degradation characteristics and the intestinal digestibility.

Using the nylon bag technique, the dietary nutrients of feeds are divided in washout (W) and non-washout (D+U) fractions. The W fraction can further be divided into two parts; a soluble (S) fraction which contains all the soluble components of dietary nutrients (Gierus *et al*., 2005) and an insoluble washout (W-S) fraction, which contains small insoluble particles which escape the nylon bags during washing or rinsing (de Jonge *et al*., 2009). As starch is insoluble by definition, there is no S fraction for starch. Therefore, the W-S fraction is considered as the W fraction for starch (Cone *et al*., 1989). In maize and grass silages, the W-S fraction for nitrogen (N) is very small or negligible, and the S fraction is considered as the

W fraction (de Jonge *et al*., 2013). The D+U fraction after nylon bag rumen incubations is divided into a potentially rumen degradable (D) fraction and rumen undegradable (U) fraction of dietary nutrients. The feeds are incubated for different time periods in the rumen of dairy cows to find the degradation of different nutrients. Microbial contamination of rumen incubated residues after specific incubation periods disturbs the accuracy of results. The degradation rate of the D fraction can be calculated using two options; one is the use of a fixed value of U at 336 h and the other is to estimate the U fraction based on the model used for calculation of degradation rate. For the calculation of the degradation rate for different nutrients (especially NDF), the use of a lag time is also important. The effective rumen degradability of nutrients can be calculated using determined degradation rate and assuming a certain rumen passage rate for each nutrient. Figure 2 shows the different steps involved in the *in situ* rumen degradation of dietary nutrients for maize and grass silages.

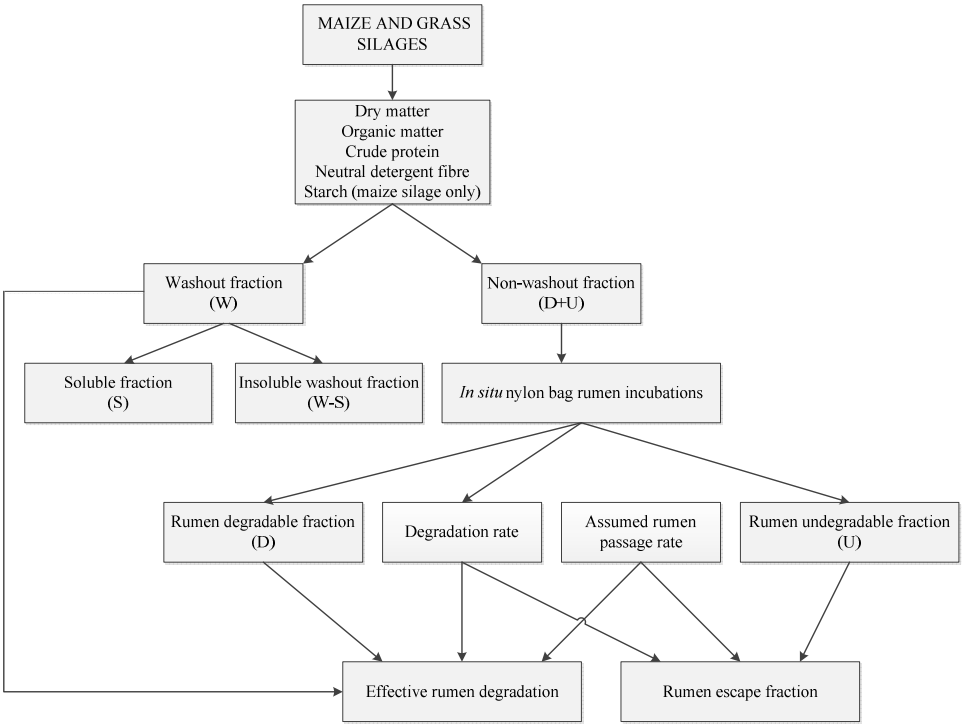

Figure 2 *In situ* rumen degradation of dietary nutrients of maize and grass silages.

In situ mobile nylon bag technique

The *in situ* MNB technique is used to estimate the intestinal digestibility of dietary nutrients of feedstuffs (Vanhatalo and Ketoja, 1995; Danesh Mesgaran *et al*., 2010; González *et al*., 2010). After a pre-incubation in the rumen and a pre-treatment in pepsin-HCl solution, mobile nylon bags are inserted in the duodenum through a cannula and are collected either at the ileum or in the faeces, determining the digestibility in the small intestine or in the total intestinal tract.

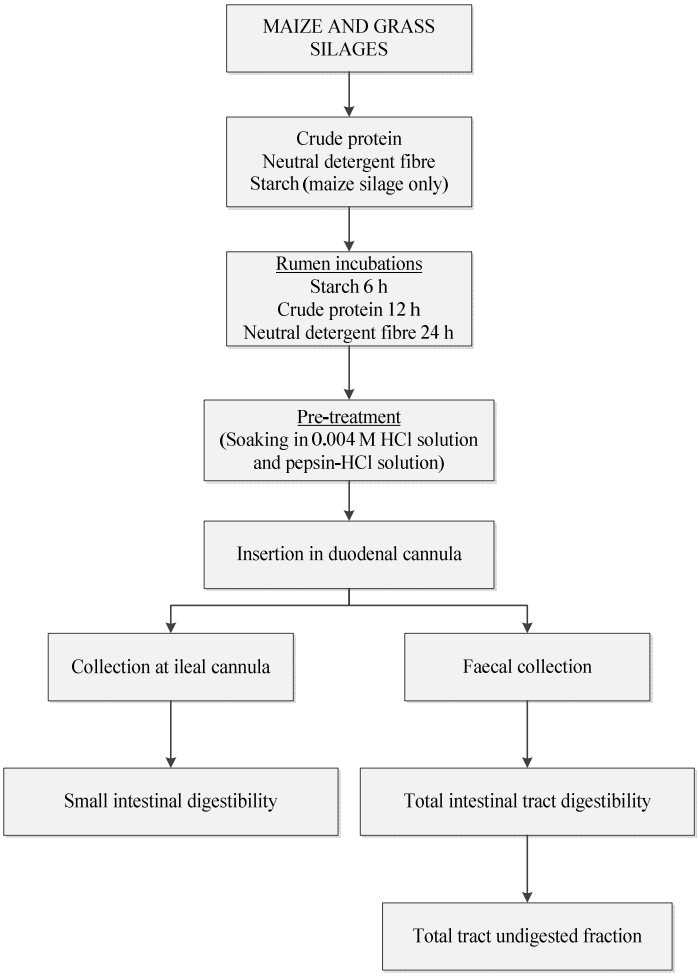

Figure 3 *In situ* mobile nylon bag intestinal digestibility of dietary nutrients of maize and grass silages.

Factors affecting the results of the MNB technique are bag size, porosity of the bags, retention time, bag recovery site, sample to surface ratio, rumen incubation period and microbial contamination (Vanhatalo *et al*., 1996; Hvelplund *et al*., 2009; Danesh Mesgaran *et al*., 2010). Different pre-incubation periods in the rumen are recommended for different nutrients to cover the rumen degradation. The rumen incubation period affects the intestinal digestion. With an increase in rumen incubation period, the intestinal digestible content decreases (Cone *et al*., 2006). Pre-treatment of mobile nylon bags containing rumen incubated residues in a pepsin-HCl solution also plays an important role in the final intestinal digestibility of dietary nutrients. Pre-treatment with a pepsin-HCl solution is recommended by feed evaluation systems to cover the abomasal digestion or digestion of nutrient between the rumen and the duodenum. Jarosz *et al*. (1994) concluded that the MNB technique provides a true measure of intestinal digestion of dietary nutrients. Figure 3 shows the different steps involved in the *in situ* MNB incubations of dietary nutrients for maize and grass silages.

Estimation of rumen degradation characteristics and intestinal digestibility

Various ruminant feed evaluation systems such as the DVE/OEB_{2010} system (Van Duinkerken *et al*., 2011) in the Netherlands, the FiM system (Thomas, 2004) in the UK, the PDI system (Vérité *et al*., 1979) in France and the Nordic NorFor system (Volden, 2011) use simple models or prediction formulas to estimate the rumen degradability and the intestinal digestibility of DM, OM, CP, starch and NDF of maize and grass silages. These prediction formulas have been derived from data of different *in situ* experiments which were performed at different institutes under various experimental conditions (incubation protocols, chemical analyses procedures, nylon bag quality, etc.) and for different scientific purposes, 20 to 50 years ago. The variability in the data, therefore, contains an unknown variation due to factors that are not related to animals. In addition, new maize and grass genotypes and varieties have been introduced over the past decades, which degradation characteristics are not included in the existing database, making the existing database less adequate for current use.

Variation in the chemical composition

To determine the feeding value of maize and grass silages for dairy cows to optimize returns for dairy farmers, the variability in the concentration of their chemical components should be taken into account. The nutritive value of maize and grass silages and the other ruminant feeds is determined by the variation in the concentration of their chemical components as well as the extent and rate of degradation of the dietary nutrients (Getachew *et al*., 2004).

Therefore, the information on relationships between the chemical composition and the *in situ* rumen degradability as well as the *in situ* MNB intestinal digestibility of dietary nutrients is very important for determining the feeding value of maize and grass silages. This information is also important for the rapid assessment of rumen degradation characteristics and intestinal digestibility of the dietary nutrients of maize and grass silages. Many *in situ* studies have been conducted to determine the effect of different individual factors, such as maturity stage at harvest (Jensen *et al*., 2005), ensiling conditions (González *et al*., 2010), bacterial inoculation (Yan *et al*., 1998) and forage species (Jančík *et al*., 2009) on rumen degradation characteristics of maize and grass silages. Few *in situ* MNB studies have also been performed to study the effect of different factors like ensiling (González *et al*., 2010), growing conditions and harvest stage (Cone *et al*., 2006) on the postruminal digestibility of CP of grass silages. To optimize diet formulation in terms of animal health and production, it is necessary to report and cover the variation in the rumen degradation characteristics and intestinal digestibility of dietary nutrients of maize and grass silages.

Variation between individual cows

Factors affecting the *in situ* results have been extensively studied. Less effort has been expended to study the variation in rumen degradability due to individual animal variation (Weakley *et al*., 1983; Van der Koelen *et al*., 1992; Castillo-Gallegos *et al*., 2012). Different numbers of animals have been used in *in situ* studies with maize and grass silages. Two (Jančík *et al*., 2009), three (Jensen *et al*., 2005), four (Fernandez *et al*., 2004) or six (Von Keyserlingk *et al*., 1996) animals have been used. In these studies, it was assumed that the number of animals used was sufficient to cover the variation between the individual cows and to obtain a representative estimate of the rumen degradation fractions of the different nutrients. Various feed evaluation systems such as the DVE/OEB_{2010} system (Van Duinkerken *et al*., 2011) and the Nordic NorFor system (Volden, 2011) recommend to use three animals for nylon bag incubations of feedstuffs. In the past, studies have been conducted to determine the variation between individual cows for concentrate ingredients. Weakley *et al*. (1983) observed significant differences between four cows for ruminal N disappearance of soybean meal and Figroid *et al*. (1972) reported significant variation between two steers for rumen DM degradation of barley and sorghum. Castillo-Gallegos *et al*. (2012) did not find significant differences between three cows in *in situ* DM degradation of kinggrass leaves. This information on variation between the individual cows is important for an accurate estimation of rumen degradation characteristics of dietary nutrients of specific feed ingredients.

Moreover, this information is important because the number of cows used and the pooling of rumen incubated residues for chemical analysis are two main factors which determine the costs of *in situ* experiments.

Fractionation methods

The fractionation of dietary nutrients of feeds into washout (W), soluble (S), potentially rumen degradable (D) and rumen undegradable (U) fractions is important to simulate the process of nutrient digestion in the rumen and intestinal track of dairy cows. The fractionation of feeds provides information about specific rumen bypass nutrients which can be absorbed in the intestinal wall to optimize milk production. Different ruminant feed evaluation systems use a washing machine for washing the nylon bags containing feed samples to determine the W fraction and to remove adhering material from the rumen incubated nylon bags. The washing machine method (WMM) has been extensively used in several *in situ* and fractionation studies (Gierus *et al*., 2005). Different washing machines, rinsing procedures, washing programs and washing times have been used in these studies, leading to variation in the results (de Jonge *et al*., 2009). Lack of a standard rinsing procedure is one of the main factors causing variation in the W fraction and the *in situ* results (Lindberg, 1985). Another disadvantage of the washing machine procedure is that next to the S fraction also small insoluble particles are washed out, behaving completely different in the rumen than the S fraction. de Jonge *et al*. (2009) introduced a new, modified method (MM) as an alternative for the WMM. Recently, de Jonge *et al*. (2013) tested different shaking speeds and suggested one shaking/rinsing procedure for concentrates and forages. This method has the advantage over washing nylon bags in a washing machine that one rinsing procedure is used. In addition, the MM uses a buffer solution as solvent instead of water for rinsing; making the procedure more "physiologically normal" and likely to provide more representative estimates. Another advantage of the MM is that the insoluble washout (W-S) fraction is measured instead of calculated by subtracting the S fraction from the W fraction. This method has already been tested for many concentrate ingredients, but not for forages.

Aims of the thesis

The main objective of the research described in this thesis was to determine relationships between the broad range in the chemical composition and the *in situ* rumen degradation characteristics and the intestinal digestibility of the dietary fractions or nutrients of maize and grass silages. In addition, the variation between the individual cows in rumen degradation

characteristics of dietary fractions or nutrients of maize and grass silages was investigated. It was evaluated whether or not the use of three cows is a sufficient number for *in situ* nylon bag experiments with maize and grass silages. Furthermore, the relationships between the chemical composition and the N and/or starch fractions of maize and grass silages were investigated. This thesis includes information on the rumen fermentation profile, intestinal digestibility and the efficiency of nutrient digestion of maize and grass silages which can be used in existing feed evaluation systems for dairy cows. Regression equations are reported to estimate the rumen degradation characteristics or intestinal digestibility of dietary nutrients from chemical characteristics in order to improve the estimation of the bioavailability of dietary fractions or nutrients and feeding value of maize and grass silages.

Outlines of the thesis

Chapters 2 and 3 describe studies on the relationships between the variation in the concentration of chemical components and the *in situ* rumen degradation characteristics of maize and grass silages, respectively. Chapter 4 describes a study to determine the variation between cows in *in situ* rumen degradation characteristics of dietary fractions or nutrients for maize and grass silages. Chapter 5 reports a study on the intestinal digestibility of CP, NDF and starch of maize silages, and the CP and NDF of grass silages, using the MNB technique. This chapter also describes the relationships between the chemical composition and the intestinal digestibility of CP, NDF and/or starch of maize and grass silages. Chapter 6 compares two fractionation methods (WMM *vs.* MM) for N and starch of maize silages and for N of grass silages. In addition, the relationships between the N and/or starch fractions and the chemical components of maize and grass silages are described. The final chapter (Chapter 7) discusses the main results and provides a comparison with the most relevant research studies. This chapter also provides the main conclusions and recommendations for further research.

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CHAPTER 2

Relationship between chemical composition and *in situ* rumen degradation characteristics of maize silages in dairy cows

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Abstract

Several *in situ* studies have been conducted on maize silages to determine the effect of individual factors such as maturity stage, chop length and ensiling of maize crop on the rumen degradation of maize silages but the information on the relationship between chemical composition and *in situ* rumen degradation characteristics of maize silages remains scarce. The objectives of this study were to determine and describe relationship between the chemical composition and the rumen degradation characteristics of dry matter (DM), organic matter (OM), crude protein (CP), starch and neutral detergent fibre (aNDFom) of maize silages. Seventy-five maize silage samples were selected, with a broad range in chemical composition and quality parameters. The samples were incubated in the rumen for 2, 4, 8, 16, 32, 72 and 336 h, using the nylon bag technique. Large variations were found in the rumen degradable fractions of DM, OM, CP, starch and aNDFom of the maize silages, due to the broad range in chemical composition. The new database with *in situ* rumen degradation characteristics of DM, OM, CP, starch and aNDFom of the maize silages was obtained under uniform experimental conditions; same cows, same incubation protocol and same chemical analysis procedures. Regression equations were developed with significant predictors ($P<0.05$) describing strong, moderate and weak relationships between the chemical composition of maize silages and the washout content, rumen undegradable content, potentially rumen degradable content, fractional degradation rate and effective rumen degradability of DM, OM, CP, starch and aNDFom. A number of the developed regression equations can be used for the rapid and accurate estimation of rumen degradation characteristics of dietary fractions or nutrients of maize silages used in practice.

Introduction

Maize silage is an important component of the diet of dairy cows in Europe and North America (Ettle and Schwarz, 2003). The chemical composition of maize varies between different cultivars, ensiling processes, growth conditions and stages of maturity (Fernandez *et al*., 2004; Jensen *et al*., 2005; González *et al*., 2010). This variation results in variable rumen degradation of dietary nutrients (Ali *et al*., 2012) which should be taken into account when determining the feeding value of maize silages. A number of techniques are commonly used to determine the nutrient availability of different feedstuffs (Habib *et al*., 2013). The *in situ* nylon bag technique has been extensively applied to determine the rumen degradation characteristics of different feedstuffs for dairy cows (Ørskov and McDonald, 1979; Vanzant *et al*., 1998; Harazim *et al*., 2002). The *in situ* nylon bag technique is preferred over *in vitro* methods because the feeds are incubated in the actual rumen environment (Nocek, 1988).

The nutritive value of a ruminant feed is determined not only by its rate and extent of degradation but also by the variation in the concentration of chemical components of that feed (Getachew *et al*., 2004). Therefore, information on the relationship between chemical composition and rumen degradation is important to determine the nutritive value of the feedstuffs. Information on the relationship between chemical composition and *in situ* rumen degradation characteristics of dry matter (DM), organic matter (OM), crude protein (CP), starch and neutral detergent fibre (NDF) of maize silages in the scientific literature is scarce. Several *in situ* studies have been conducted on maize silages to determine the effect of individual factors such as maturity stage (Johnson *et al*., 1999; Di Marco *et al*., 2002; Jensen *et al*., 2005), chop length (Fernandez *et al*., 2004) and ensiling (González *et al*., 2010) of maize crop on the rumen degradation of specific nutrients like DM (Von Keyserlingk *et al*., 1996), OM (Arieli *et al*., 1998), CP (Von Keyserlingk *et al*., 1996; Shannak *et al*., 2000), starch (Fernandez *et al*., 2004; Hindle *et al*., 2005) and NDF (Varga and Hoover, 1983; Stensig *et al*., 1994; Jensen *et al*., 2005). In addition, often in these studies only a limited number (n=1 to 12) of maize silage samples were investigated and compared to other feedstuffs. Moreover, those studies were performed under different experimental conditions (incubation protocols, chemical analyses procedures, nylon bag quality, etc.) which result in a large variation in the rumen degradation characteristics of dietary fractions or nutrients of maize silages. No comprehensive study has been conducted to determine the combined effects of parameters which caused variation in the chemical composition of maize silages. Predicting the *in situ* rumen degradation of dietary fractions or nutrients of maize silages from chemical

composition is important to allow the rapid assessment of nutritive value and correct formulation of rations for dairy cows.

The objectives of this study were to determine and describe the relationships between the chemical composition and the *in situ* rumen degradation characteristics of DM, OM, CP, starch and aNDFom (neutral detergent fibre assayed with a heat stable amylase and expressed exclusive of residual ash) of maize silages.

Materials and methods

Samples collection and processing

Seventy five maize silage samples (~5 kg per silage) were obtained from maize grown at different Dutch commercial farms located in different regions in the Netherlands during 2007, 2008 and 2009. The samples were collected by trained staff of a commercial laboratory (BLGG Research, Wageningen, The Netherlands) using a hollow drill. After collection, the samples were stored at -20°C. Frozen samples were cut using a bread slicer (JAC Duro BEL 450; ABO, Leek, The Netherlands) with a distance of 11 mm between the discs, thoroughly mixed by hand and divided into three parts. One part (~2.5 kg) was subjected to chemical analysis after freeze drying and grinding over a 3 mm sieve (Pepping, Deventer, The Netherlands 200 AN-797002), another part (~1.5 kg) was stored at -20°C for nylon bag incubations, and the third part (~1 kg) was stored (-20°C) as a reserve for possible future reanalysis. The mean chemical composition and quality parameters of the maize silage samples are presented in Table 1.

In situ *rumen incubations*

Three multiparous (second or third lactation) Holstein Friesian cows, producing >15 kg milk per day, fitted with permanent ruminal cannulas were used. The cows were fed a total mixed diet (Table 2) twice a day and had 24 h/d access to water. The maize samples were incubated in the rumen at the research farm “Waiboerhoeve” (Wageningen UR Livestock Research, Lelystad, The Netherlands) according to the procedure used by Ali *et al.* (2012). Maize silage samples (~5 g DM per sample) were weighed into 10 cm x 19 cm nylon bags (Porosity 24 %, pore size 37 µm; Nybolt, Zurich, Switzerland) and incubated in the rumen of the three cows for 2, 4, 8, 16, 32, 72 and 336 h. For 2, 4, 8, 16 and 32 h, six bags of each maize silage sample were incubated in the rumen of three cows for each rumen incubation period (2 bags per cow per incubation time). Because of a low recovery of rumen incubated residues per bag for the

rumen incubation periods of 72 and 336 h, 9 bags of each sample were incubated in the rumen of three cows for each incubation periods (3 bags per cow per incubation time). After removal from the rumen, bags were stored at -20°C for at least 24 h, after which the bags were thawed and washed in the washing machine (AEG-Electrolux Öko Turnamat 2800, Stockholm, Sweden) for 40 min using tap water at 25°C. The 0 h bags were washed in the washing machine as described above without incubation in the rumen and residues were used to calculate the washout (W) fraction. The washed bags were freeze dried and the rumen incubation residues of each maize silage sample from the three cows at one incubation time were individually weighed and afterwards pooled. The pooled rumen incubated residues were ground (Pepping, Deventer, The Netherlands 200 AN-797002) over a 3 mm sieve and stored at 4°C until chemical analyses.

Table 1 *Chemical composition of maize silages (n = 75)*

Variable	Mean	SD[1]	Minimum	Maximum
Chemical composition (g/kg DM)[#]				
Dry matter (g/kg fresh matter)	351	36	272	440
Ash	36	9	21	79
Crude protein	71	6	57	88
Crude fat	36	4	27	47
Sugar	11	5	3	43
Starch	344	38	247	427
Neutral detergent fibre (aNDFom[2])	378	37	278	452
Acid detergent fibre (ADFom[3])	212	24	152	263
Lignin (sa)[4]	17	3	11	26
Silage quality parameters[*]				
pH	3.89	0.14	3.60	4.40
NH_3-nitrogen (g N/kg DM)[5]	1.18	0.36	0.33	2.30

[#] Determined in freeze dried material.
[*] Predicted through near infrared reflection spectroscopy (NIRS) by BLGG, Wageningen, the Netherlands.
[1] Standard deviation.
[2] Neutral detergent fibre assayed with a heat stable amylase and expressed exclusive of residual ash.
[3] Acid detergent fibre expressed exclusive of residual ash.
[4] Lignin determined by solubilization of cellulose with sulphuric acid.
[5] NH_3-nitrogen is the proportion of nitrogen. It is separately presented to show range in the quality parameters of silages.

Table 2 *Diet components of the total mixed ration (TMR) fed to the cows during rumen incubations*

Feed ingredient	g/kg DM in TMR
Maize silage	237.4
Grass silage	395.8
Grass hay/wheat straw	13.5
Soybean meal	47.4
Sweet syrup	9.5
Wet distillers grains with solubles	84.3
Soybean meal (rumen protected)	18.6
Minerals and vitamin premix[1]	10.9
Concentrate[2]	182.6

[1] Contained: calcium 175 g/kg; phosphorous 0.2 g/kg; magnesium 130 g/kg; sodium 50 g/kg; chloride 78 g/kg; vitamin A 600,000 IE/kg; vitamin D 120,000 IE/kg; vitamin E 8,000 IE/kg.
[2] Contained (%): maize, 30; palm kernel, 22; rapeseed solvent extract, 21.3; citrus pulp, 10; soybean meal (rumen protected), 5; beet molasses, 5; molasses, 2; wheat, 2; chalk (CaO), 0.6; urea, 0.6; salt, 0.5; magnesium oxide (80% MgO), 0.5; palm oil, 0.2; vitamin and minerals premix, 0.3.

Chemical analysis

The ground freeze dried maize silage samples were analyzed for DM, ash, CP, crude fat (CFat), sugar, starch, aNDFom, ADFom (acid detergent fibre expressed exclusive of residual ash) and lignin (sa, lignin determined by solubilization of cellulose with sulphuric acid). The ground pooled rumen incubated residues were analyzed for DM, ash, CP, aNDFom and starch. The DM content was determined by oven drying at 103°C for 4 h (ISO 6496) and ash content by incineration at 550°C for 4 h (ISO 5984). The N content was determined using the Kjeldahl method (ISO 5983) and CP was calculated as N x 6.25. Neutral detergent fibre (aNDFom) was determined according to the modified method of Van Soest *et al.* (1991), using amylase and expressing values exclusive of residual ash (ISO 16472). Acid detergent fibre (ADFom) was determined by boiling with acid detergent reagent and expressed exclusive residual ash (ISO 13906: 2008). Lignin (sa) was determined after boiling with acid detergent reagent and pre-treatment of sulphuric acid (ISO 13906:2008). Starch was determined using the amyloglucosidase method (ISO 15914) after dissolving in 100% dimethyl sulfoxide. The CFat and sugar contents were determined according to ISO 6492 and the Luff-Schoorl method (NEN 3571:1947nl), respectively.

Calculations

The fractional degradation rate (k_d, h^{-1}) of DM ($k_{d\text{-}DM}$), OM ($k_{d\text{-}OM}$), CP ($k_{d\text{-}CP}$) and starch ($k_{d\text{-}starch}$) was calculated according to the first order model of Robinson *et al*. (1986) including U, D and k_d:

$$Y_t = U + D_t \times \exp(- k_d \times t) \quad (1)$$

where Y_t = degradation at time t, U = rumen undegradable fraction, D_t = fraction potentially degradable in the rumen at time t, t = time of incubation (h).

The k_d of aNDFom ($k_{d\text{-}aNDFom}$) was calculated by the same model including a lag time (Stensig *et al*., 1994):

$$Y_t = U + D_t \times \exp(- k_d \times (t - L)) \quad (2)$$

where L = lag time.

The effective rumen degradability of DM (ED_{DM}), OM (ED_{OM}), starch (ED_{starch}) and aNDFom (ED_{aNDFom}) of maize silages was calculated according to the equation of Ørskov and McDonald (1979):

$$ED = W + (k_d / (k_d + k_p)) \times D$$

where W = washout fraction, k_p = fractional passage rate (h^{-1}) and D = potentially rumen degradable insoluble fraction.

Five % of the W fraction of CP of forages was assumed to escape rumen degradation (Van Duinkerken *et al*., 2011) and considered to be part of rumen escape protein (REP). Therefore, the ED of CP (ED_{CP}) and REP are calculated by:

$$ED_{CP} = 0.95 \times W + (k_d / (k_d + k_p)) \times D$$

$$REP = U + (k_p / (k_d + k_p)) \times D + 0.05 \times W$$

The k_p values of 0.045 and 0.06 h^{-1} for CP and starch, respectively, were adopted from the DVE/OEB_{2010} system (Van Duinkerken *et al*. 2011). Tuori *et al*. (1998) calculated and compared values of ED_{CP} using different k_p values (0.05, 0.06, 0.08 h^{-1}) adopted from different protein evaluation systems which were also used to calculate the ED_{CP} in the present study. A k_p value of 0.020 h^{-1} was used for aNDFom of maize silages, based on the results of Pellikaan (2004). Jančík *et al*. (2009) used three k_p values for calculating the ED_{DM}; 0.02, 0.05 and 0.08 h^{-1} which are representative of low, medium and high feeding levels, respectively. The ED_{DM} in the present study was also calculated using these three k_p values; 0.02 h^{-1} (ED_{DM2}), 0.05 h^{-1} (ED_{DM5}) and 0.08 h^{-1} (ED_{DM8}). The ED_{OM} in the present study was also calculated using these three k_p values; 0.02 h^{-1} (ED_{OM2}), 0.05 h^{-1} (ED_{OM5}) and 0.08 h^{-1} (ED_{OM8}).

Statistical analysis

Rumen degradation data of DM, OM, CP, starch and aNDFom after incubation in the rumen for different periods (2, 4, 8, 16, 32, 72, and 336 h) was summarized by the Descriptive Statistics of SAS 9.2 (2009). The k_d of the DM, CP and starch of maize silages was calculated using model 1 and the k_d of aNDFom of maize silages was calculated using model 2 in SAS 9.2 (2009). Regression equations were derived to determine the relationships between the chemical composition and the *in situ* rumen degradation characteristics of maize silage samples using PROC REG procedure of SAS 9.2 (2009). The backward stepwise procedure was followed to derive the regression equations with significant predictors (P<0.05). For some parameters, no regression equation was presented as none of the predictors proved significant (P>0.05). Those parameters were U_{CP}, $U_{starch,}$ $k_{d\text{-}starch}$ and U_{aNDFom}.

Results

The nutrient composition and quality parameters of the maize silages showed a broad range (Table 1). The variation in DM, starch and aNDFom content was large compared to other nutrients. There was a negative relationship (R^2=0.40) between the starch and aNDFom content in the maize silages (Figure 1). The average rumen degradable fractions of DM, OM, CP, starch and aNDFom of the maize silages incubated in the rumen of the three cows for 2, 4, 8, 16, 32, 72 and 336 h are shown in Table 3. The range in the values for the rumen degradable fraction of DM, OM, CP and starch was smaller in case of longer (32, 72 and 336 h) incubation periods, compared to short periods (2, 4, 8, and 16 h). In case of aNDFom, the range in the rumen degradable fraction was, however, larger for 16, 32, 72 and 336 h (Table 3). The mean values and range for W, U, D, k_d and ED of DM, OM, CP, starch and aNDFom of the 75 maize silage samples are shown in Table 3. The REP fraction of the maize silage samples ranged from 0.200 to 0.462, with an average value of 0.302 (Table 4). The lag time for aNDFom ranged from 0 to 15.71 h, with an average value of 4.34 h (Table 4).

Table 3 *Average rumen degradable fractions of dry matter, organic matter, crude protein, starch and neutral detergent fibre of maize silages (n=75) incubated for different periods of time (2, 4, 8, 16, 32, 72 and 336 h)*

In situ parameter	Rumen incubation time periods (h)						
	2	4	8	16	32	72	336
Dry matter							
Mean	0.291	0.342	0.453	0.549	0.665	0.774	0.855
SD[1]	0.056	0.053	0.052	0.040	0.035	0.031	0.027
Minimum	0.159	0.188	0.314	0.420	0.582	0.710	0.793
Maximum	0.419	0.475	0.561	0.628	0.760	0.848	0.900
Organic matter							
Mean	0.280	0.333	0.449	0.545	0.666	0.774	0.855
SD	0.058	0.055	0.053	0.041	0.036	0.031	0.026
Minimum	0.145	0.169	0.319	0.413	0.584	0.713	0.801
Maximum	0.406	0.466	0.558	0.623	0.759	0.849	0.906
Crude protein							
Mean	0.473	0.491	0.572	0.685	0.811	0.927	0.971
SD	0.085	0.084	0.071	0.063	0.036	0.014	0.007
Minimum	0.199	0.181	0.436	0.452	0.719	0.884	0.940
Maximum	0.647	0.652	0.736	0.809	0.920	0.957	0.985
Starch							
Mean	0.491	0.585	0.770	0.884	0.966	0.991	0.997
SD	0.127	0.112	0.114	0.067	0.021	0.004	0.002
Minimum	0.164	0.225	0.403	0.612	0.855	0.981	0.992
Maximum	0.711	0.805	0.939	0.959	0.997	1.000	1.000
Neutral detergent fibre							
Mean	-0.020	0.010	0.050	0.151	0.335	0.568	0.744
SD	0.051	0.054	0.054	0.055	0.060	0.055	0.047
Minimum	-0.098	-0.089	-0.065	-0.018	0.218	0.427	0.642
Maximum	0.088	0.118	0.176	0.300	0.492	0.683	0.891

[1] Standard deviation.

Table 4 *Mean, standard deviation (SD), minimum and maximum dry matter, crude protein, starch and neutral detergent fibre values for the washout fraction[1] (W), rumen undegradable fraction (U), potentially rumen degradable fraction[2] (D), degradation rate (k_d, h^{-1}) and effective degradability (ED)[3] of maize silages*

Variable	Mean	SD[4]	Minimum	Maximum
Dry matter (DM)				
W_{DM}	0.271	0.067	0.091	0.420
U_{DM}	0.170	0.030	0.110	0.289
D_{DM}	0.559	0.073	0.431	0.756
$k_{d\text{-}DM}$	0.041	0.007	0.029	0.064
ED_{DM2}	0.645	0.031	0.558	0.715
ED_{DM5}	0.522	0.039	0.427	0.608
ED_{DM8}	0.460	0.043	0.343	0.559
Organic matter (OM)				
W_{OM}	0.258	0.071	0.068	0.413
U_{OM}	0.145	0.026	0.094	0.199
D_{OM}	0.597	0.083	0.444	0.797
$k_{d\text{-}OM}$	0.042	0.007	0.031	0.060
ED_{OM2}	0.662	0.033	0.585	0.742
ED_{OM5}	0.531	0.042	0.418	0.621
ED_{OM8}	0.464	0.048	0.330	0.571
Crude protein (CP)				
W_{CP}	0.497	0.088	0.194	0.687
U_{CP}	0.025	0.008	0.009	0.046
D_{CP}	0.478	0.088	0.296	0.791
$k_{d\text{-}CP}$	0.033	0.006	0.017	0.049
ED_{CP}	0.698	0.051	0.538	0.800
REP[5]	0.302	0.051	0.200	0.462
Starch				
W_{starch}	0.438	0.137	0.062	0.708
U_{starch}	0.006	0.006	0	0.021
D_{starch}	0.556	0.136	0.287	0.979
$k_{d\text{-}starch}$	0.112	0.030	0.053	0.180
ED_{starch}	0.791	0.070	0.527	0.902
Neutral detergent fibre (aNDFom)[6]				
W_{aNDFom}	-0.012	0.055	-0.098	0.099
U_{aNDFom}	0.255	0.048	0.113	0.356
D_{aNDFom}	0.755	0.083	0.559	0.926
$k_{d\text{-}aNDFom}$	0.023	0.004	0.016	0.036
Lag time (h)	4.335	3.928	0	15.709
ED_{aNDFom}	0.389	0.044	0.264	0.524

[1] The fraction disappeared by washing with tap water in a washing machine at 25°C for 40 min.
[2] The *D* fraction was calculated as D = 1 - (W + U).
[3] The ED_{DM} was calculated by using three passage rates; 0.02 h^{-1}(ED_{DM2}), 0.05 h^{-1} (ED_{DM5}) and 0.08 h^{-1} (ED_{DM8}). The ED_{OM} was also calculated by using three passage rates; 0.02 h^{-1} (ED_{OM2}), 0.05 h^{-1} (ED_{OM5}) and 0.08 h^{-1} (ED_{OM8}). The ED_{CP}, ED_{starch} and ED_{aNDFom} were calculated using k_p values of 0.045, 0.06 and 0.020 h^{-1}, respectively.
[4] Standard deviation.
[5] Rumen escape protein. 5 % of the *W* fraction was added in REP.
[6] Neutral detergent fibre assayed with a heat stable amylase and expressed exclusive of residual ash.

The regression equations describing relationships between the W, U, D and ED of DM, OM, CP, starch and aNDFom (dependent variable), and the chemical composition (independent variable) of the maize silages are presented in Table 5. The ED_{DM2}, ED_{DM5} and ED_{DM8} were mainly influenced by the contents of DM and aNDFom whereas the $k_{d\text{-}DM}$ was only affected by the DM content (Table 5). Regression analysis shows that the parameters of OM were influenced by the contents of DM, ash, CFat, aNDFom, ADFom and lignin (sa) in maize silages (Table 5). The regression analysis also identified significant relationships between the W_{CP}, D_{CP}, $k_{d\text{-}CP}$ and ED_{CP}, and the contents of CP, CFat, ADFom and lignin (sa) in the maize silages (Table 5). A positive relationship (R^2=0.72) was found between the CP content and the ED_{CP} of maize silages (Figure 2).

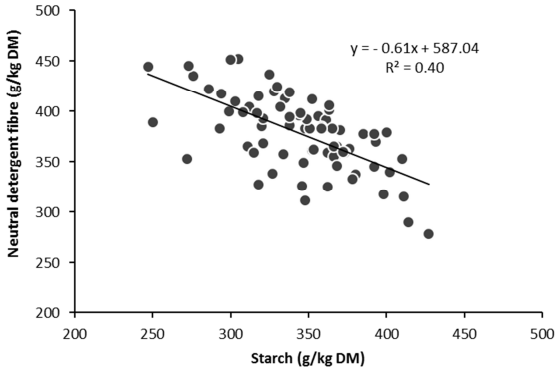

Figure 1 Relationship between starch and neutral detergent fibre (aNDFom) contents in maize silages (n=75).

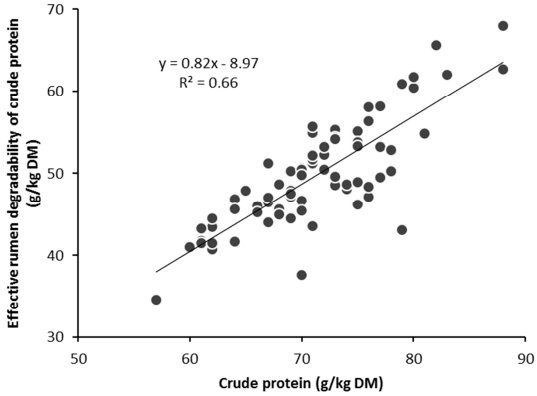

Figure 2 Relationship between crude protein content and effective rumen degradability of crude protein in maize silages (n=75).

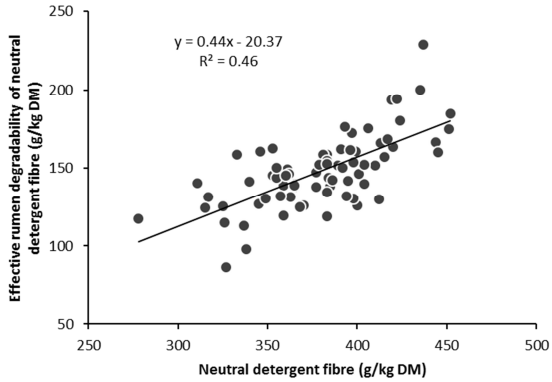

Figure 3 Relationship between neutral detergent fibre content (aNDFom) and effective rumen degradability of aNDFom in maize silages (n=75).

The W_{starch} and D_{starch} were influenced mainly by the DM content whereas the ED_{starch} was influenced by the starch content. Relationships were also found between different degradation parameters of aNDFom and the chemical composition of the maize silages (Table 5). A positive relationship (R^2=0.46) was found between the aNDFom content and the ED_{aNDFom} of maize silages (Figure 3).

The calculated average values for ED_{CP} of the maize silages using k_p values of 0.045, 0.050, 0.060 and 0.080 h^{-1} were 0.700, 0.688, 0.667 and 0.637 respectively (Table 6). The smallest range in ED_{CP} values was observed for the DVE/OEB_{2010} system (k_p, 0.045 h^{-1}) while the largest range in ED_{CP} values was observed for the British Metabolizable protein system when the k_p value of 0.080 h^{-1} was used.

Table 5 *Relationship between the washable content (W, g/kg DM), rumen undegradable content (U, g/kg DM), potentially rumen degradable content (D, g/kg DM), and effective rumen degradation (ED, g/kg DM) of dry matter (DM), crude protein (CP), starch and neutral detergent fibre (aNDFom[1]), and the chemical composition of maize silages*

Regression equation	R^2	RMSE[2]
Dry matter (g/kg fresh matter)		
W_{DM} = 352.54 (± 49.08) - 0.37 (± 0.07) starch - 0.35 (± 0.08) aNDFom	0.29	18.67
U_{DM} = - 100.05 (± 22.03) + 0.14 (± 0.03) DM + 0.10 (± 0.03) starch + 0.36 (± 0.05) ADFom[3]	0.48	6.90
D_{DM} = - 54.51(± 47.92) + 0.84 (± 0.08) DM + 0.27 (± 0.10) aNDFom - 0.67 (± 0.16) ADFom	0.74	21.46
$k_{d\text{-}DM}$ = - 4.61 (± 2.92) + 0.05 (± 0.01) DM	0.37	2.57
ED_{DM2} = 10.74 (± 4.15) + 0.59 (± 0.04) DM + 0.81 (± 0.28) sugar	0.77	12.32
ED_{DM5} = 118.95 (±28.59) + 0.36 (± 0.05) DM - 0.17 (± 0.05) aNDFom	0.60	13.56
ED_{DM8} = 137.45 (± 31.02) + 0.26 (± 0.05) DM - 0.18 (± 0.05) aNDFom	0.45	14.71
Organic matter (g/kg DM)		
W_{OM} = 692.00 (± 128.03) - 0.96 (± 0.21) DM - 0.28 (± 0.21) aNDFom	0.22	61.42
U_{OM} = - 19.88 (± 19.01) - 0.61 (± 0.10) ADFom + 1.83 (± 0.79) lignin (sa)[4]	0.51	18.01
D_{OM} = 362.59 (± 138.53) + 0.85 (± 0.23) DM + 0.95 (± 0.30) aNDFom – 2.11 (± 0.49) ADFom	0.41	62.84
$k_{d\text{-}OM}$ = 10.09 (± 1.40) - 0.04 (± 0.002) DM - 0.03 (± 0.02) CFat[5] - 0.02 (± 0.003) ADFom	0.28	0.57
ED_{OM2} = 942.72 (± 63.12) - 0.26 (± 0.10) DM - 0.63 (± 0.36) ash - 0.51 (± 0.10) aNDFom	0.28	27.48
ED_{OM5} = 874.40 (± 74.02) - 0.50 (± 0.12) DM - 0.50 (± 0.12) aNDFom	0.25	35.51
ED_{OM8} = 849.64 (± 83.27) - 0.63 (± 0.14) DM - 0.48 (± 0.14) aNDFom	0.25	39.95
Crude protein (g/kg DM)		
W_{CP} = - 48.78 (± 11.82) + 0.67 (± 0.12) CP + 0.39 (± 0.17) CFat + 0.11 (± 0.03) ADFom	0.44	6.05
D_{CP} = 40.78 (± 11.82) + 0.27 (± 0.12) CP - 0.35 (± 0.15) CFat - 0.12 (± 0.03) ADFom	0.24	6.21
$k_{d\text{-}CP}$ = 6.68 (± 0.98) + 0.004 (± 0.001) DM + 0.007 (± 0.002) aNDFom - 0.03 (± 0.003) ADFom + 0.07 (± 0.02) lignin (sa)	0.46	0.44
ED_{CP} = 1.11 (± 6.14) - 0.03(± 0.01) DM + 0.81 (± 0.06) CP + 0.17 (± 0.07) sugar	0.76	3.20
Starch (g/kg DM)		
W_{starch} = 437.96 (± 96.18) - 0.47 (± 0.16) DM - 0.34 (± 0.16) aNDFom	0.13	45.62
D_{starch} = - 95.55 (± 57.67) + 0.82 (± 0.15) DM	0.26	50.67
ED_{starch} = 164.48 (± 35.00) + 0.29 (± 0.10) starch	0.10	32.72

Neutral detergent fibre (aNDFom, g/kg DM))		
D_{aNDFom} = 153.52 (± 45.73) - 2.10 (± 0.79) CFat + 0.55 (± 0.10) aNDFom	0.34	29.59
$k_{d\text{-}aNDFom}$ = - 2.95 (± 1.88) + 0.05 (± 0.01) aNDFom + 0.16 (± 0.06) lignin (sa)	0.41	1.49
ED_{aNDFom} = - 4.30 (± 19.67) + 0.66 (± 0.07) aNDFom - 0.48 (± 0.11) ADFom	0.57	15.38

[1] Neutral detergent fibre assayed with a heat stable amylase and expressed exclusive of residual ash.
[2] Root mean square error.
[3] Acid detergent fibre expressed exclusive of residual ash.
[4] Lignin determined by solubilization of cellulose with sulphuric acid.
[5] Crude fat.

Table 6 *Effect of passage rates (k_p, h^{-1}) obtained from different protein evaluation systems on the effective rumen degradability of crude protein*[1]

Protein evaluation system	k_p	Effective rumen degradability of crude protein			
		Mean	SD[2]	Minimum	Maximum
DVE/OEB 2010[3]	0.045	0.700	0.049	0.538	0.800
AAT-PBV[4]	0.050	0.688	0.051	0.517	0.793
PDI[5]	0.060	0.667	0.054	0.483	0.781
MP[6]	0.080	0.637	0.059	0.432	0.764

[1] Effective rumen degradability of crude protein $_{was}$ calculated using same formula; $ED_{CP} = 0.95 \times W + (k_d / (k_d + k_p)) \times D$.
[2] Standard deviation.
[3] Dutch protein evaluation system.
[4] Danish protein evaluation system.
[5] French protein evaluation system.
[6] British metabolizable protein system.

Discussion

The samples used in the present study were collected from different commercial farms in the Netherlands during 2007, 2008 and 2009 to cover a broad range in quality due to growing seasons, weather conditions, soil quality, fertilisation practices, sowing dates, varieties, maturity stages and ensiling practices. The broad range in the chemical composition and quality parameters reflected a large variation in the rumen degradation characteristics between maize silages. The *in situ* rumen degradation characteristics of DM, OM, CP, starch and aNDFom of these maize silages was determined under uniform experimental conditions, i.e. the same cows, the same incubation protocol and the same chemical analysis procedures. This makes these data more representative than the data for maize silages currently used by different feed evaluation systems in Europe e.g. the DVE/OEB_{2010} system (Van Duinkerken *et al.*, 2011) in the Netherlands, Feed into Milk system (Thomas, 2004) in the UK and the NorFor Nordic feed evaluation system (Volden, 2011) used in the Denmark, Sweden, Iceland and Norway. The data used by these feed evaluation systems was compiled from different *in situ* experiments performed at different places for different scientific purposes, under different experimental conditions and with various chemical analysis procedures.

The large range in rumen degradable fractions of DM, OM, CP, starch and aNDFom between different samples at each rumen incubation period was due to the broad range in chemical composition of the maize silage samples. More than 0.95 of the starch was degraded in the rumen after 32 h of rumen incubation and after 336 h actually all the starch (0.997-1.0) was degraded. Fernandez *et al.* (2004) reported that 0.713 to 0.954 of the starch fraction of maize silages was degraded in the rumen after 24 h. The broad range in the chemical composition of the maize silage samples also contributed to the large range in the values for the *W*, *U* and *D*

fractions of DM, OM, CP, starch and aNDFom. The large range in the values of k_d and ED of DM, OM, CP, starch and aNDFom was also due to the variation in the chemical composition of the samples. In the present study, the values for D_{OM} and $k_{d\text{-}OM}$ were 0.597 and 0.042, respectively. Arieli *et al*. (1998) reported *in vivo* values of 0.292 and 0.049 for D_{OM} and $k_{d\text{-}OM}$, respectively. The difference in the observed values between the present study and the study of Arieli *et al*. (1998) might be due to the different techniques (*in situ* vs. *in vivo*) and the number of samples used (75 *vs.* 2) in both studies. Arieli *et al*. (1998) used only two maize silages whereas 75 maize silages with a broad range in chemical composition were used here. In the present study, the ED_{DM} decreased from 0.645 to 0.460 when k_p value increased from 0.020 to 0.08 h^{-1}. The higher k_p value resulted in lower value for ED_{DM} (Arieli *et al.*, 1998).

Different protein evaluation systems use different k_p values for the calculation of ED_{CP}. Tuori *et al*. (1998) compared the values of ED_{CP} using different k_p values originating from different protein evaluation systems. In the present study, these k_p values were used in the same formula to calculate the ED_{CP} for the maize silages (Table 5). Results show that the calculated ED_{CP} value of maize silages decreased when a higher k_p value was used. The higher k_p value might lead to underestimation of the ED_{CP} of maize silages. The differences in ED_{CP} values occurred due to the use of different k_p values and should be taken into account when comparing different protein evaluation systems. Tuori *et al*. (1998) concluded that the nylon bag method overestimates the true differences in the protein degradation value of feeds, especially when high k_p values were used.

During the past three decades, a number of *in situ* studies have been conducted to determine the effect of individual factors such as maturity stage (Jensen *et al.*, 2005), chop length (Fernandez *et al*., 2004) and ensiling (González *et al*., 2010) on the rumen degradation characteristics of the dietary fractions and nutrients of maize silages. Less effort has been expended to determine relationships between the chemical composition and the *in situ* rumen degradation characteristics of maize silages. This information may be important to allow rapid and accurate estimation of the rumen degradation characteristics of maize silages used in practice. In the present study, the results of the regression analysis demonstrated strong, moderate and weak relationships between the rumen degradation characteristics of DM, OM, CP, starch and aNDFom and the chemical composition of maize silages. The D_{DM} was affected by the contents of DM, aNDFom and ADFom in maize silages. The ED_{DM} at different rumen passage rates (k_p) was positively related to DM and negatively related to the aNDFom content of the maize silages, indicating that the presence of insoluble fibre content especially hemicellulose restricts the DM degradation of the maize silages. There was a

negative correlation (R=-0.38, $P<0.001$) between the DM and aNDFom content of the maize silages. The R^2 value of 0.37 shows a moderate relationship between the $k_{d\text{-}DM}$ and DM content in maize silages. The ED_{OM} values calculated using different k_p values (0.02, 0.05, 0.08 h^{-1}), were negatively related to the contents of DM, ash and aNDFom in maize silages. Regression analysis shows that the ED_{CP} was positively related to the CP and sugar contents, and negatively related to DM content of maize silages (Table 4). It means that the ED_{CP} of maize silages which were ensiled from early cut maize crop was higher than the late maturity cut maize crop. The DM content increases but the CP content of maize crop decreases with advanced maturity. In the present study, a significant positive relationship (R^2=0.72, $P<0.05$) was also found between the ED_{CP} and the CP content in maize silages (Figure 2). A high CP content in silages indicates that more N is present inside the cell which is readily degradable in the rumen of cows. Also Vik-Mo (1989) found that a higher CP content in forages resulted in higher value for ED_{CP}. The W_{starch} showed a negative relationship with DM and aNDFom contents. This is caused by difference in maturity stage of maize crop at cutting. More mature maize crop means more starch present, resulting in less DM and aNDFom contents. The negative correlation (R=-0.63, $P<0.001$) between the starch and aNDFom contents is also related to the maturity of the maize crops used for making silage. This negative relationship (R^2=0.40) between starch and aNDFom contents of maize silages is also shown in Figure 1. This relationship varied at different maturity stages of the maize crops. The DM and starch content of maize crops increase but the aNDFom content decreases with maturation of the maize crop. The D_{starch} was positively related to the DM content. The DM and starch content of the maize silages largely influenced the starch degradation in the rumen (Offner *et al.*, 2003). The R^2 value of 0.10 shows that there was a weak relationship between the ED_{starch} and the starch content. In the present study, the ED_{aNDFom} was positively related with the aNDFom content and negatively related to ADFom content of maize silages. This result may highlight the limitation of the use of stepwise regression and may be due to the hemicellulose content in the aNDFom which is readily degradable in the rumen of dairy cows compared to the cellulose content. No relationship was found between the $k_{d\text{-}aNDFom}$ and lag time of maize silages but the lag time varied between different samples of maize silages. The $k_{d\text{-}aNDFom}$ positively related to contents of aNDFom and lignin (sa) in maize silages but this relationship was moderate (R^2=0.41). The positive relationship (R^2=0.46) showing that $k_{d\text{-}aNDFom}$ increased with increasing aNDFom content in maize silages (Figure 3). Because of lower R^2 values, most of the developed regression equations are of limited use for the prediction of rumen degradation characteristics of dietary nutrients. However, a number of the developed

equations are valuable for the estimation of rumen degradation characteristics of maize silages used in practice.

Conclusions

The present study reports strong, moderate and weak relationships between the chemical composition and *in situ* degradation characteristics of dietary fractions or nutrients of maize silages used in practice. Unlike previous studies, a large number of maize silages were used (n=75) to include the variation occurs due to growing season, location and chemical composition and were tested under uniform experimental conditions. A number of the developed regression equations can be used in practice for the rapid assessment of the nutritive value of maize silages and optimal formulation of rations for dairy cows.

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CHAPTER 3

Relationship between chemical composition and *in situ* rumen degradation characteristics of grass silages in dairy cows

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Abstract

The DVE/OEB$_{2010}$ system in the Netherlands uses a large database of *in situ* rumen incubations with grass silage and grass hay samples to derive prediction formulas to estimate the rumen degradation characteristics of a number of feed value parameters. These *in situ* rumen incubations were not performed for this specific purpose and the data were generated at different research institutes over more than 40 years, using different grass management and fertilisation practices and using different protocols. The objectives of this study were to 1) generate a new database on the rumen degradability of dry matter (DM), organic matter (OM), crude protein (CP) and neutral detergent fibre (NDF) of grass silages, 2) compare this new database with the old database used in the DVE/OEB$_{2010}$ system, and 3) derive regression equations using the new database to investigate the relationship between chemical composition and *in situ* ruminal degradability of DM, OM, CP and NDF of the grass silages. Sixty nine grass silages, with a broad range in chemical composition and quality parameters, were selected and incubated using the nylon bag technique in the rumen of three lactating Holstein Friesian cows for 2, 4, 8, 16, 32, 72 and 336 h. There was a large range in the rumen degradable fractions of DM, OM, CP and NDF of the grass silages at each rumen incubation period. The rumen degradability of DM, OM, CP and NDF in the present study was determined using the same standard incubation protocol, the same cows, and the same chemical analysis procedures for all the grass silage samples. Regression analysis, using the new database, showed relationships between the washable content, rumen undegradable content, potentially rumen degradable content and effective rumen degradability of DM, OM, CP and NDF, respectively, and the chemical composition of the grass silages. The rumen escape protein was also affected by the variation in the chemical composition of grass silages.

Introduction

Grass silage is a forage biomass that is mainly used as a winter fodder for dairy cows and is the most important form of conserved forage for ruminants in many regions of Europe (Dawson *et al*., 2002). Protein in grass silage is more degradable in the rumen than protein in grass hay and fresh grass due to the fermentation processes in the silos (De Boever *et al*., 2004). Ruminal degradation of different components of grass silages is influenced by many factors such as stage of maturity, preservation method, forage species and cultivars (Hoffman *et al*., 1993; Elizalde *et al*., 1999; Beever and Mould, 2000).

To optimize diet formulation in terms of performance, nutrient losses, animal well-being and economical profitability, information is required on the nutrient requirements of animals and nutrient availability of various feedstuffs. In ruminants, the nutrient availability can be measured using various *in situ*, *in vitro* and *in vivo* techniques. The *in situ* nylon bag technique is the most frequently used technique for the determination of degradability parameters of chemical feed components such as dry matter (DM), crude protein (CP), neutral detergent fiber (NDF), minerals and trace elements (Harazim *et al*., 2002; Homolka *et al*., 2002; Jančík *et al*., 2008) and is considered a reference method to determine the rumen degradation characteristics of feedstuffs (De Boever *et al*., 2002).

Various ruminant feed evaluation systems, such as the DVE/OEB_{2010} system (Van Duinkerken *et al*., 2011) in the Netherlands and the PDI system (Vérité and Peyraud, 1989) in France, use regression equations or prediction formulas to estimate the rumen degradation characteristics of a number of feed value parameters. The Dutch DVE/OEB_{2010} feed evaluation system (Van Duinkerken *et al*., 2011) uses a database compiled from different *in situ* rumen incubations studies with grass silage and grass hay samples. These studies were conducted at various research institutes in the Netherlands and Belgium employing different protocols to estimate the degradation characteristics of certain feed components spanning several decades. These *in situ* rumen incubations studies were not performed for the specific purpose to derive regression equations for the DVE/OEB_{2010} feed evaluation system but were conducted to investigate specific scientific hypotheses. At present, these results can be considered outdated as most of these experiments were conducted before 1995 during which time a large variety in crop species and cultivars were used in the Netherlands. Nitrogen fertilization level, ensiling methods and incubation protocols have changed, compared to when the studies were conducted and which data have been used in the DVE/OEB_{2010} database. Different subsets of the DVE/OEB_{2010} database were used to derive prediction

formulas for degradation characteristics of nutrients for grass silage and grass hay samples. For example, data of a subset of 97 grass silage and grass hay samples were used to derive prediction formulas for the degradation characteristics of CP while the data of a subset of 50 samples were used to derive prediction formulas for the degradation characteristics of NDF.
The objective of this study was to improve the prediction of the feeding value of grass silages. This study reports the 1) generating of a new database on the rumen degradability of DM, OM, CP and NDF of grass silages, 2) comparison of this new database with the old database used in the DVE/OEB_{2010} system, and 3) regression equations derived using new database to investigate the relationship between chemical composition and the *in situ* ruminal degradability of DM, OM, CP and NDF of grass silages.

Materials and methods

Selection of grass silages

More than one hundred grass (mainly *Lolium perenne*) silage samples (~10 kg per silage) were obtained during 2007, 2008 and 2009 from various Dutch commercial farms located in different regions in the Netherlands. The samples were collected by trained technicians from a feed analysis laboratory (Blgg AgroXpertus, Wageningen, The Netherlands) using a hollow drill. After collection, individual silages were homogenized, divided into roughly two equal parts with one half air dried (70°C for 16 h) and subjected to chemical analyses while the other half was stored at -20°C. After chemical analyses of the air dried material, a table was developed containing information on chemical composition and quality parameters of grass silages. The information on pH and ammonia-nitrogen was used to show quality range in grass silage samples. A total of 69 grass silage samples were selected on the basis of broad range in chemical composition and silage quality parameters to use in this nylon bag study. The selected samples were stored at -20°C. Each selected grass silage stored at -20°C was located and cut using a bread slicer (JAC Duro BEL 450; ABO, Leek, The Netherlands) having a distance of 11 mm between the discs, thoroughly mixed by hand and divided into three parts; one part (~2.5 kg) was subjected to wet chemical analyses after freeze drying, another part (~1.5 kg) stored (-20°C) for later nylon bag incubations, and the third part (~1 kg) was stored (-20°C) as a reserve for possible future reanalysis.

In situ *rumen incubations*

Three multiparous (second lactation) Holstein Friesian cows, producing >15 kg milk per day, fitted with permanent ruminal cannulas were used in this experiment. Cows were fed total mixed ration (for composition see Table 2 of Chapter 2) twice a day and had 24 h/d access to fresh water. The grass silage samples were incubated in the rumen of the cows which were housed at the research farm "Waiboerhoeve" (Wageningen UR Livestock Research, Lelystad, The Netherlands) according to the procedure described by Cone *et al*. (2004). The fresh grass silage samples (~5 g DM) were weighed into 10 cm x 19 cm nylon bags (pore size 37 µm; porosity 24 %; Nybolt, Zürich, Switzerland) and the bags were stored at -20°C. The frozen bags were incubated in the rumen for 2, 4, 8, 16, 32, 72 and 336 h. The 0 h bags were washed in the washing machine (AEG-Electrolux Öko Turnamat 2800, Stockholm, Sweden) for 40 min using tap water at 25°C without incubation in the rumen and residues were used to calculate the W fraction. Six bags of each grass silage sample, combined with 2 reference samples per series, were incubated in the rumen of the three cows (2 bags per cow per incubation time) for 2, 4, 8, 16, and 32 h. Because of a low recovery of incubated residue per nylon bag for the 72 and 336 h incubation periods, 9 bags of each grass silage and 2 reference samples were incubated in the rumen of the three cows (3 bags per cow per incubation time) for these incubation periods. After removal from the rumen, bags were placed in ice water after which the bags were washed to remove the adhering stuffs. Then the bags were stored in freezer at -20°C for at least 24 h, after which the bags were thawed and washed in the washing machine as described above. The washed bags were stored at -20°C and subsequently freeze dried. For each grass silage sample, rumen incubation residues from the three cows during one incubation period were pooled and the contents were ground over a 3 mm sieve, using a hammer mill (Pepping, 200 AN-797002, Deventer, The Netherlands).

Chemical analyses

The ground (3 mm) freeze dried grass silage samples were analyzed for DM, ash, CP, crude fat (CFat), crude fibre (CF), sugar, NDF, acid detergent fibre (ADF), acid detergent lignin (ADL), neutral detergent insoluble nitrogen (NDIN) and acid detergent insoluble nitrogen (ADIN). The pooled, ground rumen incubated residues were analysed for DM, ash, CP and NDF. The DM content was determined by oven drying at 103°C for 4 h (ISO 6496) and ash content by incineration at 550°C for 4 h (ISO 5984). The NDF was determined according to the modified method of Van Soest *et al.* (1991) with the use of amylase (ISO 16472) and expressed without residual ash. The ADF was determined by boiling with acid detergent

reagent and expressed without residual ash (ISO 13906:2008). The ADL was determined after boiling with acid detergent reagent and a treatment with sulphuric acid (ISO 13906:2008). Crude fat (CFat) was determined by ISO 6492 and sugar content was determined by the Luff-Schoorl method (NEN 3571:1947nl). The N was determined using the Kjeldahl method (ISO 5983) and CP was calculated as N×6.25.

Calculations

Effective rumen degradability (ED)

The ED of DM (ED_{DM}), NDF (ED_{NDF}) and OM (ED_{OM}) was calculated according to equation of Ørskov and McDonald (1979):

$$ED = W + (k_d / (k_d + k_p)) \times D \quad (1)$$

where W = washable fraction, k_d = fractional degradation rate (h^{-1}), k_p = fractional passage rate (h^{-1}) and D = potentially rumen degradable insoluble fraction. For silages, 5% of W fraction of CP was assumed to be REP (Van Duinkerken *et al*., 2011), therefore the ED of CP (ED_{CP}) was calculated by the modified formula in the present study;

$$ED_{CP} = 0.95 \times W + (k_d / (k_d + k_p)) \times D \quad (2)$$

Rumen escape protein (REP)

The REP was calculated using the following modified model of Cone *et al.* (2009):

$$REP = U + (k_p / (k_d + k_p)) \times D + 0.05 \times W \quad (3)$$

where U = rumen undegradable fraction.

Fractional degradation rate (k_d)

The k_d of DM, OM and CP was calculated according to the first order model of Robinson *et al*. (1986) including U, D and k_d:

$$Y_t = U + D_t \times \exp(- k_d \times t) \quad (4)$$

where Y_t = degradation at time t, D_t = potentially degradable fraction in the rumen at time t, The k_d of NDF was calculated using model 4 including a lag time (Stensig *et al*., 1994):

$$Y_t = U + D_t \times \exp(- k_d \times (t - L)) \quad (5)$$

where L = lag time (h).

Fractional passage rate (k_p)

Jančík *et al*. (2009) used three k_p values for calculating the ED of DM; 0.02, 0.05 and 0.08 h^{-1} representing low, medium and high feeding amounts respectively The ED of DM in the

present study was calculated using three corresponding k_p values of 0.02 h^{-1} (ED_{DM2}), 0.05 h^{-1} (ED_{DM5}) and 0.08 h^{-1} (ED_{DM8}). In the present study, the ED of OM of grass silages was calculated using the same three k_p values as used for DM; 0.02 h^{-1} (ED_{OM2}), 0.05 h^{-1} (ED_{OM5}) and 0.08 h^{-1} (ED_{OM8}). An average k_p value for NDF of 0.025 h^{-1} (range 0.018 and 0.029 h^{-1}) was used based on data for fresh grass and grass silages of Pellikaan (2004). The k_p value (0.045 h^{-1}) for CP was adopted from the DVE/OEB_{2010} system (Van Duinkerken *et al*., 2011). Tuori *et al*. (1998) compared values of ED_{CP} using different k_p values (0.05, 0.06, 0.08 h^{-1}) adopted from different protein evaluation systems which were also used to calculate the ED_{CP} in the present study.

Statistical Analysis

Rumen degradability data of DM, CP and NDF after different rumen incubation times (2, 4, 8, 16, 32, 72, and 336 h) were summarized by descriptive statistics. The k_d was calculated using model 4 and 5 in Genstat (14th edition). Regression equations were derived to determine the relationship between the W content, U content, D content and ED of DM, OM, CP and NDF and the chemical composition of grass silage samples using the PROC REG procedure of SAS 9.2. The backward stepwise procedure was followed to derive the regression equations with significant predictors (P<0.05). For k_d of DM, OM, CP and NDF, no regression equation was presented as none of the predictors proved significant (P<0.05).

Results

Chemical composition of grass silages (n=69) used in this experiment and, grass silage (n=102) and grass hay (n=14) samples in the database used by the DVE/OEB_{2010} system is shown in Table 1. The grass silage/hay samples in the DVE/OEB_{2010} database had a larger range in DM content (143 to 909 g/kg fresh matter) than the silages evaluated in the present study (201 to 680 g/kg fresh matter). The average grass silage/hay CP content (198 g/kg DM) was higher in the samples of the DVE/OEB_{2010} database compared to the present study (169.5 g/kg DM) while the reverse was observed for the average sugar content (91 *vs.* 55 g/kg DM). No information was available on the quality parameters of the grass silages used in the DVE/OEB_{2010} database.

Table 1 *Chemical composition of grass silages (n=69) used in this experiment and, grass silage (n=102) and grass hay (n=14) samples in the database used by the DVE/OEB$_{2010}$ system*

	Grass silages used in this experiment				DVE/OEB$_{2010}$ database[1]			
Variable	Mean	SD[2]	Minimum	Maximum	Mean	SD	Minimum	Maximum
Chemical composition (g/kg DM)[#]								
Dry matter (g/kg fresh matter)	453.6	112	201	680	473	194	143	909
Ash	102.2	18.2	70	192	113	24	31	250
Crude protein	169.5	28.8	109	222	198	48	99	305
Crude fat	42	7.3	28	63	40	7	17	62
Crude fiber	256.6	26.1	162	323	265	36	201	342
Sugar	91	44.6	12	246	55	49	1	228
Neutral detergent fibre	491.4	53.4	326	611	470	68	330	669
Acid detergent fibre	267.8	28.9	157	332	287	42	220	355
Acid detergent lignin	19.3	6.6	10	40	27	13	10	57
NDIN[3] (g N/kg DM)	23.5	14.5	6.4	67	nd[4]	nd	nd	nd
ADIN[5] (g N/kg DM)	1.5	0.7	-0.1	4.6	nd	nd	nd	nd
Silage quality parameters[*]								
pH	5.01	0.63	4.00	6.67	nd	nd	nd	nd
NH_3-nitrogen (g N/kg DM)	2.40	0.94	0.93	7.94	nd	nd	nd	nd

[#] Chemical composition of grass silages used in this experiment was determined on freeze dried material.
[*], Silage quality parameters were determined on air dried material.
[1] The data were compiled from different *in situ* experiments that were performed 17 to 50 years ago for different purposes.
[2] Standard deviation.
[3] Neutral detergent insoluble nitrogen.
[4] Not determined.
[5] Acid detergent insoluble nitrogen.

Table 2 *Average rumen degradable fractions of dry matter, organic matter, crude protein and neutral detergent fibre of grass silages (n=69) incubated for different time periods (2, 4, 8, 16, 32, 72 and 336 h)*

Time periods (h)	Dry matter				Organic matter				Crude protein				Neutral detergent fibre (NDF)			
	Mean	SD[1]	Minimum	Maximum	Mean	SD	Minimum	Maximum	Mean	SD	Minimum	Maximum	Mean	SD	Minimum	Maximum
2	0.306	0.067	0.158	0.505	0.273	0.064	0.132	0.479	0.522	0.087	0.343	0.727	-0.001	0.043	-0.094	0.087
4	0.323	0.070	0.156	0.523	0.292	0.065	0.143	0.493	0.531	0.091	0.342	0.723	0.020	0.045	-0.081	0.115
8	0.389	0.076	0.226	0.564	0.367	0.074	0.209	0.547	0.560	0.090	0.341	0.743	0.129	0.070	-0.015	0.443
16	0.528	0.075	0.369	0.715	0.514	0.074	0.338	0.702	0.662	0.082	0.454	0.830	0.326	0.074	0.180	0.512
32	0.698	0.066	0.544	0.838	0.701	0.060	0.544	0.831	0.740	0.071	0.547	0.891	0.605	0.066	0.442	0.728
72	0.797	0.054	0.660	0.877	0.801	0.046	0.658	0.881	0.773	0.070	0.576	0.894	0.764	0.052	0.624	0.863
336	0.841	0.036	0.742	0.916	0.847	0.031	0.761	0.930	0.777	0.065	0.539	0.878	0.846	0.031	0.738	0.905

[1] Standard deviation.

There was a large range in the values for rumen degradable fraction of DM, OM, CP and NDF of the grass silage samples for each incubation period (Table 2). The range in the values for rumen degradable fraction of DM, OM and CP was larger in the case of short (2, 4, 8, and 16 h) compared to longer rumen incubation periods (32, 72 and 336 h). In case of NDF, the range in the rumen degradable fraction was, however, larger after 16, 32, 72 and 336 h. The mean and range in values for W, U, D, k_d and ED of DM, OM, CP and NDF are shown in Table 3. The average values for the W fraction of DM (W_{DM}), OM (W_{OM}), CP (W_{CP}) and NDF (W_{NDF}) were 0.240, 0.205, 0.405 and 0.007, respectively. The REP fraction of the grass silages ranged from 0.271 to 0.522, with an average value of 0.390 (Table 3). The lag time for NDF ranged from 3.3 to 8.3 h with an average value of 4.9 (Table 3).

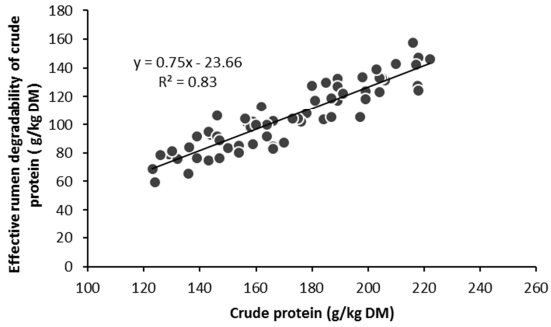

Figure 1 Relationship between crude protein and effective rumen degradability of crude protein of grass silages.

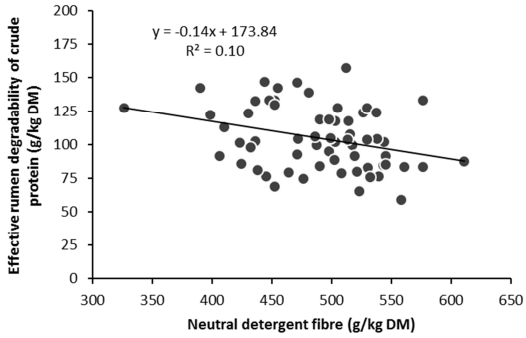

Figure 2 Relationship between neutral detergent fibre and effective rumen degradability of crude protein of grass silages.

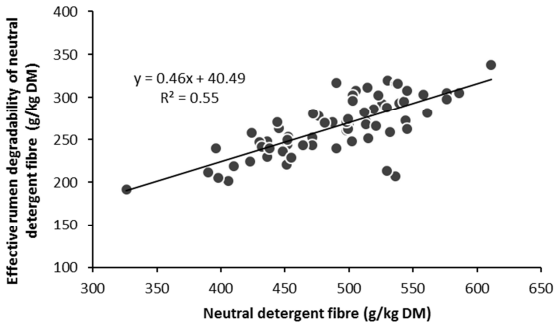

Figure 3 Relationship between neutral detergent fibre and effective rumen degradability of neutral detergent fibre of grass silages.

There was strong relationship (R^2=0.83) found between the CP content and ED_{CP} of grass silage samples (Figure 1) whereas the relationship (R^2=0.10) between the NDF content and the ED_{CP} of grass silages was weak (Figure 2). The relationship (R^2=0.55) was also found between the NDF content and the ED_{NDF} of grass silage samples (Figure 3).

The results of the multiple linear regression analyses between the W, U, D and ED of the DM, OM, CP and NDF on the one hand and the chemical composition of the grass silages on the other hand are reported in Table 4. The W_{DM}, U_{DM}, D_{DM}, ED_{DM2}, ED_{DM5} and ED_{DM8} were mainly influenced by the contents of DM, ash and NDF while the W_{OM}, U_{OM}, D_{OM}, ED_{OM2}, ED_{OM5} and ED_{OM8} were mainly influenced by the contents of ash and NDF in grass silages. The W_{CP}, U_{CP}, D_{CP} and ED_{CP} were influenced by the contents of CP and NDF in the grass silages. The D_{NDF} and ED_{NDF} were influenced by the content of NDF while the U_{NDF} was influenced by the contents of sugar and ADL. The relationship between REP and the chemical composition was explained by the contents of CP, NDF and ADF (Table 4).

The results of the ED_{CP} of 69 grass silages calculated using different k_p values from different protein evaluation systems are shown in Table 5. The average calculated values for ED_{CP} using k_p values of 0.045, 0.050, 0.060 and 0.080 h^{-1} were 0.610, 0.601, 0.585 and 0.560, respectively (Table 5). The largest range in ED_{CP} values was observed when the k_p value (0.080 h^{-1}) of the British Metabolizable protein system was used while the smaller range was observed for the DVE/OEB_{2010} system.

Table 3 *The average, minimum and maximum dry matter, organic matter, crude protein and neutral detergent fiber values for the washout fraction[1] (W), rumen undegradable fraction[2] (U), potentially rumen degradable fraction[3] (D), degradation rate (k_d, h^{-1}) and effective degradability (ED)[4] of 69 grass silages*

Variable	Mean	SD[5]	Minimum	Maximum
Dry matter (DM)				
W_{DM}	0.240	0.062	0.101	0.412
U_{DM}	0.159	0.036	0.084	0.258
D_{DM}	0.601	0.054	0.469	0.742
k_d	0.043	0.007	0.030	0.064
ED_{DM2}	0.648	0.048	0.528	0.750
ED_{DM5}	0.516	0.055	0.380	0.647
ED_{DM8}	0.449	0.057	0.314	0.592
Organic matter (OM)				
W_{OM}	0.205	0.061	0.086	0.389
U_{OM}	0.153	0.031	0.070	0.239
D_{OM}	0.642	0.056	0.498	0.780
k_d	0.043	0.007	0.029	0.062
ED_{OM2}	0.640	0.047	0.507	0.760
ED_{OM5}	0.499	0.056	0.351	0.646
ED_{OM8}	0.427	0.058	0.281	0.588
Crude protein (CP)				
W_{CP}	0.405	0.087	0.205	0.598
U_{CP}	0.222	0.065	0.122	0.461
D_{CP}	0.373	0.103	0.143	0.584
k_d	0.068	0.025	0.024	0.136
ED_{CP}	0.610	0.061	0.478	0.729
REP[6]	0.390	0.061	0.271	0.522
Neutral detergent fibre (NDF)				
W_{NDF}	0.007	0.046	0.000	0.090
U_{NDF}	0.154	0.031	0.095	0.262
D_{NDF}	0.839	0.054	0.746	0.951
k_d	0.046	0.009	0.022	0.083
Lag time (h)	4.892	1.070	3.320	8.280
ED_{NDF}	0.545	0.046	0.386	0.647

[1] The fraction disappeared by washing with tap water in a washing machine at 25°C for 40 min.
[2] The residual fraction determined after 336 h incubation in the rumen.
[3] The *D* fraction was calculated as D = 1-(W+U).
[4] The ED of DM was calculated by using three passage rates; 0.02 h^{-1}(ED_{DM2}), 0.05 h^{-1} (ED_{DM5}) and 0.08 h^{-1} (ED_{DM8}). The ED of OM was also calculated by using three passage rates; 0.02 h^{-1}(ED_{OM2}), 0.05 h^{-1} (ED_{OM5}) and 0.08 h^{-1} (ED_{OM8}). The ED_{CP} was calculated using 0.045 h^{-1} value of passage rate while the ED_{NDF} was calculated using 0.025 h^{-1} value of passage rate.
[5] Standard deviation.
[6] Rumen escape protein. 5 % of W was added in REP.

Table 4 *Relationship between the washable content (W, g/kg DM), rumen undegradable content (U, g/kg DM), potentially rumen degradable content (D, g/kg DM), and effective degradability (ED, g/kg DM) of dry matter (DM), organic matter (OM), crude protein (CP) and neutral detergent fibre (NDF), and the chemical composition of grass silages*

Regression equation	R^2	RMSE[1]
Dry matter (g/kg fresh matter)		
W_{DM} = 140.55 (± 25.55) + 0.21 (± 0.03) DM - 0.25 (± 0.05) NDF	0.49	22.60
U_{DM}= - 35.18 (± 13.17) + 0.17 (± 0.02) DM + 0.30 (± 0.10) ash	0.61	15.92
D_{DM} = 102.44 (± 24.19) + 0.64 (± 0.02) DM + 0.18 (± 0.05) NDF	0.92	21.40
ED_{DM2} = 48.41 (± 17.26) + 0.61 (± 0.02) DM - 0.32 (± 0.14) ash	0.91	20.91
ED_{DM5} = 146.94 (± 31.20) + 0.50 (± 0.02) DM - 0.40 (± 0.14) ash - 0.20 (± 0.05) NDF	0.86	21.40
ED_{DM8} = 153.50 (± 31.60) + 0.43 (± 0.03) DM - 0.36 (± 0.15) ash - 0.22 (± 0.05) NDF	0.81	21.67
Organic matter (g/kg DM)		
W_{OM} = 570.90 (± 13.91) - 0.79 (± 0.08) NDF	0.57	36.41
U_{OM} = 225.87 (± 20.17) - 0.47 (± 0.09) CP - 0.35 (± 0.06) sugar + 1.24 (± 0.37) NDF	0.53	19.34
D_{OM} = 250.28 (± 59.21) + 0.28 (± 0.13) sugar + 0.61 (± 0.06) NDF	0.32	44.73
ED_{OM2} = 993.68 (± 35.98) - 0.87 (± 0.17) ash - 0.68 (± 0.06) NDF	0.69	24.66
ED_{OM5} = 913.99 (± 40.82) - 0.71 (± 0.19) ash - 0.80 (± 0.06) NDF	0.70	27.98
ED_{OM8} = 859.19 (± 42.07) - 0.64 (± 0.19) ash - 0.84 (± 0.07) NDF	0.70	28.84
Crude protein (g/kg DM)		
W_{CP} = 54.51 (± 16.82) - 0.06 (± 0.01) DM + 0.47 (± 0.04) CP - 0.19 (± 0.04) NDF + 0.20 (± 0.08) ADF[2]	0.77	9.94
U_{CP} = 61.58 (± 5.22) - 0.09 (± 0.02) CP - 0.11 (± 0.02) sugar	0.39	5.71
D_{CP}= - 74.30 (± 10.63) + 0.10 (± 0.01) DM + 0.56 (± 0.05) CP	0.73	12.33
REP[3] = - 19.14 (± 14.29) - 0.76 (± 0.04) CP + 0.17 (± 0.03) NDF - 0.14 (± 0.06) ADF	0.44	8.58
ED_{CP} = 19.17 (± 14.29) + 0.76 (± 0.04) CP - 0.17 (± 0.03) NDF + 0.14 (±0.06) ADF	0.88	8.58
Neutral detergent fibre (NDF, g/kg DM))		
U_{NDF} = 68.33 (± 8.39) - 0.15 (± 0.04) sugar + 1.10 (± 0.31) ADL[4]	0.33	15.94
D_{NDF} = 76.13 (± 28.89) + 0.68 (± 0.06) NDF	0.67	25.64
ED_{NDF} = 47.30 (± 26.08) + 0.45 (± 0.05) NDF	0.52	23.14

[1] Root mean square error.
[2] Acid detergent fibre.
[3] Rumen escape protein. 5 % of W was also added in REP.
[4] Acid detergent lignin.

Table 5 *Effect of passage rates (k_p, h^{-1}) obtained from different protein evaluation systems on the effective rumen degradability of crude protein (ED_{CP})*[1]

Protein evaluation system	k_p	ED_{CP}			
		Mean	SD[2]	Minimum	Maximum
DVE/OEB 2010[3]	0.045	0.610	0.061	0.478	0.729
AAT-PBV[4]	0.050	0.601	0.062	0.471	0.722
PDI[5]	0.060	0.585	0.064	0.452	0.711
MP[6]	0.080	0.560	0.066	0.424	0.693

[1] The ED_{CP} was calculated using same formula; $ED_{CP} = 0.95 \times W + (k_d / (k_d + k_p)) \times D$.
[2] Standard deviation.
[3] Dutch protein evaluation system.
[4] Danish protein evaluation system.
[5] French protein evaluation system.
[6] British metabolizable protein system.

Discussion

The DVE/OEB_{2010} database consisted of data obtained from different *in situ* experiments performed at different institutes in the Netherlands 17 to 50 years ago. These studies used different grass species, ensiling methods, fertilisation practices and incubation protocols compared to the present study. In addition, different numbers of samples were used to derive prediction formulas for the degradation characteristics of CP (n=97) and NDF (n=50). Moreover, different chemical analyses procedures were used across the different *in situ* experiments and these experiments were performed to investigate specific scientific hypotheses. In contrast, the database generated in the present study included 69 grass silages which were selected on the base of a large range in chemical composition and quality parameters and purposely used to derive regression equations for the prediction of the feeding value of grass silages. The same standard incubation protocol, same cows, and same chemical analysis procedures were used for all the grass silages in the new database. In addition, the regression equations were developed to predict the degradation characteristics of DM, OM, CP and NDF of grass silages. The main disadvantage of DVE/OEB_{2010} database is that it is combined database of grass silage and grass hay samples. The chemical composition of grass silage and grass hay samples is different. This variation in chemical composition due to combination of grass silage and hay samples makes this database unreliable.

The large range in the DM content of the samples in the DVE/OEB_{2010} database was due to the inclusion of grass silage and grass hay samples, which vary highly in their DM content. The average CP content in the DVE/OEB_{2010} database was higher than the grass silages used in the present study, which is likely caused by a much higher N fertilization level on grasslands 30 years ago. Nowadays, the N fertilization level is much lower due to effective

legislation implemented in 1984 to reduce environmental pollution (Jongbloed and Lenis, 1998). The higher average sugar content in the grass silage samples used in the present study might be due to lower N fertilization and lower NDF and CF contents in combination with higher DM content of the samples. The average U_{CP} fraction (0.222) in the new database obtained in the present study was higher than the U_{CP} fraction (0.109) in the DVE/OEB_{2010} database. The higher U_{CP} fraction might be due to different CP content of samples (169.5 g/kg DM) used in the present study compared to the samples (198 g/kg DM) used in DVE/OEB_{2010} database. High CP content of grass silage samples used in DVE/OEB_{2010} database means that more N was present inside the cell. That N was readily degradable in the rumen and leaving small fraction for U_{CP}. It also might be due to microbial contamination of the rumen incubated residues. The results of this study were not corrected for microbial contamination. The average k_d value (0.068) of CP was also higher in the new database compared to the k_d value (0.058) of DVE/OEB_{2010} database. This may be due to the use of only grass silages in the present study. De Boever *et al*. (2004) also concluded that the CP content in grass silage is more degradable in the rumen than that of grass hay and fresh grass due to the fermentation processes in the silos. The average U_{NDF} fraction (0.154) of grass silages used in the new database was close to the average U_{NDF} fraction (0.180) of grass silages and grass hay used in the DVE/OEB_{2010} database but the range in U_{NDF} fraction was larger (0.088 to 0.383 *vs.* 0.095 to 0.262) in the DVE/OEB_{2010} database than the new database. This larger range in the DVE/OEB_{2010} database may be due to the use of grass silage and grass hay samples together, different incubation protocols, and different number of grass silage samples compared to the new database.

The range in the rumen degradable fractions of DM, OM, CP and NDF of the grass silages after different incubation periods in the rumen was due to the large range in the chemical composition of the grass silage samples. In the present study, the average values for W_{DM}, W_{OM}, W_{CP} and W_{NDF} fractions were 0.240, 0.205, 0.405 and 0.007, respectively. Gosselink *et al*. (2004) reported a W_{DM} fraction of 0.250 which is similar to the results obtained in the present study. The W_{CP} fraction reported by Cone *et al*. (2004) was 0.535 for low DM grass silages (± 250 g DM/kg) and 0.408 for high DM grass silages (± 450 g DM/kg). The average U_{CP} fraction in the present study was 0.222, whereas Cone *et al*. (2004) reported 0.181 average value for U_{CP} fraction of low DM grass silages and 0.184 average value for U_{CP} fraction of high DM grass silages. The difference in W_{CP} and U_{CP} fractions might be due to the high CP content present in the grass silages (208 g/kg DM for low DM grass silages and

209 g/kg DM for high DM grass silages) used by Cone *et al*. (2004) compared to the present study (169.5 g/kg DM).

The large range in the ED values of DM, OM, CP and NDF was also due to larger range in the chemical composition of the grass silages. In the present study, average ED_{DM2}, ED_{DM5} and ED_{DM8} values were 0.648, 0.516 and 0.449, respectively. Jančík *et al*. (2009) reported values of 0.665, 0.539 and 0.478 for ED_{DM2}, ED_{DM5} and ED_{DM8}, respectively, for grass silages. The difference in the values could be due to the low average ash content (84.5 g/kg DM) of grass silages used by Jančík *et al*. (2009) compared to the ash content (102.2 g/kg DM) of the grass silages used in the present study and the different number of grass silages (n=40) studied by Jančík *et al*. (2009). The average value for ED_{NDF} in the present study was 0.545, which is lower than the ED_{NDF} value of 0.660 reported for grass cubes (Stensig *et al*., 1994). Lag time varied between the different samples of grass silages. In the present study, there was no relationship found between the lag time and k_d of NDF of grass silages. Varga and Hoover (1983) also did not find relationship between the lag time and k_d of NDF. The average ED_{CP} was 0.610 in the present study which is comparable to the result (0.629) reported by Von Keyserlingk *et al*. (1996). Castillo *et al*. (2001) reported a lower value (0.571) for ED_{CP} of grass silages. The higher ED_{CP} value in the present study might be due to the higher average CP content (169.5 g/kg DM) in grass silages compared to the grass silages (123 g/kg DM) investigated by Castillo *et al*. (2001). Cone *et al*. (2004) calculated the REP, using the same REP formula as used in the present study, and reported average values of 0.270 and 0.316, for low and high DM grass silages, respectively. These values are lower than the 0.390 obtained in the present study. The difference in REP values is likely to be due to the larger range in CP content (128-305 g/kg DM) in the grass silages used by Cone *et al*. (2004) and the grass silages (108-222 g CP/kg DM) used in the present study. De Boever *et al.* (2004) reported an average REP fraction of 0.244 for grass silages**.** The low REP fraction in case of De Boever *et al*. (2004) might be due to the variation in CP content.

Different protein evaluation systems use different k_p values for the calculation of ED_{CP}. Tuori *et al*. (1998) compared the values of ED_{CP} calculated using different k_p values originating from different protein evaluation systems. In the present study, these k_p values were used in equation 2 to calculate the ED_{CP} for the grass silages (Table 5). The use of different k_p values led to different calculated values of ED_{CP} for the same grass silages. The use of different k_p values might lead to underestimation or overestimation of the ED_{CP} in different protein evaluation systems.

The regression analyses showed that there are relationships between W, U, D and ED of DM, OM, CP and NDF and the chemical composition of the grass silages (Table 4). The regression equations show that the W_{DM} and D_{DM} contents were influenced by the contents of DM and NDF in the grass silage samples. A similar relationship was reported by Jančík *et al.* (2009) for grass silages. The ED_{CP} is affected by the CP content in the forage. The forage with a higher CP content resulting in a higher value for ED_{CP} (Vik-Mo, 1989). In the present study, a strong relationship was found between the ED_{CP} and the CP content of grass silages. The regression analysis showed that the ED_{CP} was affected by the contents of CP but also NDF and ADF in the grass silages. The ED_{CP} was positively related to NDF content and negatively related to ADF content. It might be due to presence of hemicellulose content in NDF that restrict the CP degradation. The regression analysis showed that the W_{CP} and D_{CP} were mainly influenced by the content of CP while the U_{CP} was influenced by the contents of CP and sugar in the grass silages. The REP was affected by the DM and CP contents in the grass silage samples. The D_{NDF} content influenced by the NDF content in the grass silages while the U_{NDF} content was influenced by the contents of sugar and ADL in the grass silages.

Table 6 *Regression equations[1] derived using DVE/OEB$_{2010}$ database (Personal communication, Product Board Animal Feed, The Netherlands)*

Regression equations
Crude protein (CP)
% W = 94.35 - 0.10 DM[2]
% U = - 0.03 CP + 0.03 NDF
k_d = 38.40 + 0.02 CP - 0.14 NDF + 0.0001 NDF[3]
Neutral detergent fibre (NDF)
% U = 40.90 - 0.65 DOM[4] + 0.05 NDF
k_d = 6.13 - 0.006 NDF

[1] These are unpublished regression equation received from the product board animal feed, the Netherlands. No regression equations were derived for dry matter and organic matter. Information about R^2 values and root mean square error is not available.
[2] Dry matter.
[3] Neutral detergent fibre.
[4] Digestion of organic matter.

The new regression equations can be used for the estimation of W, U, D and ED of DM, OM, CP and NDF of grass silages. The old regression equations were derived for only CP and NDF (Table 6). The information about R^2 values of those equations was also missing. New regression equations derived from new database can be used as acceptable estimates for rumen degradation characteristics of DM, OM, CP and NDF of grass silages.

Conclusions

The rumen degradable fractions of DM, OM, CP and NDF were influenced by the large range in the chemical composition of the grass silages. The new database is more reliable because it is obtained under uniform experimental conditions and the variation in the chemical composition of grass silages used in practice is covered. The present study reports relationships between the chemical composition and *in situ* rumen degradation characteristics of dietary fractions or nutrients of grass silages. A number of the developed regression equations with high R^2 values give acceptable estimates of the rumen degradation characteristics of the dietary fractions or nutrients of grass silages.

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CHAPTER 4

Variation between individual cows in *in situ* rumen degradation characteristics of maize and grass silages

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Abstract

Factors affecting *in situ* rumen degradation characteristics of different feedstuffs have been extensively studied. Data on the variability in rumen degradation of maize and grass silages between individual animals remain scarce. The objective of this study was to determine either three cows are sufficient or not to cover the variation between individual cows in *in situ* rumen degradation characteristics of dry matter (DM), organic matter (OM), crude protein (CP), starch and neutral detergent fibre (aNDFom) for maize and grass silages. Fifteen maize and 15 grass silage samples, with a broad range in chemical composition, were selected. The maize and grass silage samples were incubated in the rumen for 2, 4, 8, 16, 32, 72 and 336 h, using the nylon bag technique. Three cows were used for nylon bag incubation of maize silages and three other cows for grass silages. The variation between individual cows was significant for degradation rate (k_d) of DM (P=0.02), OM (P=0.03) and CP (P=0.005), and the effective rumen degradability (ED) of DM (P=0.01) and CP (P<0.001) of maize silages whereas no significant (P>0.05) differences were found for the rumen undegradable (U) fraction and potentially rumen degradable (D) fraction of DM, OM and CP, and all the parameters of starch and aNDFom. The variation between individual cows was not significant (P>0.05) for U, D, k_d and ED of DM, OM, CP and aNDFom of grass silages. The data indicate that the use of three animals for nylon bag incubations of grass silage samples is sufficient. In addition, the pooling of rumen incubated residues of three cows after each rumen incubation period for grass silages is allowed to get a representative sample. For maize silages, a number of estimates related to DM, OM and CP were significantly different between cows, necessitating the use of four or more cows for *in situ* nylon bag incubations of maize silages.

Introduction

Dietary nutrient availability is essential information to ensure that the nutrient requirements of animals are met. Several *in situ*, *in vitro* and *in vivo* techniques have been developed to measure nutrient availability and over the past decades, much research has focussed on determining the nutritive value of major forages and feed ingredients used in dairy cow nutrition. The *in situ* (*in sacco*) technique has been extensively used for the determination of the degradation of different chemical components in the rumen (De Boever *et al*., 2002; Homolka *et al*., 2007; Jančík *et al*., 2008). Factors affecting the results of these *in situ* techniques include bag and pore size, sample size, bag material, bag insertion and removal procedures, rumen incubation time, number of replicate animals, animals, diet of the experimental animals, feeding level, feeding frequency, rinsing procedure, mathematical models, and microbial contamination (Michalet-Doreau *et al*., 1992; Vanzant *et al*., 1998). A number of these factors have been extensively studied (Weakley *et al*., 1983; Van der Koelen *et al*., 1992; Castillo-Gallegos *et al*., 2012). Less focused has been directed on investigating the variation between individual animals for nylon bag incubations of forages.

In the past, many studies have been conducted to determine the *in situ* rumen degradability of dietary fractions or nutrients of maize and grass silages. In many of these studies, an arbitrary assumption was made that chemical analysis of pooled rumen incubated residues of two (Ranjbari *et al*., 2007; Jančík *et al*., 2009), three (Volden *et al*., 2002; Cone *et al*., 2004; Jensen *et al*., 2005), four (Fernandez *et al*., 2004; Lund *et al*., 2007) or six (Narasimhalu *et al*., 1989; Von Keyserlingk *et al*., 1996; Shannak *et al*., 2000) cows is sufficient to provide a representative sample. However, little data exists in the scientific literature to determine the validity of this assumption for nylon bag incubations of forages. Various ruminant feed evaluation systems such as the DVE/OEB_{2010} system (Van Duinkerken *et al*., 2011) in the Netherlands and the NorFor - the Nordic feed evaluation system (Volden, 2011) used in Denmark, Norway, Iceland and Sweden recommend to use three cows for rumen incubation of feeds and allow pooling of rumen incubated residues to perform chemical analysis on representative samples. The use of three cows for nylon bag incubations has not been validated for maize and grass silages which are commonly fed to dairy cows in the Europe. In the past, few studies were performed with other feeds to determine the variation between individual cows. Weakley *et al*. (1983) observed no significant differences between individual animals (n=4), days of incubation and periods of experimentation on ruminal disappearance of dry matter (DM) of soybean meal whereas significant differences between individual

animals in *in situ* nitrogen (N) disappearance were observed. Figroid *et al*. (1972) found significant differences between steers (n=2) in *in situ* rumen DM degradability for barley and sorghum. Recently, an *in situ* study conducted by Castillo-Gallegos *et al*. (2012) with kinggrass leaves showed no significant differences between cows (n=3) for DM disappearance and concluded that two cows are sufficient. As the experimental costs increase significantly with increasing animal numbers, between-animal variation is essential to obtain cost-effective estimates for *in situ* rumen degradation of maize and grass silages.

This study was designed to determine either three cows are sufficient or not to cover the variation between the individual cows in *in situ* rumen degradation characteristics of DM, organic matter (OM), crude protein (CP), starch and neutral detergent fibre (aNDFom) for maize and grass silages.

Materials and methods

Samples selection and rumen incubations

Fifteen maize and 15 grass (mainly *Lolium perenne*) silage samples (~5 kg per silage) were obtained during 2007, 2008 and 2009 from various Dutch commercial farms located in different regions in the Netherlands. The samples were collected by trained technicians from a feed analysis laboratory (Blgg Research, Wageningen, The Netherlands) using a hollow drill. After collection, the samples were stored at -20°C until processing. Next, frozen samples were cut using a bread slicer (JAC Duro BEL 450; ABO, Leek, The Netherlands) having a distance of 11 mm between the discs, thoroughly mixed by hand and divided into three parts; one part (~2.5 kg) was subjected to chemical analyses after freeze drying and grinding (3 mm), another part (~1.5 kg) was stored at -20°C for later nylon bag incubations, and the third part (~1.0 kg) was stored (-20°C) as a reserve for possible future analysis. The chemical composition of the maize and grass silage samples is presented in Table 1.

Six multiparous (second or third lactation) Holstein Friesian cows, producing >15 kg milk per day and fitted with permanent rumen cannulas, were used in this experiment. Cows were fed a total mixed ration (for composition see Table 2 in Chapter 2) and had 24 h/day access to fresh water. Information on age, lactation number, milk production, milk protein, and milk fat production of the six cows used for the maize and grass silage rumen incubations is presented in Table 2. Three cows (Cow 1, 2 and 3) were used for maize silages nylon bag incubations and other three cows (Cow 4, 5 and 6) were used for grass silages nylon bag incubations.

Table 1 *Chemical composition and quality parameters of maize and grass silages*

Variable	Maize silage (n=15)				Grass silage (n=15)			
	Mean	SD[1]	Minimum	Maximum	Mean	SD	Minimum	Maximum
Chemical composition (g/kg DM)[#]								
Dry matter (g/kg fresh matter)	348.1	49.3	272.2	478.8	464.9	126.7	237.0	685.0
Ash	34.0	6.4	22.0	43.0	109.6	19.8	85.5	163.0
Crude protein	68.2	8.1	55.1	81.0	158.9	32.9	102.0	220.0
Crude fat	36.5	5.1	29.0	47.0	40.5	9.5	27.0	65.0
Starch	339.8	36.7	286.0	402.0	nd[2]	nd	nd	nd
Sugar	10.0	4.1	5.8	22.0	62.6	37.6	11.0	135.0
Neutral detergent fibre[3]	394.7	37.9	326.0	451.0	545.4	44.5	464.0	611.0
Acid detergent fibre[4]	218.2	25.4	180.0	263.0	297.1	20.1	254.0	316.0
Lignin (sa)[5]	19.4	3.6	14.0	26.0	23.2	7.1	14.0	39.0
Silage quality parameters[*]								
pH	3.89	0.17	3.60	4.30	5.14	0.63	4.23	6.12
NH_3-nitrogen (g N/kg DM)[6]	0.39	0.16	0.66	0.11	2.62	1.28	0.86	5.68

[#] Determined on freeze dried material.
[*] Predicted through near infrared reflection spectroscopy (NIRS) by BLGG, Wageningen, the Netherlands.
[1] Standard deviation.
[2] Not determined.
[3] Neutral detergent fibre assayed with a heat stable amylase and expressed exclusive of residual ash.
[4] Acid detergent fibre expressed exclusive of residual ash.
[5] Lignin determined by solubilization of cellulose with sulphuric acid.
[6] NH_3-nitrogen determined on fresh material and then recalculated to content in DM of air dried material.

Table 2 *Information about the cows used for maize and grass silage rumen incubations*

Variable	Maize silage rumen incubations			Grass silage rumen incubations		
	Cow 1	Cow 2	Cow 3	Cow 4	Cow 5	Cow 6
Age (days)	1754	1717	1654	1388	1397	1271
Lactation number	2	3	2	2	2	2
Milk production, 305 days (kg)	9051	8299	9425	8528	9068	8413
Milk protein, 305 days (kg)	323	336	301	297	319	291
Milk fat, 305 days (kg)	459	428	363	369	392	392

The maize and grass silage samples (~5 g DM) were weighed into 10 cm x 19 cm nylon bags (porosity 24 %; pore size 37 µm; Nybolt, Zürich, Switzerland) and incubated in the rumen for 2, 4, 8, 16, 32, 72 and 336 h according to the procedure used by Ali *et al.* (2012). The 0 h bags were washed in a washing machine (AEG-Electrolux Öko Turnamat 2800, Stockholm, Sweden) for 40 min using tap water at 25°C without incubation in the rumen. The washed bags were stored at -20°C for at least 24 h, subsequently freeze dried and the residues were used to calculate the washout (W) fraction. Six bags of each maize and grass silage sample were incubated in the rumen of the three cows (2 bags per cow per incubation time) for 2, 4, 8, 16, and 32 h. Because of the low recovery of incubated residues per nylon bag for the 72 and 336 h incubation periods, 9 bags of each silage sample were incubated in the rumen of the three cows (3 bags per cow per incubation time) for these incubation periods. After removal from the rumen, bags were frozen at -20°C for at least 24 h, after which the bags were thawed and washed in the washing machine as described above for 0 h bags. The washed bags were stored at -20°C and subsequently freeze dried. For each maize and grass silage sample, rumen incubation residues from each cow after each rumen incubation period were ground separately over a 3 mm sieve, using a hammer mill (Pepping, 200 AN-797002, Deventer, The Netherlands).

Chemical analysis and calculations

The ground freeze dried maize silage samples were analyzed for DM, ash, CP, crude fat (CFat), sugar, starch, aNDFom, ADFom (acid detergent fibre expressed exclusive of residual ash) and lignin (sa). The ground freeze dried grass silage samples were analyzed for DM, ash, CP, CFat, sugar, aNDFom, ADFom and lignin (sa). The ground rumen incubated residues of maize silages were analysed for DM, ash, CP, starch and aNDFom, whereas the ground rumen incubated residues of grass silages were analysed for DM, ash, CP and aNDFom.

The DM content was determined by oven drying at 103°C for 4 h (ISO 6496) and ash content by incineration at 550°C for 4 h (ISO 5984). The N content was determined using the Kjeldahl method (ISO 5983) and CP was calculated as N×6.25. Starch was determined using the amyloglucosidase method (ISO 15914) after dissolving in 100% dimethyl sulfoxide. Neutral detergent fibre (aNDFom) was determined according to the modified method of Van Soest *et al*. (1991), using amylase and expressing without residual ash (ISO 16472). Acid detergent fibre (ADFom) was analysed by boiling with acid detergent reagent and expressed without residual ash (ISO 13906:2008). Lignin (sa) was determined after boiling with acid detergent reagent and solubilization of cellulose with sulphuric acid (ISO 13906:2008). The CFat was determined by ISO 6492 and sugar content was determined by the Luff-Schoorl method (NEN 3571:1947nl).

The fractional degradation rate (k_d) of DM ($k_{d\text{-}DM}$), OM ($k_{d\text{-}OM}$), CP ($k_{d\text{-}CP}$) and starch ($k_{d\text{-}starch}$) was calculated according to the first order model of Robinson *et al*. (1986) including U, D and k_d:

$$Y_t = U + D_t \times \exp(-k_d \times t) \quad (1)$$

where Y_t = degradation at time t, U = the rumen undegradable fraction, D_t = fraction potentially degradable in the rumen at time t, t = time of incubation (h).

The k_d of aNDFom ($k_{d\text{-}aNDFom}$) was calculated by the same model including a lag time:

$$Y_t = U + D_t \times \exp(-k_d \times (t - L)) \quad (2)$$

where L = lag time.

The effective rumen degradability (ED) of DM (ED_{DM}), OM (ED_{OM}), starch (ED_{starch}) and aNDFom (ED_{aNDFom}) was calculated according to the equation of Ørskov and McDonald (1979):

$$ED = W + (k_d / (k_d + k_p)) \times D$$

where W = washout fraction, k_p = fractional passage rate (h^{-1}) and *D* = potentially rumen degradable insoluble fraction.

The ED_{DM} and ED_{OM} of maize and grass silages was calculated using an assumed k_p value of 0.05 h^{-1}. For maize silages, a k_p value of 0.06 h^{-1} was used for starch, based on Van Duinkerken *et al*. (2011). For maize and grass silages, k_p values of 0.020 and 0.025 h^{-1}, respectively, were used for aNDFom, based on data of Pellikaan (2004).

For forages, 5% of the W fraction of CP was assumed to escape rumen degradation (Van Duinkerken *et al*., 2011) and is considered to be part of the rumen escape protein (REP).

Therefore, the ED of CP (ED_{CP}) was calculated by the modified equation used for the calculation of ED_{DM}, ED_{OM}, ED_{starch} and ED_{aNDFom}:

$ED_{CP} = 0.95 \times W + (k_d / (k_d + k_p)) \times D$

The k_p value of 0.045 h^{-1} for CP was adopted from the DVE/OEB_{2010} system (Van Duinkerken *et al*., 2011).

It was assumed that 5% of the W fraction was a part of REP and REP was calculated using the equation;

$REP = U + (k_p / (k_d + k_p)) \times D + 0.05 \times W$

Statistical analysis

Rumen degradability data of DM, OM, CP, starch and aNDFom of maize and grass silages after incubation in the rumen of the three cows for 2, 4, 8, 16, 32, 72 and 336 h were summarized by descriptive statistics. The $k_{d\text{-}DM}$, $k_{d\text{-}OM}$, $k_{d\text{-}CP}$ and $k_{d\text{-}starch}$ of maize silages and the $k_{d\text{-}DM}$, $k_{d\text{-}OM}$, and $k_{d\text{-}CP}$ of grass silages were calculated using model 1 and the $k_{d\text{-}aNDFom}$ of maize and grass silages was calculated using model 2 in SAS® (2009). Variation between individual cows was determined for *U*, *D*, k_d and ED of DM, OM, CP, starch and aNDFom of maize silages, and the DM, OM, CP and aNDFom of grass silages by PROC MIXED procedure of SAS® (2009). Variation between individual cows for different incubation periods was determined by PROC MIXED procedure of SAS® (2009). Data were analysed for the fixed effect of cow and random effect of samples using the PROC MIXED procedure of the SAS.

$Y_{ijk} = \mu + PS_i + S_j + e_{ijk}$

where, Y_{ijk} is the dependent variable; μ the general mean; PS_i is the fixed effect of cows *i* (*i* = 1, 2, 3); S_j is the random effect of samples *j* (*j* = 1 to 15 for maize silage samples and 1 to 15 for grass silage samples); ϵ_{ijk} is the residual. Values were considered to differ significantly when $P<0.05$.

Results

The average values for U, D, k_d and ED of DM, OM, CP, starch and aNDFom of the 15 maize silages for individual cows are presented in Table 3. Standard error of the mean (SEM) for $k_{d\text{-}CP}$ was higher compared to the k_d values of DM, OM, starch and aNDFom of the maize silages (Table 3). The range in the average values of ED_{DM} was larger compared to those for ED_{OM}, ED_{CP}, ED_{starch} and ED_{aNDFom} of the maize silages.

Table 3 *Average values, standard error of the mean and confidence interval for rumen undegradable (U, %) fraction, potentially rumen degradable (D, %) fraction, fractional degradation rate (k_d, h^{-1}) and effective rumen degradability (ED, %)[1] of maize silages (n=15) for three cows*

Variable	Cow 1	Cow 2	Cow 3	SEM[2]	95% Confidence interval		Significance[3] level for cows
					Lower limit	Upper limit	
Dry matter (DM)							
U_{DM}	16.21	16.49	17.26	0.31	15.74	17.56	NS
D_{DM}	55.70	55.42	54.65	0.31	54.35	56.17	NS
$k_{d\text{-}DM}$	0.042	0.039	0.046	0.002	0.035	0.049	*
ED_{DM}	53.13	52.09	54.21	0.61	51.36	54.94	*
Organic matter (OM)							
U_{OM}	16.36	17.55	17.93	0.47	15.90	18.66	NS
D_{OM}	55.57	54.37	54.00	0.47	53.26	56.02	NS
$k_{d\text{-}OM}$	0.042	0.039	0.046	0.002	0.035	0.049	*
ED_{OM}	53.21	51.76	53.74	0.59	51.18	54.62	NS
Crude protein (CP)							
U_{CP}	29.72	28.58	29.90	0.42	28.19	30.61	NS
D_{CP}	18.41	19.56	18.24	0.42	17.53	19.95	NS
$k_{d\text{-}CP}$	0.029	0.021	0.035	0.004	0.016	0.040	**
ED_{CP}	57.46	56.44	58.23	0.52	55.86	58.90	***
REP[4]	42.54	43.56	41.77	0.52	41.10	44.14	***
Starch							
U_{starch}	0.51	0.41	0.32	0.05	0.27	0.57	NS
D_{starch}	55.02	55.12	55.20	0.05	54.96	55.26	NS
$k_{d\text{-}starch}$	0.101	0.096	0.096	0.002	0.093	0.103	NS
ED_{starch}	78.61	77.92	77.35	0.36	76.90	79.02	NS
Neutral detergent fibre (aNDFom[5])							
U_{aNDFom}	25.25	24.15	23.72	0.46	23.04	25.70	NS
D_{aNDFom}	75.13	74.02	73.59	0.46	72.92	75.58	NS
$k_{d\text{-}aNDFom}$	0.026	0.026	0.029	0.001	0.024	0.030	NS
Lag time (h)	4.88	5.55	4.88	0.22	4.47	5.75	NS
ED_{aNDFom}	41.28	41.27	42.30	0.34	40.63	42.61	NS

[1] The ED_{DM} and ED_{OM} was calculated using a value of 0.05 h^{-1} for the passage rate. The ED_{CP}, ED_{starch} and ED_{aNDFom} were calculated using passage rate values of 0.045 h^{-1}, 0.06 h^{-1} and 0.020 h^{-1}, respectively.
[2] Standard error of the mean.
[3] NS, not significant ($P > 0.05$); *, $0.01 < P < 0.05$; **, $0.001 < P < 0.01$; ***, $P < 0.001$.
[4] Rumen escape protein. 5 % of the *W* fraction was added in REP.
[5] Neutral detergent fibre assayed with a heat stable amylase and expressed exclusive of residual ash.

The range in average values for REP of maize silages between the three cows was 41.8 to 43.6% (Table 3). The lag time for aNDFom degradation of maize silages for the three cows ranged from 4.88 to 5.55 h (Table 3). The W fraction for DM, OM, CP, starch and aNDFom of maize silages was 28.1, 28.1, 51.9, 44.5 and 0.2%, respectively. There was a significant difference between individual cows for $k_{d\text{-}DM}$ (P=0.02), ED_{DM} (P=0.01), $k_{d\text{-}OM}$ (P=0.03), $k_{d\text{-}CP}$ (P=0.005), ED_{CP} (P<0.001) and REP (P<0.001) when fed the maize silages whereas no significant difference (P>0.05) was observed for others parameters. The average rumen degradability of DM, OM, CP, starch and aNDFom of 15 maize silages after incubation in the rumen of the three cows for 2, 4, 8, 16, 32, 72, and 336 h are presented in Figure 1. The differences in rumen degradability between individual cows were found significant for DM and OM of maize silages at rumen incubated periods of 16 (0.001<P<0.01) and 72 h (P<0.001). The differences in CP rumen degradability between individual cows were found significant at rumen incubation periods of 16 (P=0.005) and 72 h (P<0.001). The differences in aNDFom rumen degradability between individual cows were found significant at rumen incubation periods of 16 (P=0.007) and 72 h (P=0.001).

The average values for individual cows for U, D, k_d and ED of DM, OM, CP and aNDFom of 15 grass silages are shown in Table 4. Variation between individual cows was not significant (P>0.05) for U, D, k_d and ED of DM, OM, CP and aNDFom of grass silages. The between-cow variation in the k_d and ED values was somewhat larger for aNDFom compared to those of DM, OM and CP of grass silages. The variation between the cows was relatively small for all the parameters of DM, OM, CP and aNDFom of grass silages, compared to those of maize silages. The range in average REP values of 15 grass silage samples for three cows was 43.3 to 43.6% (Table 4). The average lag time for aNDFom degradation was 5.24, 3.75 and 4.18 h for cow 4, 5 and 6, respectively (Table 4). The W fraction for DM, OM, CP and aNDFom of grass silages was 19.3, 14.9, 38.3 and 0.8%, respectively. The average rumen degradability of DM, OM, CP, and aNDFom of the 15 grass silages after incubation in the rumen of three cows for 2, 4, 8, 16, 32, 72, and 336 h are presented in Figure 2. The differences in rumen degradability between individual cows were found significant for CP at 4 h (P=0.006) and aNDFom at 2 h (P=0.04). The differences between the cows in DM rumen degradability of grass silages were tend to be significant at 2 h (P=0.054).

Table 4 *Average values, standard deviation, confidence interval limits values for rumen undegradable (U, %) fraction, potentially rumen degradable (D, %) fraction, fractional degradation rate (k_d, h^{-1}) and effective rumen degradability (ED, %)[1] of grass silages (n=15) for three cows*

Variable	Cow 4	Cow 5	Cow 6	SEM[2]	95% Confidence interval		Significance[3] level for cows
					Lower limit	Upper limit	
Dry matter (DM)							
U_{DM}	20.79	21.26	21.53	0.21	20.57	21.81	NS
D_{DM}	59.92	59.45	59.18	0.21	58.90	60.14	NS
$k_{d\text{-}DM}$	0.040	0.040	0.041	0.001	0.038	0.042	NS
ED_{DM}	44.83	45.35	44.93	0.16	44.58	45.50	NS
Organic matter (OM)							
U_{OM}	20.29	20.99	21.04	0.24	20.06	21.48	NS
D_{OM}	64.76	64.06	64.02	0.24	63.57	64.99	NS
$k_{d\text{-}OM}$	0.037	0.039	0.040	0.001	0.036	0.042	NS
ED_{OM}	41.57	42.80	42.26	0.35	41.18	43.24	NS
Crude protein (CP)							
U_{CP}	29.75	29.24	28.53	0.35	28.14	30.20	NS
D_{CP}	31.96	32.47	33.19	0.35	31.51	33.57	NS
$k_{d\text{-}CP}$	0.073	0.074	0.076	0.001	0.071	0.077	NS
ED_{CP}	56.45	56.57	56.71	0.08	56.36	56.80	NS
REP[4]	43.55	43.43	43.29	0.08	43.20	43.64	NS
Neutral detergent fibre (aNDFom[5])							
U_{aNDFom}	21.08	21.84	21.58	0.23	20.84	22.16	NS
D_{aNDFom}	78.11	77.35	77.62	0.23	77.03	78.35	NS
$k_{d\text{-}aNDFom}$	0.039	0.052	0.036	0.005	0.027	0.057	NS
Lag time (h)	5.24	3.75	4.18	0.44	3.09	5.69	NS
ED_{aNDFom}	48.02	48.21	47.03	0.36	46.69	48.81	NS

[1] The ED_{DM} and ED_{OM} was calculated using a value of 0.05 h^{-1} for the passage rate. The ED_{CP} and ED_{aNDFom} were calculated using passage rate values of 0.045 h^{-1} and 0.025 h^{-1}, respectively.
[2] Standard error of the mean.
[3] Not significant ($P > 0.05$).
[4] Rumen escape protein. 5 % of the W fraction was added in REP.
[5] Neutral detergent fibre assayed with a heat stable amylase and expressed exclusive of residual ash.

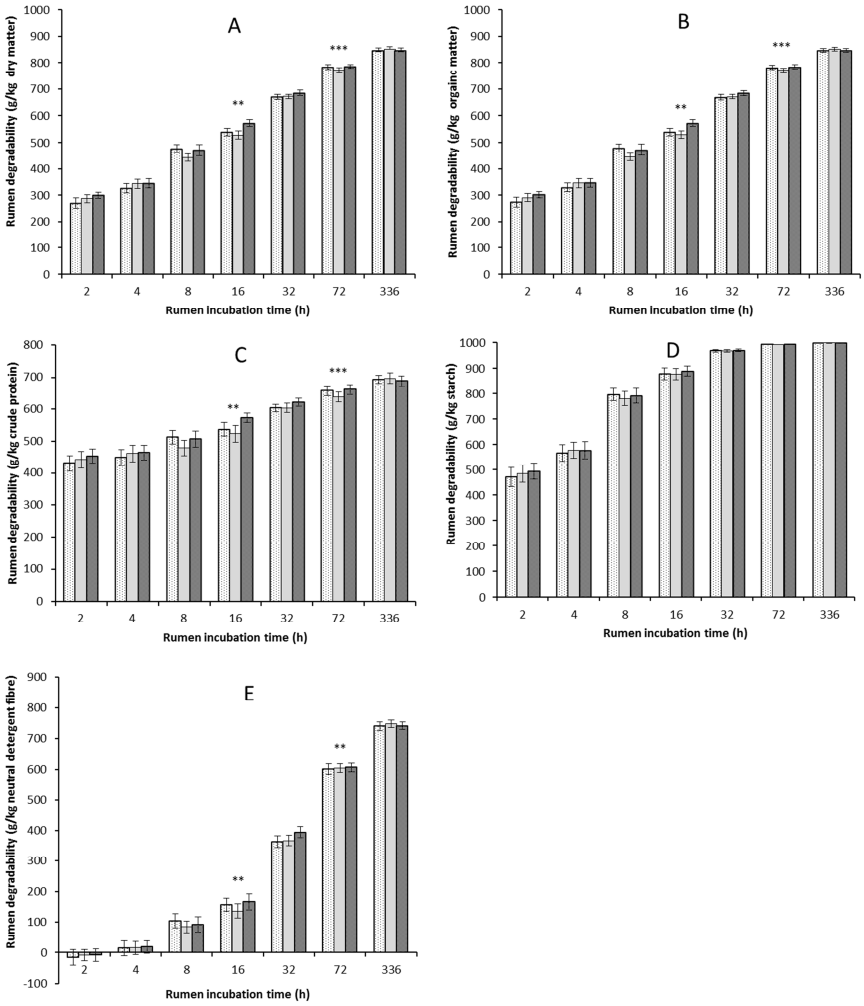

Figure 1 Average rumen degradability of (A) dry matter, (B) organic matter, (C) crude protein, (D) starch and (E) neutral detergent fibre of 15 maize silages after incubated for 2, 4, 8, 16, 32, 72, and 336 h in the rumen of cow 1 (▯), 2 (▯) and 3 (▯). Error bar represents ± standard error of the mean. **, ($0.001<P<0.01$) and ***, ($P<0.001$) indicate significant differences between the cows.

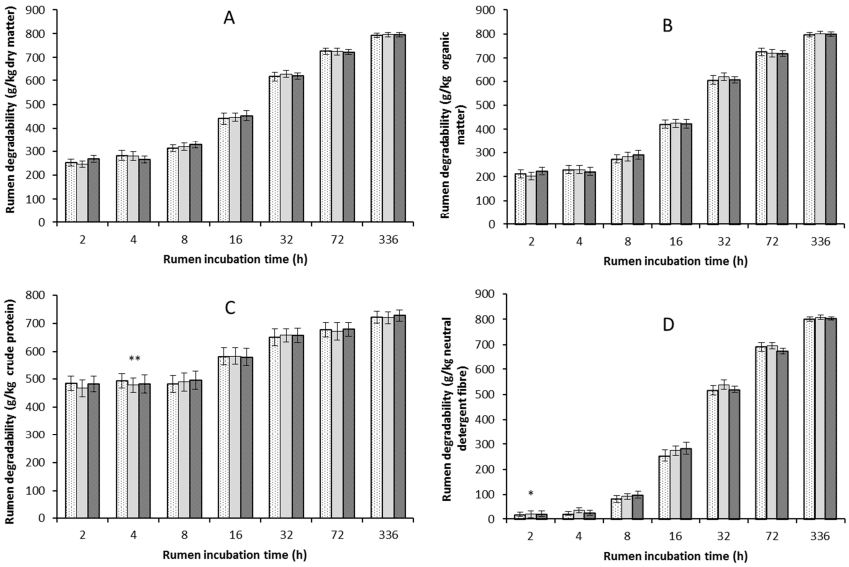

Figure 2 Average rumen degradability of (A) dry matter, (B) organic matter, (C) crude protein and (D) neutral detergent fibre of 15 grass silages after incubated for 2, 4, 8, 16, 32, 72, and 336 h in the rumen of cow 4 (▯), 5 (▯) and 6 (▮). Error bar represents ± standard error of the mean. *, ($0.01<P<0.05$) and **, ($0.001<P<0.01$) indicate significant differences between the cows.

Discussion

Factors affecting the *in situ* rumen degradation characteristics of DM, OM, CP, starch and aNDFom of different feedstuffs have been extensively well studied. There are much less scientific publications, however, reporting the variation between individual cows in rumen degradation characteristics of forages. In many *in situ* studies, an arbitrary assumption is made that chemical analysis of pooled rumen incubated residues of two (Ranjbari *et al*., 2007; Jančík *et al*., 2009), three (Volden *et al*., 2002; Cone *et al*., 2004; Jensen *et al*., 2005), four (Fernandez *et al*., 2004; Lund *et al*., 2007) or six (Narasimhalu *et al*., 1989; Von Keyserlingk *et al*., 1996; Shannak *et al*., 2000) animals is sufficient to provide a representative sample. Pooling of samples is predominantly done to reduce the costs of chemical analyses. Authoritive feeding tables such as those of the CVB in the Netherlands (CVB, 2003) and the NorFor - the Nordic feed evaluation system used in Denmark, Norway, Iceland and Sweden suggest pooled samples of three cows (Volden, 2011). Information on the variation between animals is important for providing rapid estimates which can be used with confidence in practical feed formulation.

Maize and grass silages are used extensively to feed dairy cows in the Europe and Latin America. The difference between individual cows in the present study was significant (P<0.05, P<0.01 and P<0.001) for a number of parameters ($k_{d\text{-}DM}$, ED_{DM}, $k_{d\text{-}OM}$, $k_{d\text{-}CP}$ and ED_{CP}) in the case of maize silages. Others have also found significant differences between the cows in rumen degradation of dietary nutrients. In the case of grass silages, no significant (P>0.05) difference between cows was found for U, D, k_d and ED of DM, OM, CP and aNDFom. Weakley *et al*. (1983) found no signficant differences (P>0.1) between *in situ* DM disappearance between the four cows but found significant differences (P<0.1) in *in situ* N disappearance of soybean meal. Figroid *et al*. (1972) found that the *in situ* DM disappearance of barley and sorghum was significantly different (P<0.01) between two steers. Castillo-Gallegos *et al*. (2012) reported a smaller variance between cows for *in situ* DM disappearance of kinggrass leaves than the variance between bags after different rumen incubation periods.

The average values for D_{DM}, $k_{d\text{-}DM}$ and ED_{DM} of the three cows in the present study were 55.7%, 0.042 h^{-1} and 53.1%, respectively. Ranjbari *et al*. (2007) reported values of 48.9%, 0.040 h^{-1} and 48.0% for D_{DM}, $k_{d\text{-}DM}$ and ED_{DM} of maize silages, respectively. The differences in the D_{DM} and ED_{DM} between the two studies may be due to the high average DM content (348 g/kg fresh matter) of the maize silages in the present study compared to those used (234 g/kg fresh matter) by Ranjbari *et al*. (2007). The reason for the differences between cows in the CP rumen degradability observed here may be due to the effect of microbial contamination of rumen incubated residues as nylon bag residues were not corrected for microbial contamination in the present study. Microbial contamination is one of the main factors that affect the results of the *in situ* CP degradation of forages (Olubobokun *et al*., 1990; González *et al*., 2010).

In the present study, the differences between the cows for all parameters (DM, OM, CP and aNDFom) of grass silages were relatively small compared to the maize silages. The average values of the three sows for D_{CP}, $k_{d\text{-}CP}$ and ED_{CP} for the grass silages were 29.8%, 0.073 h^{-1} and 56.5%, respectively. Von Keyserlingk *et al*. (1996) reported values of 20.7%, 0.088 h^{-1} and 33.0% for D_{CP}, $k_{d\text{-}CP}$ and ED_{CP}, respectively. The differences in the values between both studies could be due to the different average CP content of the grass silages (159 *vs*. 131 g/kg DM) as a high CP content of forages results in higher ED_{CP} values (Vik-Mo, 1989).

Differences between individual cows in rumen degradability were also assessed for specific rumen incubation periods. There were significant differences in rumen degradability between individual cows for DM, OM, CP and aNDFom of maize silages at rumen incubation periods of 16 and 72 h. The starch rumen degradability of maize silages between individual cows was

not significant ($P>0.05$) at all rumen incubation time periods. The rumen degradability of CP of grass silages also shows significant ($0.01<P<0.05$) differences between individual cows at 4 h. The significant differences only for 4 h might be due to the microbial contamination of CP residues. The differences between the cows in rumen degradability were relatively small for grass silages compared to maize silages at specific rumen incubation periods.

Conclusions

Pooling of rumen incubated residues of three cows in *in situ* degradation studies after each rumen incubation period is sufficient to obtain estimates for degradability of dietary fractions or nutrients for grass silages. For maize silages, a number of estimates related to DM, OM and CP were significantly different between cows, necessitating the use of four or more cows.

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CHAPTER 5

Postruminal degradation of crude protein, neutral detergent fibre and starch of maize and grass silages in dairy cows

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Abstract

The Dutch feed evaluation system for ruminants uses assumptions and regression equations to estimate the intestinal digestibility of crude protein (CP), neutral detergent fibre (NDF) and starch. These assumptions and equations are based on many different studies, obtained over a very long period. The objective of this study was to develop a unique dataset on the ruminal degradability and the postruminal digestibility of CP, NDF (aNDFom, amylase neutral detergent fibre organic matter basis) and starch in maize and grass silages, using the mobile nylon bag technique. Twenty samples of maize silage and 20 samples of grass silage were used. The samples were selected to represent a broad range in digestibility and chemical composition. Prior to the intestinal incubations, samples were incubated in the rumen for 6 h (starch), 12 h (CP) or 24 h (aNDFom) using the rumen nylon bag technique. Residues from the rumen incubations were transferred to mobile nylon bags and inserted in the duodenum through a cannula. Half of the bags for CP and starch were collected from the ileal cannula and the remaining half of the bags from the faeces. For aNDFom, all the bags were collected from faeces. There was a large variation in the rumen degradability and the intestinal digestibility (small and/or large intestine) of CP, aNDFom and starch. The rumen degradable fractions, the intestinal digestible fractions and the total tract undigested fractions of CP, aNDFom and starch were influenced by their proportions in the maize and grass silages. The results proved the assumption of the Dutch feed evaluation system that the rumen undegraded starch is completely digested in the small intestine of dairy cows. Regression showed that the rumen degradability, the intestinal digestibility and the total tract undigested contents were influenced by the chemical composition of the maize and grass silages.

Introduction

Different ruminant feed evaluation systems, such as the DVE/OEB$_{2010}$ system in the Netherlands (Van Duinkerken *et al*., 2011), the Feed into Milk (FiM) system in the UK (Thomas, 2004) and the Cornell Net Carbohydrate and Protein System (CNCPS) in the USA (Fox *et al*., 2004), use assumptions and simple models to calculate the intestinal digestibility of rumen undegraded feed protein. The Dutch DVE/OEB$_{2010}$ system uses data of different *in situ* experiments to derive regression equations for a number of feed value parameters. These data are derived from experiments performed at different institutes, 20 to 50 years ago with crop varieties and methods of ensiling which differ from current practices and incubation protocols have changed over time. However, data on intestinal digestibility of CP of maize and grass silages remain scarce (Van Straalen *et al*., 1993; Tamminga, 1994; Cone *et al*., 2006) and even less is known about the intestinal digestibility of neutral detergent fibre (NDF) and starch of maize and grass silages (CVB, 2007). Furthermore, the DVE/OEB$_{2010}$ assumes that the rumen undegraded starch from maize silage is completely digested in the small intestine (Van Duinkerken *et al*., 2011). These limitations highlight the need for more accurate regression equations on rumen degradability and intestinal digestibility as well as new knowledge on the intestinal digestibility of CP, aNDFom (neutral detergent fibre assayed with a heat stable amylase and expressed exclusive of residual ash) and starch of maize and grass silages to improve predictive power of the DVE/OEB$_{2010}$ system.

The aim of this study was to investigate the relationship between the chemical composition and the determined intestinal digestibility for CP and aNDFom in maize and grass silages as well as starch in maize silages, in order to develop new regression equations for the Dutch DVE/OEB$_{2010}$ system.

Materials and Methods

Selection of maize and grass silages

One hundred maize and 100 grass silage samples (~10 kg per silage) were obtained during the growth seasons in 2007, 2008 and 2009 from different Dutch commercial farms located in different regions in the Netherlands using a hollow drill by trained technical assistants from a commercial company (Blgg AgroXpertus, Wageningen, The Netherlands). After collection, individual silages were thoroughly mixed, divided in two parts with one part air dried and subjected to chemical analyses while the other half was stored (-20°C) at Wageningen UR Livestock Research, Lelystad, The Netherlands.

Table 1 *Chemical composition of the maize and grass silages*

Variable	Maize silages (n=20)				Grass silages (n=20)			
	Mean	SD[1]	Minimum	Maximum	Mean	SD	Minimum	Maximum
Chemical composition (g/kg DM)								
Dry matter (g/kg fresh matter)	358	39.5	292	440	494	134.2	229	722
Ash	41	11.7	21	59	104	26.4	77	195
Crude protein	73	6.2	61	84	168	35	108	222
Crude fat	37	4.2	29	46	41	8.8	27	60
Starch	311	69.9	176	427	nd[2]	nd	nd	nd
Sugar	12.9	3.5	8	22	96	65.2	12	246
Neutral detergent fibre (aNDFom[3])	396	64.7	278	503	508	71	358	610
Acid detergent fibre (ADFom[4])	224	39.8	152	289	275	41.2	174	334
Lignin (sa)[5]	17	3.4	13	27	22	6.3	11	35
Silage quality parameters								
pH	3.96	0.16	3.80	4.40	5.15	0.65	3.90	6.20
NH_3-Nitrogen (g N/kg DM)	1.23	0.31	0.55	1.79	1.66	0.72	0.42	2.94

[1] Standard deviation.
[2] Not determined.
[3] Neutral detergent fibre assayed with a heat stable amylase and expressed exclusive of residual ash.
[4] Acid detergent fibre expressed exclusive of residual ash.
[5] Lignin determined by solubilization of cellulose with sulphuric acid.

Table 2 *Composition (g/kg DM in TMR[1]) of diets fed to cows during the rumen nylon bag incubations (Diet 1) and mobile nylon bag incubations (Diet 2)*

Feed ingredient	Diet 1	Diet 2
Maize silage	237.4	297.9
Grass silage	395.8	199.4
Grass hay/wheat straw	13.5	-
Rapeseed cake	-	85.4
Soybean meal	-	111.9
Barley (rolled)	-	99
Barley whole crop silage	-	122
Soybean expeller	47.4	-
Dried sugar beet pulp	-	67.6
Sweet syrup	9.5	-
Wet distillers grains	84.3	-
Soybean expeller (rumen protected)	18.6	-
Minerals	10.9	11.1[2]
Salt	-	2.2
Vitamin mixture	-	1.3[3]
Lime	-	2.2
Concentrate	182.6	

[1] Total mixed ration.
[2] Calcium, 7 g/kg DM in TMR; phosphorus, 4.1 g/kg DM in TMR.
[3] Vitamin A 1000 (606 IU), vitamin D 1000 (42.5 IU), vitamin E (2343 mg).

After chemical analyses of the air dried material, 20 maize silage and 20 grass silage samples were selected to provide a range in chemical composition and silage quality parameters (Table 1). Each selected sample (maize or grass silage) was cut using a bread slicer, thoroughly mixed and divided into three parts; one part was subjected to chemical analysis after freeze drying, the other part stored (-20°C) for later nylon bag incubations, and the third part was stored (-20°C) as a reserve.

Ruminal incubations

Three multiparous Holstein Friesian cows (producing >15 kg milk per day), fitted with ruminal cannulas were used for rumen incubations at Wageningen UR Livestock Research, Lelystad, The Netherlands. Cow were fed diet 1 (Table 2) twice a day and had 24 h access to water. All the maize and grass silage samples were incubated in the rumen according to the CVB (2003) protocol. Dry matter content of individual maize and grass silage samples was determined prior to rumen incubations. Fresh sample (~5 g DM of maize or grass silage) was weighed into 10 cm x 19 cm nylon bags (Pore size 41 µm; Nybolt, Zurich, Switzerland) to

determine rumen degradability. The maize silage samples were incubated in the rumen for 6 h for starch degradation, 12 h for CP degradation and 24 h to determine aNDFom degradation. The grass silage samples were incubated in the rumen for 12 h for CP degradation (Cone et al., 2006) and 24 h to determine aNDFom degradation. After removal from the rumen, the bags were washed for 40 min in a washing machine using tap water at 25°C where after the bags were frozen at -20°C for at least one day before freeze drying. The freeze dried rumen incubated residues of the three cows for each maize and grass silage sample were pooled and ground (Pepping, Deventer, The Netherlands 200 AN-797002) over a 1 mm sieve. The ground rumen incubated residues were transported to Denmark for mobile nylon bag incubations.

Pretreatment and intestinal incubations

Three multiparous Holstein Friesian cows (producing >15 kg milk per day), fitted with duodenal and ileal cannula were used for intestinal incubations at the Department of Animal Science, Aarhus University, Foulum, Denmark. Cows were fed diet 2 (Table 2) twice a day and had 24 h access to water. Approximately 0.5 g of ground rumen incubated residue along with two iron balls (6 mm diameter) were put into 6 cm x 6 cm mobile nylon bags (pore size 12 µm; polyester, Saatitech S.p.A., 22070 Veniano, Como, Italy) and heat sealed (Elwis-pack A/S, Denmark). The bags were soaked in a 0.004 M HCl solution at pH 2.4 for 1 h where after the bags were incubated for 2 h in a pepsin-HCl solution (100 mg pepsin A/S Orthana, 1:10.000 dissolved in 1 L of 0.004 M HCl) at 40°C in a shaking water bath. After that, maximum 12 bags were daily inserted (two bags with 20-30 min intervals) via the duodenal cannula into each cow. Six bags per sample (maize or grass silage) were inserted in the duodenal cannulas of three cows (2 bags per cow) for aNDFom. All bags were collected from the faeces by washing in a sieve bucket. Twelve bags per sample (maize or grass silage) were incubated in the intestinal tract of the three dairy cows (4 bags per cow) for CP and starch. Half of the bags for CP and starch incubation were collected from the ileal cannula by placing a magnet (round-ended cylindrical, 7.5 cm x 1.5 cm), held by a rubber stopper into the ileal cannula. The remaining half of the bags was collected from the faeces by washing in the sieve bucket. After recovery, bags were washed in a washing machine using tap water at 25°C and then the washed bags were frozen at -20°C for at least one day. After that, the bags were freeze dried and transported back to the Netherlands. The mobile nylon bag residues of each maize and grass silage sample were pooled and again ground to 1 mm sieve in a small grinder (Tecator, Sweden, serial no. 2671) for chemical analysis.

Chemical analysis

Dry matter (DM) was determined by drying at 103°C for 4 h (ISO 6496) and ash by incineration at 550°C for 4 h (ISO 5984). Ash free NDF (aNDFom) was determined by a modified method of Van Soest *et al*. (1991) with the use of heat stable amylase but without sodium sulphite (ISO 16472). Acid detergent fibre (ADFom) was determined by boiling with ADF reagent and expressed without residual ash (ISO 13906:2008). Lignin (sa) was also determined after boiling with ADF reagent and a treatment with sulphuric acid (ISO 13906:2008). The N was determined by the Kjeldahl method (ISO 5983) and CP was calculated as N×6.25. Starch was determined using the amyloglucosidase method (ISO 15914) after dissolving in 100% dimethyl sulfoxide.

Calculations

The intestinal digestion of rumen undegraded feed protein (DRUP, g/kg DM) was calculated using the following equation from the Dutch protein evaluation system DVE/OEB_{2007} (Tamminga *et al*., 2007):

$DRUP = CP \times RUP/100 \times dRUP/100$

where CP = Crude protein (g/kg DM), RUP = Rumen undegraded protein (g/kg DM), dRUP = Intestinal digestibility of the rumen undegraded feed protein (g/kg DM).

The values of RUP and dRUP obtained in the present study were used in the above equation for the calculation of DRUP. In the DVE/OEB_{2007} system, the RUP is calculated as $U + (k_p / (k_d + k_p)) \times D$ where U is a CP fraction not degradable by rumen microbes, D is a potentially degradable CP fraction, k_d is the rate of degradation of the D fraction and k_p is the rate of passage. In the present study, RUP was the undegraded CP content after 12 h rumen incubation. Calculated values of DRUP were compared with the determined intestinal digestion values of rumen undegraded feed protein using the mobile nylon bag technique.

Statistical analysis

Data on rumen degradability, small intestinal digestibility, large intestinal digestibility and total tract undigested fractions of CP and starch, as well as the data on rumen degradability, intestinal digestibility and total tract undigested aNDFom fraction were summarized by Descriptive Statistics of SAS 9.2. Pearson correlation coefficients were determined between the rumen degradable, intestinal digestible and total tract undigested fraction by the PROC CORR procedure in SAS 9.2. Regression equations were derived to estimate the rumen

degradable, the intestinal digestible (small and large intestinal) and the total tract undigested contents of CP, aNDFom and starch from the chemical composition of the maize and grass silages using PROC REG procedure in SAS 9.2. The backward stepwise procedure was followed to derive regression equations based on chemical composition, and presented equations are with significant predictors (P<0.1). For some parameters, no regression equation was presented as none of the predictors proved to be significant. These parameters were large intestinal digestible content of CP of maize silages, total tract undigested content of CP of grass silages, intestinal digestible and total tract undigested content of aNDFom of maize and grass silages, and the rumen degradable content of starch of maize silages.

Results

Crude protein

The rumen undegraded protein (RUP) fraction after 12 h of incubation of the maize and grass silages ranged from 0.328 to 0.580 and from 0.168 to 0.575, respectively. The small intestinal CP digestibility ranged from 0.250 to 0.503 for maize silages and from 0.109 to 0.451 for grass silages (Table 3). The RUP fraction affected the intestinal digestible fraction and the total tract undigested fraction of CP for the maize and grass silage samples (Figure 1). The regression equations between the chemical composition of the silages and the rumen degradable content, the small intestinal digestible content and the total tract undigested content of CP for maize silages and the rumen degradable content, the small intestinal digestible content and the large intestinal digestible content of CP for the grass silages are shown in Table 4. The regression equations show that the disappearance of CP in the gastrointestinal tract was influenced by the CP and aNDFom contents in the silages.

The relationship between the calculated intestinal digestion of rumen undegraded feed protein (calculated DRUP) using the equation of DVE/OEB_{2007} system and the determined intestinal digestion of rumen undegraded feed protein (determined DRUP) measured after intestinal incubation in the present study was checked by regression. These regression lines resulted in equations for DRUP of maize (eq. 1) and grass (eq. 2) silages.

Calculated DRUP = 0.898 × Determined DRUP + 7.448 (R^2 = 0.989) (1)

Calculated DRUP = 1.067 × Determined DRUP + 6.044 (R^2 = 0.990) (2)

Table 3 *Rumen degradable, small intestinal and/or large intestinal digestible and total tract undigested fractions of crude protein, neutral detergent fibre and starch of the used maize and grass silages*

Variable	Maize silages				Grass silages			
	Mean	SD[1]	Minimum	Maximum	Mean	SD	Minimum	Maximum
Crude protein			n=19				n=19	
Rumen degradable (12 h)	0.555	0.067	0.420	0.672	0.678	0.116	0.425	0.832
Small intestinal digestible	0.353	0.065	0.250	0.503	0.237	0.098	0.109	0.451
Large intestinal digestible	-0.033	0.010	-0.046	-0.015	0.004	0.014	-0.026	0.050
Total tract undigested	0.124	0.016	0.095	0.152	0.082	0.026	0.049	0.150
Neutral detergent fibre (aNDFom[2])			n=18				n=20	
Rumen degradable (24 h)	0.329	0.081	0.156	0.447	0.513	0.055	0.437	0.625
Intestinal digestible	0.075	0.018	0.042	0.107	0.065	0.019	0.028	0.105
Total tract undigested	0.596	0.074	0.501	0.762	0.423	0.059	0.279	0.511
Starch			n=18					
Rumen degradable (6 h)	0.654	0.107	0.500	0.815	-	-	-	-
Small intestinal digestible	0.335	0.104	0.183	0.490	-	-	-	-
Large intestinal digestible	0.005	0.009	-0.013	0.024	-	-	-	-
Total tract undigested	0.005	0.005	0.000	0.016	-	-	-	-

[1] Standard deviation.

[2] Neutral detergent fibre assayed with a heat stable amylase and expressed exclusive of residual ash.

Table 4 *Regression equations for rumen degradable (RD, g/kg DM), small intestinal digestible (SID, g/kg DM), large intestinal digestible (LID, g/kg DM) and total tract undigested content (TTU, g/kg DM) of crude protein (CP) and neutral detergent fibre (aNDFom[1]) based on chemical composition of the maize and grass silages*

Maize silages	R^2 value	Grass silages	R^2 value
Crude protein (g/kg DM)			
RD = 118.76 - 0.14 DM[2] - 0.19 ash - 1.21 lignin (sa)[3]	0.688	RD = 94.05 + 0.70 CP - 0.19 aNDFom	0.865
SID = - 84.40 + 0.11 DM + 0.20 ash + 0.64 CP + 0.98 lignin (sa)	0.660	SID = - 281.82 - 0.09 DM + 0.49 ash + 0.36 CP + 0.31 sugar + 0.45 aNDFom	0.720
TTU = 4.44 + 0.15 sugar + 0.01 aNDFom	0.476	LID = 51.11 + 0.02 DM - 0.07 ash - 0.03 CP - 0.08 sugar - 0.07 aNDFom	0.778
Neutral detergent fibre (g/kg DM)			
RD = - 155.83 + 0.72 aNDFom	0.808	RD = - 145.70 + 0.39 aNDFom + 0.60 ADFom[4]	0.902

[1] Neutral detergent fibre assayed with a heat stable amylase and expressed exclusive of residual ash.
[2] Dry matter.
[3] Lignin determined by solubilization of cellulose with sulphuric acid.
[4] Acid detergent fibre expressed exclusive of residual ash.

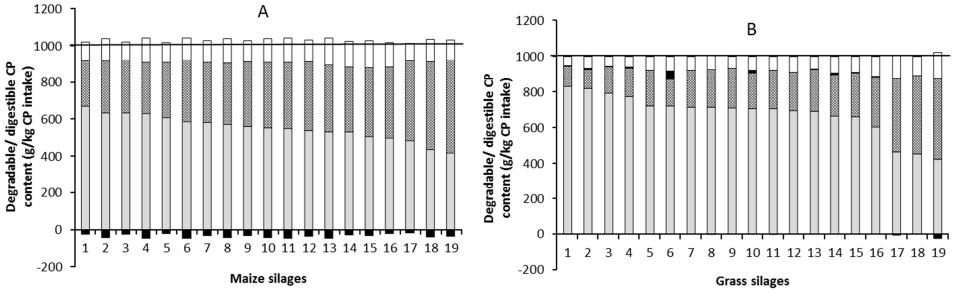

Figure 1 Ruminal degradable content (▒, incubated in the rumen for 12 h), small intestinal (▩) and large intestinal (■) digestible content, and total tract undigested content (□) of crude protein (CP, g/kg CP) of (A) maize (n=19) and (B) grass silage (n=19).

Neutral detergent fibre (aNDFom)

Mean values for 24 h rumen degradation, the intestinal degradable and the total tract undigested fraction of aNDFom of the maize and grass silage samples are presented in Table 3. The disappearance of aNDFom in the rumen was higher for the grass silages than for the maize silages. On average, the aNDFom content in grass silages (494 g/kg DM) was higher than in maize silages (358 g/kg DM). The ruminal degradable, the intestinal degradable and the total tract undigested content of aNDFom (g/kg aNDFom) for all the maize and grass silages used in this experiment are shown in Figure 2. Regression analysis showed that the rumen aNDFom degradability was influenced by the aNDFom content in the maize and grass silage samples (Table 4).

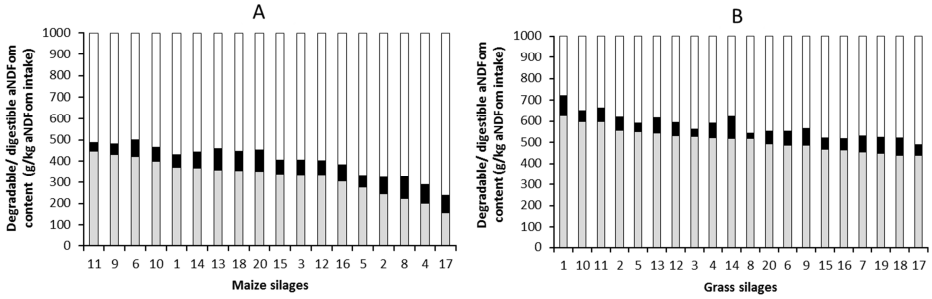

Figure 2 Ruminal degradable content (▒, incubated in the rumen for 24 h), intestinal (■) digestible content, and total tract undigested content (□) of aNDFom (g/kg aNDFom) of (A) maize (n=18) and (B) grass silage (n=20).

Starch

The fraction of starch degraded in the rumen plus small intestine was 0.984 to 1.00. The fraction degraded in the large intestine and the total tract undigested fraction of starch were on average less than 0.01 (Table 3). The ruminal degradable, the small intestinal and large intestinal starch degradable, and the total tract undigested contents were compared for the different samples of maize silages used in the present study (Figure 3).

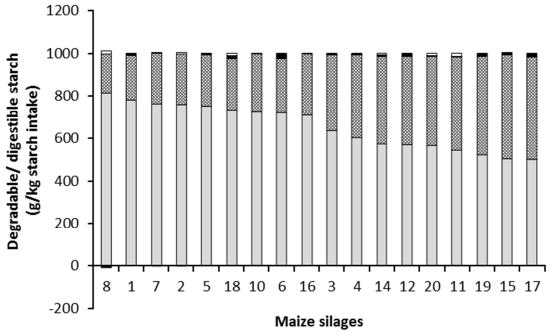

Figure 3 Ruminal degradable (▒, incubated in the rumen for 6 h), small intestinal (▨) and large intestinal (■) digestible, and total tract undigested contents (□) of starch (g/kg starch) of maize silage (n=18).

Discussion

Crude protein

The intestinal CP digestible fraction in the present study was 0.241 for grass silages. Cone *et al*. (2006) used low and high DM grass silages in a mobile nylon bag experiment and reported 0.255 intestinal CP digestible fraction for low DM grass silages and 0.224 for high DM grass silages. In the present study, the total tract undigested fraction of CP from the total tract was 0.124 for maize silages and 0.082 for grass silages, whereas Cone *et al*. (2006) reported a value for grass silage of 0.091 which is slightly higher than the average total tract undigested fraction of the 19 grass silages investigated in this study. This might be due to the high average CP content of the grass silages used by Cone *et al*. (2006), which was 198 g CP/kg DM, while in the present study the average was only 168 g CP/kg DM. In the present study, the whole tract CP digestibility for maize and grass silages was 0.876 and 0.918, respectively. Třináctý *et al*. (2003) reported almost similar results for maize (0.879) and grass silage (0.914). With an increase in rumen degradable content, the intestinal digestible content decreased (Hvelplund *et al*., 1992; Cone *et al*., 2006). Similar results were observed in the

present study (Figure 2). The ruminal degradation of maize silages was negatively correlated with the small intestinal digestion (r=-0.978, $P<0.001$) and the large intestinal digestion (r=-0.048, $P=0.843$), and the ruminal degradation of grass silages was negatively correlated with the small intestinal digestion (r=-0.988, $P<0.001$) and positively correlated with the large intestinal digestion (r=0.424, $P=0.071$).

The regression lines for maize and grass silages showed a strong linear relationship between the calculated (used values of present study in equation of DVE/OEB_{2007}) and determined (present study) values of DRUP. In the DVE/OEB_{2007} system, the RUP is calculated as $U + (k_p / (k_d + k_p)) * D$ but in the present study, RUP was calculated as 1000 - CP (g/kg) degraded after 12 h rumen incubation. This different approach to calculate the RUP can make a difference in the calculated and determined DRUP. The linear regression observed however means that the old DVE/OEB_{2007} approach of calculating RUP is still valid. The difference in absolute values of intestinal digestion may be caused by the different approaches for RUP.

Regression equations showed that the chemical composition of silages influenced the disappearance of CP from the gastrointestinal tract. The DM, ash, CP and aNDFom content in the maize and grass silages affected the ruminal degradability, intestinal (small intestine + large intestine) digestibility and total tract undigested content of CP in the gastrointestinal tract of the dairy cows.

Neutral detergent fibre (aNDFom)

As animals do not secrete enzymes with fibrolytic activity, rumen undegraded aNDFom cannot be digested in the small intestine, but might be degraded in the hindgut. Therefore, all the duodenal inserted bags for aNDFom measurement were collected from the faeces. The 24 h rumen degradability of aNDFom was 0.156 to 0.447 for maize silages and 0.437 to 0.625 for grass silages. The rumen degradability was different compared to studies of Fernandez *et al.* (2004), Jensen *et al.* (2005) and Van Vuuren *et al.* (2010) because of the use of different rumen incubation times in these studies. The reason to use the short rumen incubation period (24 h) in the present study was to determine the clear relationship between the chemical composition and intestinal digestibility of aNDFom. A longer rumen incubation period would have lowered the aNDFom in-flow in the hindgut and would have reduced the power of the study to determine the intestinal digestibility of aNDFom. But the intestinal digestibility (0.042 to 0.107) of aNDFom of maize silages was consistent with the *in vivo* results (0.07 to 0.09) reported by Jensen *et al.* (2005). The range of intestinal digestibility in this study was higher because of the variation in the proportion of aNDFom in the maize silages and the

higher number of samples (n=20) used, compared to the number of samples (n=3) used by Jensen *et al*. (2005). The aNDFom content was negatively correlated (R=-0.912, P<0.001) with the starch content of the maize silages. A high content of readily degradable carbohydrate in the diet decreases the pH in the rumen and the activity of cellulolytic microbes in the rumen. Therefore, the rate of NDF degradation decreased with an increase in the proportion of starch in the animal's diet (Robinson *et al*., 1987). However, such an effect of the rumen environment is not expected using the *in situ* technique. The intestinal digestibility of maize silages was negatively correlated with the rumen degradation (R=-0.483, *P*=0.042) and positively correlated with the total tract undigested fraction (R=0.293, *P*=0.238). The intestinal digestibility of grass silages was positively correlated (R=0.031, *P*=0.896) with the rumen degradation and negatively correlated (R=-0.356, *P*=0.123) with the total tract undigested fraction. The contribution of the postruminal digestion of the total tract digestion of aNDFom varied between 0.09 and 0.35 for maize silages and between 0.05 and 0.17 for grass silages. Tamminga (1993) reported that hindgut fermentation made up 0 to 0.20 of the total tract digestion of NDF. The regression equations show that the rumen degradability of the aNDFom of maize and grass silage samples was influenced by the proportion of aNDFom in these silages.

Starch

Starch is a main source of energy in the ration of high producing dairy cows. The starch, escaping rumen fermentation is digested by enzymes and absorbed as glucose in the small intestine (Noberg *et al*., 2007). An attractive strategy to overcome a negative energy balance is to feed rations in which the starch utilization is shifted from the rumen to the small intestine (Larsen *et al*., 2009). It is assumed that milk production is more supported by starch digested in the small intestine than starch degraded in the rumen (McDonald *et al*., 1995). Owens *et al*. (1986) found that starch digested in the small intestine provided up to 42% more energy than starch digested in the rumen. Starch is almost completely degraded in the entire gastrointestinal tract of dairy cows (Hindle *et al*., 2005). In this study, the average rumen undegraded starch fraction was 0.245 and the total tract undigested fraction was 0.005. Jensen *et al*. (2005) reported a low rumen undegraded starch fraction (0.19) for maize silages having average DM content of 310 g/kg. The rumen undegraded starch fraction reported by Jensen *et al*. (2005) was low because of a high rumen incubation time (16 h) compared to the present study (6 h). A longer incubation time in the present study would have lowered the starch in-flow in the small intestine and would have reduced the power of the study to determine the

small intestinal digestibility of starch. The *in vivo* average rumen undegraded starch fraction was 0.08 and total tract undigested fraction was 0.01 for maize silages (Jensen *et al*., 2005). In the present study, the total tract digestibility of starch was 0.984 to 1 which is consistent with the total tract digestibility of starch (0.940 to 0.994) reported by Fernandez *et al*. (2004) and *in vivo* total tract digestibility of starch (0.98 to 1.00) reported by Jensen *et al*. (2005). The present study shows that the amount of starch present in the feed affected the ruminal and small intestinal degradation of starch. The *in vivo* study of Van Vuuren *et al*. (2010) also showed that the amount of starch present in the feed or forage significantly affected the ruminal, small intestinal and large intestinal degradation of starch. The intestinal digestion of starch was negatively correlated with the rumen degradation (R=-0.483, *P*=0.042).

The Dutch feed evaluation system (DVE/OEB_{2007}) assumes that the rumen undegraded starch of maize silages is completely digested in the small intestine. The results of the present study have proven this assumption for maize silage starch and the results show nearly complete degradation of starch in the rumen plus small intestine (0.984 to 1.00). But there might be some effect of sample pre-treatment (milling to 1mm before incubations in the intestinal tract) on the small intestinal digestibility of rumen undegraded starch of maize silages. The *in vivo* results may be slightly different from the *in situ* results because of no sample pre-treatment in case of *in vivo* study.

Conclusions

Rumen degradability, intestinal digestibility and the total tract undigested content of CP, aNDFom and starch in maize and grass silages were affected by the chemical composition of the silages. The present study proved the assumption of the Dutch feed evaluation systems that rumen undegraded starch from maize silage is completely digested in the small intestine of dairy cows. Equations based on chemical composition can give acceptable estimates of the intestinal digestion of CP and aNDFom.

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CHAPTER 6

Comparison of fractionation methods for nitrogen and starch in maize and grass silages

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Animal (submitted)

Abstract

Using the nylon bag technique, many feed evaluation systems use a washing machine method (WMM) to determine the washout (W) fraction and to wash the rumen incubated nylon bags. As this WMM has some disadvantages, an alternate modified method (MM) was recently introduced. The aim of this study was to determine and compare the W and non-washout (D+U) fractions of nitrogen (N) and starch of grass and maize silages, using the WMM and the MM. In addition, regression equations were derived to estimate the N and starch fractions out of the chemical composition of the silages, using both methods. Ninety-nine maize silage and 99 grass silage samples were selected with a broad range in chemical composition and silage quality parameters. The results showed a large range in the W, soluble (S) and D+U fractions of N of maize and grass silages and the W, insoluble washout (W-S) and D+U fractions of starch of maize silages, determined by both methods, due to variation in their chemical composition. Differences in the values of the different fractions, obtained with both methods were caused by different methodological approaches, such as different rinsing procedures (washing *vs.* shaking), duration of rinsing (60 min *vs.* 40 min) and different solvents (water *vs.* buffer solution). The positive linear relationships between both methods, for the D+U fractions of N and starch show that the D+U values for both methods show a similar trend between the samples. Regression analysis shows relationships, with an R^2 ranging from 0.31 to 0.86, between the N and starch fractions and the chemical composition of maize and grass silages. A number of these regression equations can be used for the prediction of N and starch fractions from chemical characteristics of maize and grass silages used in practice.

Introduction

Materials and methods

Different ruminant feed evaluation systems, such as the DVE/OEB_{2010} system (Van Duinkerken *et al*., 2011) in the Netherlands, the Feed into Milk (FiM) system (Thomas, 2004) in the UK, the North American NRC system (NRC, 2001), the PDI system (Vérité *et al*., 1979) in France and the Nordic NorFor system (Volden, 2011) in Denmark, Sweden, Iceland and Norway use data of *in situ* nylon bag experiments to estimate rumen degradation characteristics of dietary nutrients in different feedstuffs. The *in situ* nylon bag technique (Ørskov and McDonald, 1979) divides feed or feed ingredients into a washout (W) fraction, a potentially rumen degradable fraction (D) and a rumen undegradable fraction (U). Within the *in situ* nylon bag technique, the washing machine method (WMM) has been extensively used to determine the W fraction and to wash the rumen incubated nylon bags to remove contaminations (Bruinenberg *et al*., 2004; Gierus *et al*., 2006). The washing of non-incubated nylon bags with this method separates the feed into a W and a non-washout (D+U) fraction. Different types of washing machines, rinsing procedures, washing programmes and washing times have been used in different *in situ* studies, causing variation in the results (Lindberg, 1985; Vanzant *et al*., 1998; de Jonge *et al*., 2009). The W fraction of feed or feed ingredients can be divided into a soluble (S) fraction (Gierus *et al.*, 2005) and an insoluble washout (W-S) fraction of small particles (de Jonge *et al*., 2009). Using the WMM, the S fraction has to be determined separately by an additional analysis and the W-S fraction can be calculated by subtraction of the S fraction from the W fraction (Van Duinkerken *et al*., 2011). The calculated W-S fraction, however is determined by difference and as such is impossible to verify the accuracy, based on total recovery. Recently, de Jonge *et al*. (2009) introduced a modified method (MM) for the nitrogen (N) and starch fractionation in concentrates and forages. The rinsing procedure of the MM involves gentle washing (shaking) of nylon bags in a buffer solution, using a mechanical shaker. With this MM, the S, W-S and D+U fraction of chemical components of feed or feed ingredients are determined directly unlike with the WMM. As all the fractions are separated with one rinsing procedure, the accuracy of the MM can be verified, based on total recovery.

The chemical composition of maize and grass silages varies between different cultivars, growing conditions, fertilisation level, harvesting practices, ensiling conditions, and stage of maturity at harvest (Cone *et al*., 1999; Fernandez *et al*., 2004; González *et al*., 2010). The information on the relationships between chemical composition and the W and D+U fractions

of dietary nutrients of maize and grass silages in the scientific literature remains scarce. This information is important for an accurate estimation of the W and D+U fractions to determine the feeding value of maize and grass silages, without doing laborious experiments.
The aim of this study was to compare the nylon bag N and starch fractions of maize and grass silages using the standard WMM and a new MM, as recently described by de Jonge *et al*. (2009). In addition, the fractionation values determined by both methods were used to derive regression equations to predict the N and starch fractions from the chemical composition of the maize and grass silages.

Sample selection and processing

Ninety-nine maize silage and 99 grass (mainly *Lolium perenne*) silage samples were obtained during 2007, 2008 and 2009 from various Dutch commercial farms, located in different regions in the Netherlands. The samples were collected by trained technicians from a feed analysis laboratory (BLGG AgroXpertus, Wageningen, The Netherlands) using a hollow drill. After collection, individual silages were stored at -20°C. Each frozen silage (maize or grass) was cut using a bread slicer (JAC Duro BEL 450; ABO, Leek, The Netherlands) having a distance of 11 mm between the discs and then thoroughly mixed by hand. The chemical composition and silage quality parameters of the maize and grass silages are presented in Table 1.

Standard washing machine method (WMM)

Approximately 5 g DM of each maize and grass silage sample was weighed into 10 cm x 19 cm nylon bags (Porosity 24%, pore size 37 µm; Nybolt, Zurich, Switzerland), in duplicate. The bags were washed in a washing machine (AEG-Electrolux Öko Turnamat 2800, Stockholm, Sweden) for 40 min, using tap water at 25°C, according to the method described by Rodrigues *et al*. (2009), to determine D+U fraction and the W fraction was calculated as 1-(D+U). The washed bags were stored at -20°C and subsequently freeze dried. For each maize and grass silage, the washed residues were pooled and the contents were ground over a 1 mm sieve, using a hammer mill (Pepping, 200 AN-797002, Deventer, The Netherlands). The washed maize silage residues were analysed for DM, N and starch, while grass silage residues were analysed for DM and N.

Table 1 *Chemical composition of the maize and grass silages*

Variable	Maize silages (n=99)				Grass silages (n=99)			
	Mean	SD[1]	Minimum	Maximum	Mean	SD	Minimum	Maximum
Chemical composition (g/kg DM)								
Dry matter (g/kg)	350.9	35.9	272.2	440.4	445.0	116.2	201.0	685.0
Ash	37.3	9.6	21.0	79.0	103.4	19.4	70.0	192.0
Crude protein	65.9	6.2	52.6	81.0	165.8	31.1	102.0	222.0
Crude fat	35.9	4.4	27.0	47.0	42.0	7.6	27.0	65.0
Starch	332.2	48.7	176.0	427.0	-	-	-	-
Sugar	11.5	4.7	3.0	43.0	87.8	46.2	11.0	246.0
Neutral detergent fibre	387.1	44.1	278.0	503.0	498.8	54	326.0	611.0
Acid detergent fibre	216.0	26.2	152.0	289.0	272.6	30.6	157.0	347.0
Lignin	17.5	3.4	11.0	27.0	19.6	6.6	10.0	40.0
Silage quality parameters								
pH	3.90	0.15	3.60	4.40	4.98	0.62	3.90	6.67
NH_3-nitrogen (g N/kg DM)	1.20	0.35	0.33	2.30	2.51	0.96	0.93	7.94

[1] Standard deviation.

Modified method (MM)

Approximately 5 g DM of maize or grass silage was weighed into 10 cm x 19 cm nylon bags (Porosity 24%, pore size 37 µm; Nybolt, Zurich, Switzerland). Two bags of each maize or grass silage were placed in a glass vessel (Ø 19 cm, 7 cm height), containing 500 ml buffer solution at room temperature. The buffer solution contained 12.2 g/l $NaH_2PO_4.H_2O$ and 8.9 g/l $Na_2B_4O_7.10H_2O$ (Merck, Darmstadt Germany) and the pH was adjusted to 6.2 with HCl (de Jonge *et al*., 2009). The glass vessel, containing the buffer solution with the bags, was placed in a mechanical water shaker (160 rpm) for 1 h. Separation of the different fractions was done according to the procedure described by de Jonge *et al*. (2013). The S fraction of each maize silage was analysed for N and starch, and the D+U fraction was analysed for DM, N and starch. The S fraction of each grass silage was analysed for N and the D+U fraction was analysed for DM and N. For maize silages, the analysis of the W-S fraction was limited to starch, which was the most abundant nutrient in this fraction (de Jonge *et al*., 2013; Yang *et al*., 2005).

Chemical analyses

The DM content was determined by oven drying at 103°C for 4 h (ISO 6496) and ash content was determined by incineration at 550°C for 4 h (ISO 5984). The N content was determined using the Kjeldahl method (ISO 5983-2) and CP was calculated as N×6.25. Neutral detergent fibre (aNDFom) was determined according to the modified method of Van Soest *et al*. (1991), using amylase and expressing values exclusive of residual ash (ISO 16472). Acid detergent fibre (ADFom) was determined by boiling with acid detergent reagent and expressed exclusive of residual ash (ISO 13906: 2008). Lignin (sa) was determined after boiling with acid detergent reagent and treatment with sulphuric acid (ISO 13906:2008). Starch was determined using the amyloglucosidase method (ISO 15914) after dissolving in dimethyl sulfoxide. Crude fat (CFat) and sugar contents were determined according to ISO 6492 and the Luff-Schoorl method (NEN 3571:1947nl), respectively.

Statistical analyses

The data on the N and starch fractions of the maize silages and N fractions of the grass silages, determined using the WMM and the MM were summarized by Descriptive Statistics using SAS 9.2 (2009). Regression equations were derived to determine the relationships between the N or starch fractions determined using both fractionation methods, and the

chemical composition of maize and grass silages using the PROC REG procedure of SAS 9.2 (2009). The backward stepwise procedure was followed to derive the regression equations with significant predictors ($P<0.05$).

Results

The results of the N fractionation of the 99 maize silages into W and D+U fractions, determined with the WMM, and into S and D+U fractions, determined using the MM, are presented in Table 2. The results of the starch fractionation into W and D+U fractions by the WMM and into W-S and D+U fractions, determined by the MM, are also presented in Table 2. A large range was found in the W, S, and D+U fractions of N and the W, W-S, and D+U fractions of starch of the maize silages, using both methods. The D+U fraction of N of the maize silages, determined with the WMM, ranged from 0.266 to 0.796, whereas the range in the S fraction, determined with the MM, ranged from 0.335 to 0.754. Figure 1 shows a significant ($P<0.001$) positive linear relationship between the two methods to determine the D+U fraction of N of the maize silages.

The range in the D+U fractions of starch of maize silages determined with the WMM was 0.270 to 0.938, whereas the range in the S fraction determined with the MM was 0.502 to 0.948. Figure 2 shows a significant ($P<0.001$) positive relationship ($R^2=0.46$) between the D+U fractions of starch of the maize silages, determined by both methods. Similar to N of the maize silages, the slope for the D+U fractions of starch was significant but a weak relationship ($R^2=0.46$) was found.

The N fractionation of the 99 grass silages into W and D+U fractions by the WMM and into S and D+U fractions using the MM is also presented in Table 2. A large range was also obtained in the W, S and D+U fractions of N of the grass silages using both methods (Table 2). The range in the D+U values of N of the grass silages, determined with the WMM, was 0.362 to 0.884, whereas the range in the D+U values determined with the MM, was 0.361 to 0.763. The positive linear relationship between the two methods for the D+U fractions of N of the grass silages is shown in Figure 3.

Table 2 *Nitrogen and starch fractions of maize and grass silages, determined using the washing machine and a modified method*

Variable	Maize silages (n=99)				Grass silages (n=99)			
	Mean	SD[1]	Minimum	Maximum	Mean	SD	Minimum	Maximum
Nitrogen								
Washing machine method								
W fraction[2]	0.531	0.091	0.204	0.734	0.435	0.099	0.116	0.638
D+U fraction[3]	0.469	0.091	0.266	0.796	0.565	0.099	0.362	0.884
Modified method								
S fraction[4]	0.509	0.085	0.246	0.665	0.467	0.085	0.237	0.639
D+U fraction	0.491	0.085	0.335	0.754	0.533	0.085	0.361	0.763
Starch								
Washing machine method								
W fraction	0.440	0.142	0.062	0.730	-	-	-	-
D+U fraction	0.560	0.142	0.270	0.938	-	-	-	-
Modified method								
W-S fraction[5]	0.232	0.089	0.052	0.498	-	-	-	-
D+U fraction	0.768	0.089	0.502	0.948	-	-	-	-

[1] Standard deviation.
[2] Washout fraction; the fraction disappeared from non-incubated nylon bags after washing in the washing machine.
[3] Non-washout fraction.
[4] Soluble fraction.
[5] Insoluble washout fraction, the insoluble small particles which escape from the nylon bag during incubation in the buffer solution.

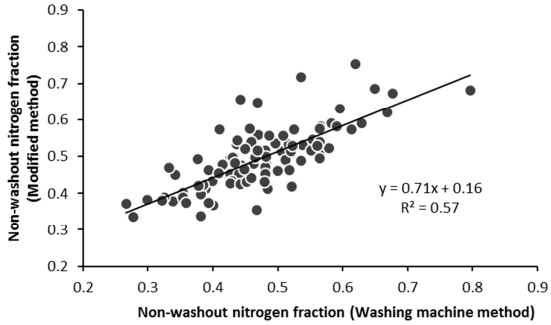

Figure 1 Relationship between the non-washout nitrogen fractions of maize silages (n=99), determined using the washing machine method and a modified method.

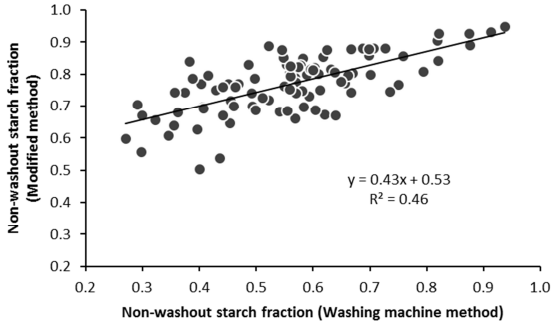

Figure 2 Relationship between the non-washout starch fractions of maize silages (n=99), determined using the washing machine method and a modified method.

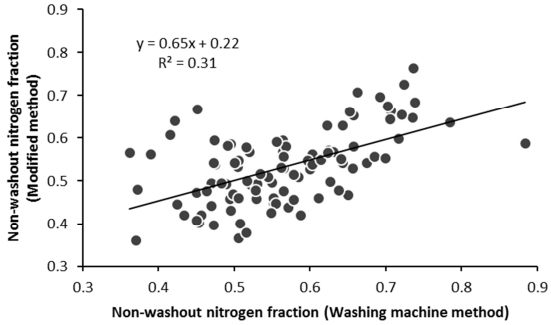

Figure 3 Relationship between the non-washout nitrogen fractions of grass silages (n=99), determined using the washing machine method and a modified method.

Regression equations describing the relationship between the chemical composition and the N and starch fractions of maize silages, determined using the WMM and the MM, are presented in Table 3. The D+U fraction of N in maize silages, determined with the WMM, was positively related to the CP and aNDFom contents, while it was negatively related with the CFat and ADFom content. The D+U fraction of N in maize silages, determined with the MM, was influenced by the contents of CP, CFat, aNDFom, ADFom and lignin (sa). The D+U fraction of starch, determined using the MM, was negatively related to CP, CFat and ADFom, but positively related to starch, DM, aNDFom and lignin (sa) contents in maize silages. The regression equations describing the relationships between the chemical components and the N fractions in grass silages, using both fractionation methods, are presented in Table 4. The D+U fraction of N determined with the WMM was influenced by the contents of DM, CP, CFat and aNDFom, whereas the D+U fractions of N, determined by the MM, was influenced by the contents of DM, CP and aNDFom in grass silages.

Table 3 *Regression equations for the nitrogen (g N /kg DM) and starch (g starch/ kg DM) fractions, determined using the washing machine and a modified method, based on the chemical composition (g/kg DM) of the maize silages (n=99)*

Regression equations	R^2	RMSE[1]
Nitrogen		
Washing machine method		
W^2 = - 6.13 (± 1.59) + 0.11 (± 0.02) CP + 0.06 (± 0.002) CFat - 0.01 (± 0.004) aNDFom + 0.03 (± 0.006) ADFom	0.47	0.95
$D+U^3$ = 6.23 (± 1.58) + 0.05 (± 0.01) CP - 0.06 (± 0.02) CFat + 0.01 (± 0.004) aNDFom - 0.03 (± 0.006) ADFom	0.31	0.95
Modified method		
S^4 = - 3.70 (± 1.48) + 0.08 (± 0.02) CP + 0.05 (± 0.02) CFat - 0.01 (± 0.004) aNDFom + 0.03 (± 0.01) ADFom - 0.07 (± 0.03) Lignin (sa)	0.40	0.88
D+U = 3.84 (± 1.49) + 0.07 (± 0.02) CP - 0.05 (± 0.02) CFat + 0.01 (± 0.003) aNDFom - 0.03 (± 0.01) ADFom + 0.07 (± 0.03) Lignin (sa)	0.41	0.88
Starch		
Washing machine method		
W = 303.37 (± 66.65) - 0.37 (± 0.12) DM - 0.51 (± 0.15) aNDFom + 0.77 (± 0.27) ADFom	0.22	38.57
D+U = - 133.56 (± 115.14) + 0.41 (± 0.12) DM + 0.73 (± 0.15) starch - 1.01 (± 0.30) ADFom	0.68	38.12
Modified method		
$W\text{-}S^5$ = - 234.82 (± 104.20) - 0.14 (± 0.07) DM + 1.06 (± 0.42) CP + 1.33 (± 0.53) CFat + 0.37 (± 0.10) starch - 0.27 (± 0.09) aNDFom + 1.23 (± 0.14) ADFom - 2.70 (± 0.81) Lignin (sa)	0.44	21.25
D+U = 234.82 (± 104.20) + 0.14 (± 0.07) DM - 1.06 (± 0.42) CP - 1.33 (± 0.53) CFat + 0.62 (± 0.10) starch + 0.27 (± 0.09) aNDFom - 1.23 (± 0.14) ADFom + 2.70 (± 0.81) Lignin (sa)	0.86	21.25

[1] Root mean square error.
[2] Washout fraction; the fraction disappeared from non-incubated nylon bags after washing in the washing machine.
[3] Non-washout fraction.
[4] Soluble fraction.
[5] Insoluble washout fraction.

Table 4 *Regression equations for the washout (W), soluble (s) and non-washout (D+U) nitrogen fractions (g N/kg DM), based on the chemical composition (g/kg DM) of grass silages*

Regression equations	R^2	RMSE[1]
Washing machine method		
W = 17.19 (± 3.77) - 0.01 (± 0.003) DM + 0.09 (± 0.01) CP - 0.10 (± 0.05) CFat - 0.02 (± 0.01) aNDFom	0.69	2.21
D+U = - 17.70 (± 3.72) + 0.02 (± 0.003) DM + 0.06 (± 0.01) CP + 0.13 (± 0.05) CFat + 0.02 (± 0.01) aNDFom	0.65	2.18
Modified method		
S = 18.16 (± 2.09) - 0.01 (± 0.002) DM + 0.06 (± 0.006) CP - 0.02 (± 0.003) aNDFom	0.75	1.64
D+U = - 15.92 (± 2.34) + 0.01 (± 0.002) DM + 0.01 (± 0.006) CP + 0.02 (± 0.004) aNDFom	0.78	1.84

[1] Root mean square error.

Discussion

Different procedures have been used with the nylon bag technique for the fractionation of ruminant feeds into W, D and U fraction (Ørskov and McDonald, 1979), and there are many limitations with these procedures (Licitra *et al*., 1996). In the present study, the results of the WMM and MM were compared to determine the effect of the methodological approaches used in these methods on the N and starch fractions in maize and grass silages. Different values were obtained for the D+U fraction of N and starch of maize silages, using the WMM and MM, as well as for the N fraction of grass silages. The different rinsing procedures (washing *vs*. shaking), different solvents (water *vs*. buffer solution), different washing/shaking times (40 min *vs*. 60 min) and different experimental conditions were used in the WMM and MM. All these differences resulted in different values for the non-washout N and starch of the maize silages and for non-washout N of the grass silages. The solubility of CP is largely determined by the pH of the solvent, as shown by Cone (1993). In the MM, the pH of the buffer solution was adjusted to 6.2 (de Jonge *et al*., 2009 and 2013), which may result in a different solubility of CP compared to the WMM where tap water is used, without pH control. The significant positive (P<0.001) linear relationship was found between the D+U values of N, determined by both methods (Figure 1). A R^2 value of 0.57 indicates that both methods are not strongly aligned with each other, resulting in different D+U values for both methods. The different values obtained for non-washout N by both methods might be due to different solvents and the pH of solvent used in both methods. The positive slope in Figure 1 indicates that the D+U fraction determined by the MM increases with an increase in the D+U fraction determined by the WMM.

Starch is insoluble in water by definition, and as such there is no S fraction of starch and the W fraction determined by the WMM contain only the W-S fraction. Therefore, the W-S fraction is considered to represent the W fraction for starch (Cone *et al*., 1989 and 2006; Chai *et al*., 2004). In the present study, the higher D+U fraction and the lower W-S fraction of starch determined with the MM indicates that the loss of insoluble small particles of starch in maize silages was less when nylon bags were shaked in the buffer solution using the MM, compared to washing bags in the washing machine. A significant linear relationship (P<0.001) was found between the D+U fractions of starch of maize silages determined by the WMM and the MM (Figure 2). The R^2 value of 0.31 indicates a weak relationship between the D+U fractions, determined by both methods for N of grass silages. The positive slope indicates that the D+U values determined by both methods show a similar increasing or

decreasing trend between different samples but many data points away from the regression line shows that the relationship was different for different samples.

In the present study, the average W fraction for N of maize silages, determined by the WMM, was 0.531 ranging from 0.204 to 0.734. Average values of 0.632, 0.618 and 0.637 for the W fraction of crude protein (CP) of maize silages were reported by Von Keyserlingk *et al*. (1996), De Boever *et al*. (2005) and González *et al*. (2010), respectively. The higher values for the W fraction of CP, obtained in these studies, might be due to the high CP content present in those maize silages and different numbers of samples used, compared with the mean CP content of 66 g/kg DM of the 99 maize silages used in the present study. The CP content of the maize silages used by Von Keyserlingk *et al*. (1996), De Boever *et al*. (2005) and González *et al*. (2010) were 80 (n=12), 73 (n=26) and 69 g/kg DM (n=30), respectively.

In the present study, the average value for the W fraction of starch of maize silages was 0.440. A high but comparable value of 0.529 was reported by De Boever *et al*. (2005). The higher value may be explained by 50 min washing program with no spin cycle used by De Boever *et al*. (2005), whereas a 40 min wool washing program was used in the present study.

The average W fraction of N of the grass silages, using the WMM in the present study, was 0.428. Gierus *et al*. (2005) reported a W fraction of 0.258 for low DM grass silages (251 g/kg fresh matter) and 0.480 for high DM grass silages (529 g/kg fresh matter). The large difference in the W fraction due to different DM contents of the grass silages, although the CP contents (295 and 288 g/kg DM) were comparable (Gierus *et al*., 2005). As the different methodological approaches of both methods resulted in different values of the different fractions, the question arises which method provides more reliable results. In the MM, one recommended shaking procedure, performed under identical and controlled experimental conditions, gives more reliable results compared to the WMM, using different washing programs and times. In addition, the use of a buffer solution in the MM makes it physiological more close to the situation in the rumen. The MM can become a standard or reliable method to determine the W, S and D+U fractions.

Besides the effect of different methodological approaches, a large range was found in the W, S and D+U fractions of N (maize and grass silages) and the W, W-S and D+U fractions of starch (maize silages), determined by both methods, which is likely due to the broad range in the chemical compositions of maize and grass silage samples. Figure 1, 2 and 3 show that the D+U values of samples varies for both methods, but the relationship differs between different samples of maize and grass silages.

The relationships between the chemical composition and the N and starch fractions of maize and grass silages were investigated by regression analysis. Regression analysis showed that the D+U fraction of N determined by both methods was positively related to the CP and aNDFom contents in maize silages. The R^2 values of 0.31 and 0.41 show weak relationships between the chemical components and the D+U fractions determined by the WMM and the MM, respectively. A moderate relationship (R^2=0.68) was found between the D+U fraction of starch determined with the WMM and the chemical composition, whereas a more strong relationship (R^2=0.86) was found between the D+U fraction of starch determined with the MM and the chemical composition of maize silages. The equations for the D+U fraction of starch determined by both methods shows that a higher starch content in maize silages results in higher values for non-washout starch. Contrary to maize silages, a moderate relationship (R^2=0.65) was found between the D+U fractions of N, determined by the WMM, and the DM, CP and aNDFom content of the grass silages. A strong relationship (R^2=0.78) was found between the D+U fraction, determined with the MM, and the DM, CP, CFat and aNDFom contents in grass silages. The relationships between the D+U fraction of N and the chemical composition were stronger for grass silages than for maize silages. The S and W fractions of N were negatively related to the aNDFom content and positively related to the CP content in grass silages, which is related to the maturity of the grass at harvest (Cone *et al*., 1999). Young grass has a high content of CP and a low content of NDF (cell walls). Upon maturation the CP content decreases and the aNDFom content increases. Moreover, a higher CP content in grass silages means that more N is present in the cytoplasm of the cells, which is always soluble at a neutral pH (Cone *et al*., 1993). The developed regression equations can be used to predict the D+U fraction of starch from the chemical composition of maize silages used in practice. Similarly, the developed regression equations for the W, S and D+U fractions of N of grass silages can also be used for the prediction of N fractions from chemical characteristics of grass silages.

Conclusions

Different values of N and starch fractions were obtained for maize and grass silages due to different methodological approaches of two fractionation methods (WMM and MM). The MM give more reliable results compared to the WMM because of the use of one recommended shaking procedure, performed under identical and controlled experimental conditions. The developed equations show relationships between N and starch fractions and

the chemical composition of maize and grass silages (R^2 ranged from 0.31 to 0.86). Some of the regression equations with a high R^2 value can be used for the prediction of N and starch fractions of maize and grass silages used in practice.

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CHAPTER 7

General discussion

Introduction

The aim of this thesis was to investigate the relationships between the chemical composition and the *in situ* rumen degradation characteristics as well as the mobile nylon bag (MNB) intestinal digestibility of dietary nutrients of maize and grass silages. Currently, maize and grass silages are fed to high yielding dairy animals in Europe and other parts of the world. Maize and grass silage composition can be highly variable which can result in variable rumen degradation characteristics and intestinal digestibility of dietary nutrients when fed to dairy cows. This variability must be taken into account when determining the nutritive and feeding value of maize and grass silages. The relationships between the chemical composition and the *in situ* rumen degradation characteristics (Chapters 2 and 3) and intestinal digestibility (Chapter 5) were studied in this thesis. The MNB intestinal digestibility of CP and starch was mainly affected by the rumen pre-incubation period and the rumen bypass fraction (Danesh Mesgaran *et al*., 2010; Vanhatalo *et al*., 1996). In this thesis, the relationship between the rumen degradability and the intestinal digestibility was also evaluated for dietary nutrients of maize and grass silages. Factors affecting the *in situ* nylon bag degradability have been extensively studied, but the information on variation between indvidual cows remain scarce (Castillo-Gallegos *et al*., 2012). Various feed evaluation systems suggested to use three cows for nylon bag incubations of feeds and feed ingredients. It was tested if the recommended number of three cows was sufficient or not for maize and grass silages to cover the variation in rumen degradation characteristics (Chapter 4). Different rinsing procedures, which are commonly used in the *in situ* nylon bag technique, have many limitations. The washing machine method (WMM) has been extensively used in *in situ* studies and the results of this method were compared with a newly introduced modified method (MM) for nitrogen (N) and starch of maize and grass silages (Chapter 6). The effect of rinsing procedures and the solvents used in these fractionation methods were compared. Standardisation of the rinsing procedure is important to obtain accurate *in situ* results. A lack of a standard rinsing procedure is considered as one of the main factors affecting the *in situ* results (Lindberg, 1985).

Importance of maize and grass silages in dairy ration

Maize and grass silages are major parts of dairy rations in Europe, New Zealand, Australia and North America (Dawson *et al*., 2002; Ettle and Schwarz, 2003; Keady *et al*., 2008), especially during the indoor feeding periods. To achieve an optimum dairy ration balance in terms of protein and energy content to yield a high milk production, a mixed forage diet

containing 50% maize silage and 50% grass silage can be used (O'Mara *et al*., 1998). Grass silage is the most important conserved forage in Europe, especially for winter feeding (Jančík *et al*., 2009). The inclusion of maize silage in a grass silage based diet increases the metabolizable energy intake, resulting in a higher milk production. The forage dry matter (DM) intake increases with the inclusion of maize silage in grass silage based diets, resulting in a higher milk protein concentration and lower milk fat concentration (Mulligan *et al*., 2002). The inclusion of maize silage, upto 33%, in grass silage based diets tended to reduce milk fat but the differences were not significant. Mulligan *et al*. (2002) reported no depression in milk fat concentration with the inclusion of maize silage higher than 33% in grass silage based diets, whereas Phipps *et al*. (1995) reported a decline in milk fat concentration when 75% of grass silage was replaced by maize silage in the dairy ration. O'Mara *et al*. (1998) achieved maximum milk fat and protein concentration by the inclusion of 67% maize silage in a mixed forage diet. Maize silage of moderate quality can replace high quality grass silage up to a certain level without a decline in milk production (O'Mara *et al*., 1998; Keady *et al*., 2008).

***In situ* rumen degradation characteristics**

The increasing importance of maize and grass silages for dairy rations has highlighted the need to study factors which influence their nutritive values and to develop prediction equations from chemical characteristics (Garcıa-Rodriguez *et al*., 2005) to allow rapid evaluation of their nutritive value. The chemical composition of maize and grass silages is highly variable due to different ensiling conditions, growing conditions, forage genotype and cultivar, and maturity stage at harvest, etc. (Cone *et al*., 1999; Fernandez *et al*., 2004; Khan *et al*., 2012). Limited information is available in the scientific literature on the relationship between the chemical composition and rumen degradation characteristics of maize and grass silages. In the past, different *in situ* studies were performed to determine the effect of different individual factors such as maturity stage, particle size, forage species, harvesting strategy and chop length on specific nutrients of maize and grass silages (Fernandez *et al*., 2004; Jensen *et al*., 2005; González *et al*., 2010). Moreover, different numbers of samples were used in these studies for different nutrients and furthermore, these studies were performed for specific scientific purposes. The results reported in Chapters 2 and 3 showed that there was a large variation in *in situ* rumen degradation characteristics of maize and grass silages. The large variation in rumen degradation characteristics was due to the broad range in the concentration of chemical components of maize and grass silages.

Ruminant feed evaluation systems such as the DVE/OEB_{2010} system (Van Duinkerken *et al*., 2011) in the Netherlands and the PDI system (Vérité *et al*., 1979) in France use relatively simple models and prediction equations to estimate the rumen degradation characteristics of DM, organic matter (OM), crude protein (CP), neutral detergent fibre (NDF) and/or starch of maize and grass silages. These systems use data of different *in situ* experiments, which were performed at different locations with different incubation protocols, experimental conditions, crop cultivars and ensiling methods. Moreover, different numbers of samples were used in these studies. In the present work, the maize and grass silages were incubated in the rumen (Chapters 2 and 3) of dairy cows for 2, 4, 8, 16, 32, 72 and 336 h to obtain data under uniform experimental conditions, thereby reducing the variation due to differences in methodological approaches. As such the values reported here are more representative of the actual variation due to uncontrollable factors such as weather conditions and ensiling method as experienced in practical situations.

Regression analysis showed relationships between the chemical composition and the rumen degradation characteristics of DM, OM, CP, NDF and/or starch of maize and grass silages. The W fraction, the potentially rumen degradable (D) fraction, the rumen undegradable (U) fraction, the fractional degradation rate (k_d) and the effective rumen degradability (ED) are the parameters of DM, OM, CP, NDF and starch which were investigated for relationships with the chemical composition of maize and grass silages. Different models have been considered to calculate the k_d; 1) a model without a lag time and U fixed at 336 h, 2) a model with a lag time and with U fixed at 336 h, 3), a model without a lag time and U estimated by the model and 4) a model with a lag time and with U estimated by the model. The k_d presented in this thesis for DM, OM, CP and starch of maize and grass silages was calculated with model 3 and the k_d for NDF of maize and grass silages was calculated with model 4 (Chapters 2, 3 and 4). The lag time is associated with a variable NDF rumen degradation of forages (Varga and Hoover, 1983). If a lag time is not included in the model then the D fraction is overestimated and the k_d value is underestimated (Stensig *et al*., 1994). The lag time for NDF of maize and grass silages varied between different samples, due to the variable NDF content present in the samples. There was no significant relationship between the lag time and the k_d values for NDF of maize and grass silages (Chapters 2 and 3). Varga and Hoover (1983) also found no relationship between the lag time and the k_d values of NDF.

Different values for rumen passage rate (k_p) have been used in different *in situ* studies to calculate the ED of dietary nutrients. Various feed evaluation systems recommend different k_p values for different nutrients of forages and concentrates. In Chapters 2 and 3, the effective

rumen CP degradation (ED_{CP}) was calculated using k_p values of 0.045, 0.05, 0.06 and 0.08 h^{-1}, adopted from the Dutch protein evaluation (DVE/OEB_{2010}) system (Van Duinkerken *et al*., 2011), the Danish protein evaluation (AAT-PBV) system (Madsen, 1985), the French protein evaluation (PDI) system (Vérité *et al*., 1979) and the British metabolizable protein (MP) system (AFRC, 1992), respectively. Figure 1 shows strong negative linear relationships between the different k_p values and the calculated ED_{CP} values of maize (R^2=0.99) and grass (R^2=0.96) silages. High R^2 values show that the higher k_p value resulted in lower calculated values for ED_{CP} of maize and grass silages. Similarly, a negative relationship between k_p and ED values was reported for CP of agro-industrial by-products by Habib *et al*. (2013) and for DM of grass and grass silages by Jančík *et al*. (2009). The use of different k_p values for the calculation of ED_{CP} shows that not only the different approaches in different protein evaluation systems, but also the different k_p values lead to different ED_{CP} values of feeds. The different ED_{CP} values indicate that the nylon bag method might overestimate the true differences in the protein degradation value of feeds, especially when high k_p values are used (Tuori *et al*., 1998). The results described in this thesis for ED of DM (ED_{DM}) were calculated using k_p values of 0.02, 0.05 and 0.08 h^{-1}, proving that the increase in k_p values resulted in lower ED values (Chapters 2 and 3).

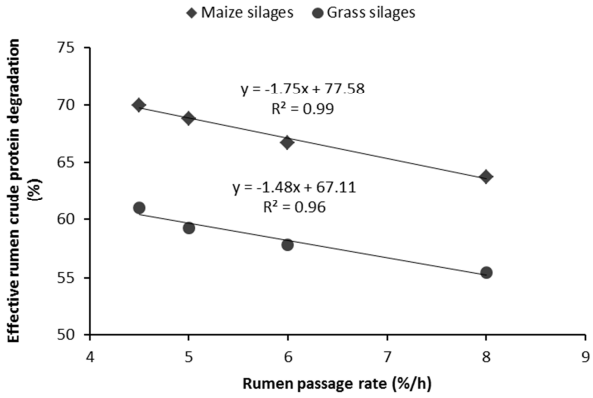

Figure 1 Relationship between rumen passage rate and effective rumen crude protein degradation of maize and grass silages.

In situ mobile nylon bag intestinal digestibility

Regression analysis in Chapter 5 showed relationships between the chemical composition and the MNB intestinal digestibility of dietary nutrients of maize and grass silages. The intestinal digestibility of specific dietary nutrients depends on the content of that nutrient in the silage as

well as the length of the pre-incubation period and the rumen degradability (Chapter 5). The information on the relationships between the chemical composition and the rumen degradation characteristics and the intestinal digestibility is important for an accurate estimation of the bioavailability of dietary nutrients. Some of the developed regression equations presented in Chapters 2, 3 and 5 can be used for a rapid estimation of the rumen degradation and intestinal digestibility characteristics of dietary nutrients of maize and grass silages used in practice.

To optimize the use of maize and grass silages in dairy ration in terms of animal health and production performance, accurate estimates of ruminal degradation and postruminal digestibility of the dietary nutrients are required. The data on intestinal digestibility of CP and NDF of maize and grass silages and even less is known about the intestinal digestibility of rumen undegraded starch of maize silages. New data on intestinal digestibility of CP, NDF and/or starch of maize and grass silages were obtained, using *in situ* MNB technique, under uniform experimental conditions (Chapter 5). Before intestinal incubations, the maize silages were incubated in the rumen (pre-incubation) for 6 (starch), 12 (CP) and 24 h (NDF) and the grass silages were incubated for 12 (CP) and 24 h (NDF). The reason to select to pre-incubation time of 6 h for starch, 12 h for CP and 24 h for NDF was to determine the clear relationships between the chemical composition and the intestinal digestibility of CP, NDF and starch of maize and grass silages. Longer rumen incubation times would have lowered the inflow of these nutrients in the intestinal tract which would reduce the power of the study.

The average apparent degradation/ digestibility (%) of CP, starch and NDF of maize silages in different parts of the gastrointestinal tract of dairy cows is presented in Figure 2A. On average, 71% (range 63-82%) of the rumen undegraded CP of maize silages was digested in the intestinal tract (small + large intestine) of dairy cows. The negative value for large intestinal CP digestibility for maize silages (Figure 2A) is likely due to microbial contamination of N in the hindgut of the dairy cows. The effect of microbial contamination on the large intestinal CP digestibility was more pronounced in the maize silages, compared to the grass silages. Microbial contamination affects the rumen degradation and intestinal digestibility of dietary nutrients, especially the CP fraction of maize and grass silages. Olubobokun *et al*. (1990) and González *et al*. (2010) also reported that microbial contamination is the main factor that affects the results of the *in situ* CP degradation of forages. The results described in this thesis on *in situ* rumen degradability and *in situ* MNB digestibility of dietary nutrients of maize and grass silages were not corrected for microbial contamination. As such values reported here are underestimates, rather than overestimates, for

rumen and small intestinal degradation but not large intestinal degradation. The average total tract CP apparent degradation of maize silages was 88%, ranging from 85 to 90%.

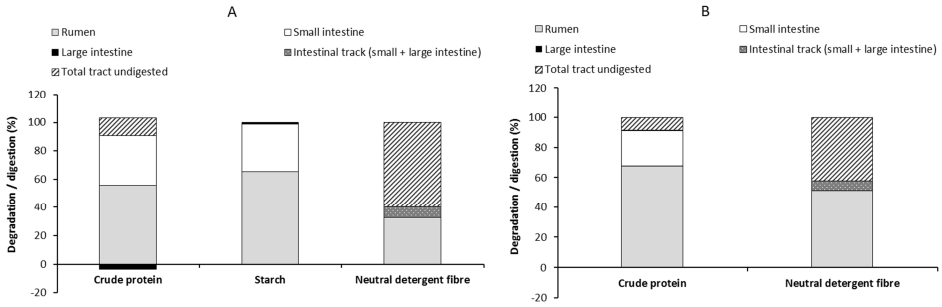

Figure 2 Apparent degradation (fermentation and digestion) or digestion of crude protein, neutral detergent fibre and/or starch of (A) maize and (B) grass silages in the gastrointestinal tract of dairy cows.

The results presented in Chapter 5 indicate that rumen undegraded starch in maize silages is almost completely digested in the intestinal tract of dairy cows. On average 97% (ranged from 91 to 100%) of the rumen undegraded starch was digested in the small intestine of the dairy cows. The rumen undegraded starch is subjected to enzymatic digestion with subsequent absorption of the glucose in the small intestine (Norberg *et al*., 2007). Owens *et al.* (1986) observed that starch digestion provides more energy in the small intestine, compared to fermentation in the rumen, which means that the net energy of feed can be increased by shifting starch digestion from the rumen to the small intestine in the dairy cows (Nocek and Tamminga, 1991). The results presented in Chapter 5 for small intestinal digestibility of starch of maize silages validate the assumption in the Dutch DVE/OEB_{2010} system (Van Duinkerken *et al*., 2011) that rumen undegraded starch is completely digested in the small intestine of dairy cows. Ruminants do not secrete fibrolytic enzymes, which mean that the rumen undegraded fibre is mainly degraded in the hindgut by microorganisms. Therefore, the intestinal NDF digestibility was determined for the total tract (small + large intestine), and not for the small and large intestine separately (Chapter 5). The results of the MNB incubations show that, on average, 11% (range 8-16%) of the rumen undegraded NDF of the maize silages was digested in the total intestinal tract (mainly in the hindgut) of the dairy cows. The average NDF degradation of maize silages in the total gastrointestinal tract (rumen and intestines) of dairy cows was 40% with the range of 24-50%. Figure 2B shows the apparent CP and NDF degradation/digestion (%) of grass silages in the gastrointestinal tract of dairy cows. The

average values for the CP degradation of grass silages in the rumen, small intestine and large intestine were 68, 24 and 0%, respectively. The results show that, on average, 74% (range 65-81%) of the rumen undegraded CP of grass silages was digested in the intestinal track of dairy cows (Chapter 5). The total tract digestibility of CP determined by the MNB technique, after 12 h incubation in the rumen, was 92% with the range of 85-95%. These results for total tract digestibility of CP were supported by Cone *et al*. (2006). The latter author reported an average value of 91% for the total tract CP digestibility of grass and grass silage samples determined by the MNB technique. These similar results for apparent CP digestibility in the gastrointestinal tract of dairy cows suggest that the entire CP is digested, except for the endogenous losses involving N and a small total tract undigested proportion. It may well be that the total tract undigested proportion is the CP that is incorporated in the cell walls and which can only be degraded after the cell wall (NDF) is degraded (Cone *et al*., 2006). The CP proportion bound to NDF was found higher in case of air drying (70°C) of incubated residues because of Maillard reactions during air dying compared to freeze drying (Cone *et al*., 1996). On average 13% (range 6-26%) of the rumen undegraded NDF of grass silages was degraded in the intestinal tract of dairy cows. The average NDF degradation of grass silages in the total gastrointestinal tract of dairy cows was 58% with the range of 49-72%. The large range in the intestinal digestibility of CP, NDF and starch was due to the broad range in the chemical composition of the maize and grass silage samples (Chapter 5).

The *in situ* results of the intestinal digestion of CP, NDF and starch of maize and grass silages may be slightly different from the *in vivo* situation, because of the pre-treatment of the rumen incubated residues (milling to 1 mm), and the chosen length of the pre-incubation in the rumen, before the intestinal incubations. Grinding rumen incubated residues helps the intestinal microbes to attack and degrade the nutrients more easily, which may lead to higher MNB intestinal digestibilities of CP, NDF and starch of maize and grass silages. In addition, the use of a nylon bag may also affect the results to a certain extent as there may be potentially less interaction between the microbiota and the ingredients/substrate compared to *in vivo* studies (Berthiaume *et al*., 2000).

Relationship between rumen degradability and intestinal digestibility

The results presented in Chapters 2, 3 and 5 show that the rumen degradation characteristics and intestinal digestibility of maize and grass silages were affected by the broad range in chemical composition. In addition, the results described in Chapter 5 show that the intestinal digestibility of CP and/or starch of maize and grass silages were also affected by their ruminal

degradation. A very strong negative and significant linear relationship was observed (Figure 3) between the CP rumen degradability and intestinal digestibility for maize (P<0.001, R^2=0.94) and grass silages (P<0.001, R^2=0.99). Cone *et al.* (2006) also found that the amount of rumen undegraded CP of grass silages largely affects the intestinal digestibility. Actually the digestibility in the small intestine compensates for a decreased degradation in the rumen.

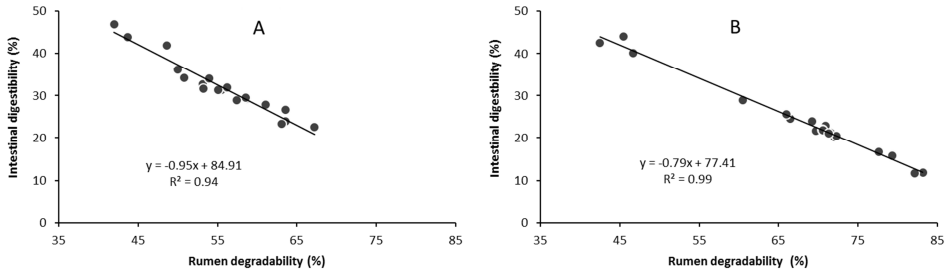

Figure 3 Relationship between rumen degradability and intestinal digestibility of crude protein of maize silages (A, n=19) and grass silages (B, n=19).

The negative relationship between the extent of ruminal degradation and the postruminal digestibility of N and amino acids of legumes was also observed by Mupangwa *et al.* (2003).

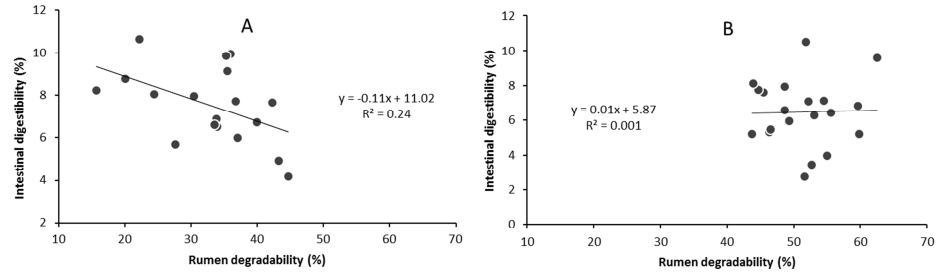

Figure 4 Relationship between rumen degradability and intestinal digestibility of neutral detergent fibre of maize silages (A, n=18) and grass silages (B, n=19).

Figure 4 shows linear relationships between the rumen degradability and intestinal digestibility of NDF of maize and grass silages. The R^2 value of 0.24 shows a very weak, but significant relationship (P=0.04) between the rumen degradability and intestinal digestibility of NDF of maize silages. The R^2 value of 0.001 (P=0.89) shows no relationship between the rumen degradability and intestinal digestibility of NDF in grass silages. Figure 5 shows a very

strong linear relationship (R^2=0.998, P<0.001) between the rumen degradability and intestinal digestibility of starch of maize silages. The intestinal digestibility of starch varies between different samples of maize silages, due to variation in the chemical composition of maize silage samples. Very strong relationships (Figure 3) for CP of maize and grass silages and for starch (Figure 5) of maize silages between the *in situ* rumen degradability and intestinal digestibility indicate that the intestinal digestibility of these nutrients was mainly affected by the rumen bypass content. The rumen bypass content of CP and starch was affected by their concentrations in the maize and grass silages.

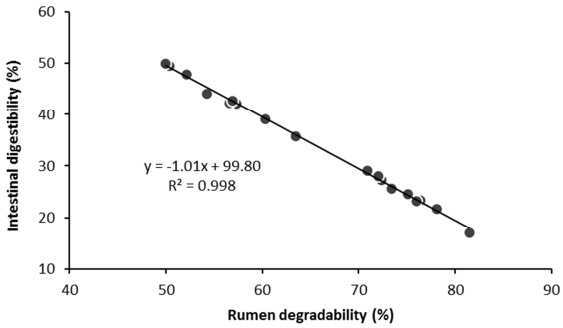

Figure 5 Relationship between rumen degradability and intestinal digestibility of starch of maize silages (n=18).

Number of animals required for *in situ* nylon bag incubations

Various ruminant feed evaluation systems such as the DVE/OEB_{2010} system (Van Duinkerken *et al*., 2011) in the Netherlands, the FiM system (Thomas, 2004) in the UK, the PDI system (Vérité *et al*., 1979) in France and the Nordic NorFor system (Volden, 2011) used in Nordic countries, developed protocols for *in situ* nylon bag incubations. These protocols recommend to use a minimum of three animals for *in situ* nylon bag incubations of ruminant feeds and feed ingredients. In the past, different studies were performed with concentrates and forages to determine the variation between individual animals in *in situ* rumen degradation characteristics of dietary nutrients. Significant differences between cows were observed for N disappearance for soybean meal (Weakley *et al*., 1983) and DM disappearance for barley and sorghum (Figroid *et al*., 1972). Various *in situ* studies were conducted with maize and grass silages, using different number of cows (2, 3, 4 and 6) and the rumen incubated residues after

specific incubation periods were pooled to obtain a representative sample (Volden *et al*., 2002; Fernandez *et al*., 2004; Lund *et al*., 2007; Ranjbari *et al*., 2007). To the author's knowledge, no study has validated the assumption that using three cows for *in situ* nylon bag incubations of maize and grass silages is sufficient to cover the variation between individual cows in rumen degradation characteristics.

Table 1 *Variation between cows in apparent rumen degradability of dry matter, organic matter, crude protein, starch and neutral detergent fibre of maize and grass silages, incubated for different time periods*[1]

Variable	Variation between cows[2]
Maize silages (n=15)	
Dry matter	*
Organic matter	*
Crude protein	***
Neutral detergent fibre	NS
Starch	NS
Grass silages (n=15)	
Dry matter	NS
Organic matter	NS
Crude protein	NS
Neutral detergent fibre	NS

[1] Rumen incubation time periods were 2, 4, 8, 16, 32, 72 and 336 h.
[2] NS, not significant ($P > 0.05$); *, $0.01 < P < 0.05$; **, $0.001 < P < 0.01$; ***, $P < 0.001$.

With this background, a research interest arose to test whether three cows are sufficient or not to cover the variation between individual cows in *in situ* rumen degradability of DM, OM, CP, NDF and/or starch of maize and grass silages. Table 1 shows the significant ($P<0.05$) and non-significant ($P>0.05$) variation in apparent rumen degradability of DM, OM, CP, NDF and/or starch of maize and grass silages incubated for 2, 4, 8, 16, 32, 72 and 336 h in the rumen of three cows. Significant differences between the cows were observed for apparent rumen degradability of DM, OM and CP of maize silages, indicating that three cows are not sufficient to cover the variation between individual cows for maize silages. Therefore, more than three cows should be used for nylon bag incubations to estimate the rumen degradation characteristics of DM, OM and CP of maize silages. To precisely determine the lowest number of cows that can be used for maize silages, further research with more than three cows is required. The results in the present study showed that the differences ($P>0.05$) between the three cows were not significant for the rumen degradability of DM, OM, CP and NDF of grass silages, indicating that three cows are sufficient for grass silages to cover the variation

between individual cows. The results also allow the pooling of rumen incubated residues of grass silages after specific incubation periods to obtain a representative sample (Chapter 4).

Nitrogen and starch fractionation of maize and grass silages

Different procedures for the *in situ* nylon bag technique (Ørskov and McDonald, 1979) have been used for the fractionation of ruminant feeds into a washout (W) fraction, a potentially rumen degradable (D) fraction and a rumen undegradable (U) fraction. The WMM has been extensively used in different *in situ* procedures to determine the W fraction and to remove the ruminal contamination from rumen incubated nylon bags (Gierus *et al*., 2005; Van der Koelen *et al*., 1992). A new method, not involving a washing machine and using buffers instead of water, was recently introduced by de Jonge *et al*. (2009) for the fractionation of concentrates and forages under more physiological conditions. In the MM, one rinsing procedure is used to separate the soluble (S), the insoluble washout (W-S), and the non-washout (D+U) fractions, using identical experimental conditions. The use of a standard shaking procedure using this MM leads to more reliable results for the W fraction and the *in situ* residues (de Jonge *et al*., 2013). In Chapter 6, the standard WMM and MM procedures were compared for the N and/or starch fractions of maize and grass silages. A large range was found for the N and starch fractions determined by both methods, which is likely due to the broad range in the chemical composition of the maize and grass silages. The results presented in Chapter 6 show that different values for N and/or starch fractions of the maize and grass silages were obtained using the standard WMM and the MM procedure. The difference in these values can be directly attributed to the different rinsing procedures and solvents used in both methods. The largest differences were found in the D+U fractions of starch compare to D+U fractions of N. The loss of insoluble small particles of starch was less during shaking of nylon bags in buffer solution, compared to washing nylon bags in the washing machine. Therefore, large differences were found between the D+U fractions of starch determined by both methods compared to the D+U fractions of N. The different linear relationships between the starch content in maize silages and the D+U fractions of starch determined by both methods also shows that the methodological approaches in both methods cause different fractionation values (Figure 6).

Regression equations were developed describing the relationship between the N and/or starch fractions and the chemical composition of the maize and grass silages (Chapter 6). As the samples were selected to obtain a broad range in chemical composition, a number of the developed equations can be used for the predictions of N and/or starch in maize and grass

silages from their chemical characteristics. The information on relationships between the chemical composition and the N and starch fractions can be used for the estimation of feeding values of maize and grass silages.

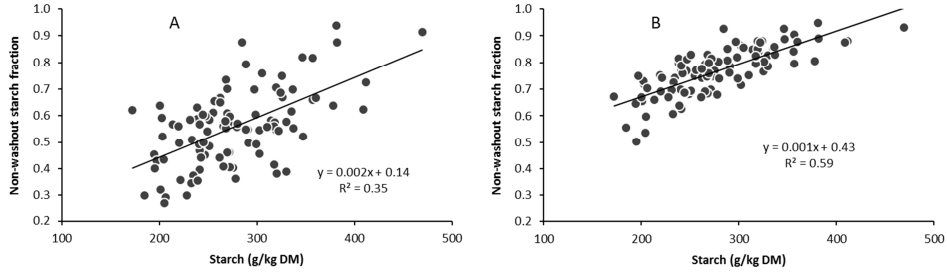

Figure 6 Relationship between the starch in maize silages (n=99) and the non-washout fractions of starch determined with the standard washing machine method (A) and the modified method (B) of de Jonge *et al*. (2009).

Conclusions

The research described in this thesis shows that the *in situ* rumen degradation characteristics and the MNB intestinal digestibility of dietary nutrients of maize and grass silages are highly variable due to the broad range in chemical composition. The developed regression equations presented in Chapters 2 and 3 provide better understanding of the relationships between chemical composition and *in situ* rumen degradation characteristics of maize and grass silages. Some of these regression equations can be used for the estimation of rumen degradation characteristics of dietary nutrients from chemical characteristics of maize and grass silages used in practice. The regression equations presented in Chapter 5 for intestinal CP digestibility can be used for the estimation of the intestinal digestion of CP from the chemical characteristics of maize and grass silages. The results show that the intestinal digestibility of CP and starch of maize and grass silages is not only influenced by the chemical composition but also by the rumen incubation period and the extent of rumen degradation.

The significant differences between individual cows for rumen degradability of DM, OM and CP of maize silages indicate that the use of more than three cows is required for an accurate estimation of the rumen degradation characteristics of DM, OM and CP in maize silage. Non-significant differences were found for all nutrients of grass silages indicating that three cows are sufficient to cover the variation between the cows and to obtain reliable estimates. The

information on variation between the cows in rumen degradation characteristics of these feedstuffs is important for estimation of their feeding values.

Different values were obtained using the standard WMM and a newly developed MM to determine the W, S and D+U fractions of N of maize and grass silages and the W, W-S and D+U fractions of starch of maize silages. The developed regression equations describe relationships between the chemical composition and the N and starch fractions determined by both fractionation methods. A number of these developed regression equations can be used for the prediction of the W and D+U fraction of the N and starch fractions from the chemical composition of maize and grass silages, without conducting expensive experiments.

Practical implications

The data for rumen degradation characteristics and intestinal digestibility of maize and grass in the present work were obtained using uniform experimental conditions and as such are relevant to nutritionists, farmers or feed companies for ration formulation to meet the nutrient requirements of dairy cows in terms of their health and production. The newly developed regression equations provide more accurate estimates of the rumen degradation and intestinal digestibility characteristics of maize and grass silages, used in practice. A number of these equations can be used by feed analysis laboratory and nutritionists for the rapid estimation of the nutritive and feeding values of maize and grass silages, without the necessity of doing laborious and time consuming *in situ* experiments. The research described in this thesis reports the efficient utilization of dietary nutrients, contributing to the enhancement of the economic profitability of dairy farms and feed companies. In addition, it can contribute to the further reduction of nutrient losses and the ecological footprint of dairy production.

Recommendations for future research

Although the data in the present study were obtained under uniform experimental conditions, the results for rumen incubated and intestinal incubated residues of dietary nutrients were not corrected for microbial contamination. Microbial contamination is one of the main factors which can affect the *in situ* results of dietary nutrients especially concerning CP in forages. This subject requires further study. Further research should also focus on the different methods available such as the diaminopimelic acid and ^{15}N-Leucine method to determine the microbial mass and to correct for microbial contamination of rumen and intestinal residues. Corrected values would provide more accurate estimates of the CP utilised from feed ingredients by dairy cows.

The results presented in this thesis on the variation between cows in *in situ* rumen degradation characteristics (Chapter 4) indicate that three cows are not sufficient for nylon bag incubations of maize silages. The studies conducted here did not determine the minimum number of cows required for nylon bag incubations of maize silages. Further research with the use of four or more cows should be conducted to determine the minimum number of cows required for nylon bag incubation of maize silages.

The use of one rinsing (shaking) procedure under identical experimental conditions and a buffer solution as solvent, make the MM "physiologically more normal" and can be expected to yield more representative estimates compared to the traditional standard WMM (Chapter 6). However, before the MM can replace the traditional WMM in the *in situ* nylon bag technique, this method should be evaluated for rumen incubated residues of maize and grass silages. The effect of the rinsing (shaking) procedure of the MM on the residual fractions, k_d and ED of dietary nutrients should also be investigated.

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Summary

The increasing importance of maize and grass silages in dairy rations in various parts of the world makes our understanding of factors which affect their nutritive value of paramount importance. This nutritive value extensively varies due to variation in chemical composition of these ensiled forages, which occurs due a combination of different factors such as use of various cultivars, fertilisation practices, growing conditions, harvesting technology, maturity at harvest and ensiling conditions. Different individual factors have been studied in different *in situ* studies, but information on the relationships between the chemical composition and the *in situ* ruminal and postruminal degradation of dietary nutrients remains scarce in the scientific literature. This information is important for the accurate estimation of the feeding value, in order to optimize ration formulation in terms of animal health and production performance, without the need to perform expensive and time-consuming experiments involving animal studies.

The first aim of this thesis was to investigate relationships between the chemical composition and the *in situ* rumen degradation characteristics and *in situ* mobile nylon bag (MNB) digestibility of dietary nutrients of maize and grass silages. The second aim of this thesis was to determine whether three cows are sufficient or not to cover the variation between individual cows in *in situ* rumen degradation characteristics of dietary nutrients of maize and grass silages. The third aim of this thesis was to compare two fractionation methods; the washing machine method and a modified method, for nitrogen (N) and starch fractions of maize silages and N fractions of grass silages. In addition, the prediction of N and/or starch fractions of maize and grass silages from chemical analyses was investigated.

In Chapter 2 and 3, the relationships between the chemical composition and the *in situ* rumen degradation characteristics of dry matter (DM), organic matter (OM), crude protein (CP), neutral detergent fibre (NDF) and/or starch of maize and grass silages were investigated. The maize and grass silage samples were obtained from different commercial farms, located in different regions in the Netherlands. After chemical analyses, 75 maize and 69 grass silage samples were selected to provide a broad range in chemical composition and silage quality parameters. The maize silage samples were selected on the basis of CP, NDF and starch content and the grass silage samples were selected on the basis of CP and NDF content. The maize and grass silages were incubated in the rumen of three dairy cows, using the nylon bag technique. The data for maize and grass silages were obtained under uniform experimental conditions (same cows, same incubation protocol and same chemical analysis procedures) therefore being free of variation of assay conditions. The broad range in the chemical composition of the maize and grass silages resulted in a large variation in the rumen

degradable fractions of DM, OM, CP, NDF and starch. These data were used to derive regression equations, describing the relationships between the chemical composition and *in situ* rumen degradation characteristics of DM, OM, CP, NDF and/or starch of maize and grass silages. Some of these equations can be used for a rapid estimation of the rumen degradation characteristics of maize and grass silages used in practice.

Various ruminant feed evaluation systems, such as the DVE/OEB_{2010} system in the Netherlands, the PDI system in France and the Nordic NorFor system used in Scandinavian countries, recommend the use of three animals for *in situ* nylon bag incubation of feeds. In Chapter 4, it was evaluated whether the recommended minimum number of (three) cows was sufficient or not to cover the variation between individual cows in *in situ* rumen degradation characteristics of maize and grass silages. Fifteen maize and 15 grass silage samples were used in this study. The results showed that significant differences (P<0.05) were found between individual cows for a number of parameters of DM, OM and CP, indicating that three cows are not sufficient for *in situ* nylon bag incubations of maize silages and that four or more cows should be used. For grass silages, non-significant differences (P>0.05) between individual cows were found for all parameters of DM, OM, CP and NDF. The results of this study suggest that using three cows are sufficient for nylon bag incubations of grass silages and pooling of rumen incubated residues is allowed to obtain a representative sample. The information on variation between individual cows is important for the estimation of the rumen degradation characteristics.

In Chapter 5, the relationships between the chemical composition and the *in situ* MNB intestinal digestibility of CP, NDF and/or starch of maize and grass silages, are described. There was a large range in rumen degradability after specific incubation times and the intestinal digestibility of CP, NDF and starch, due to the broad range in the chemical composition of the maize and grass silages. The intestinal digestibility of CP, NDF and/or starch was affected by the concentration of these components in the maize and grass silages, by the rumen incubation time and the rumen escape content. The developed regression equations provide acceptable estimates for ruminal and postruminal degradation of CP and NDF of maize and grass silages.

With the *in situ* nylon bag technique, different rinsing procedures have been used for the fractionation of feeds into washout (W) and non-washout (D+U) fractions. The D+U fraction after rumen incubation, using the nylon bag technique, is divided into a potentially rumen degradable (D) fraction and a rumen undegradable (U) fraction. The washing machine method has been used in many different *in situ* studies to determine the W fraction and to wash

adhering material from rumen incubated nylon bags. Different washing machines, washing programmes and washing times have been used in these studies, causing variation in the W fraction and in the *in situ* results. A new modified method was recently introduced with one rinsing procedure under controlled experimental conditions, using a buffer solution instead of water. In Chapter 6, the N and/or starch fractions of maize (n=99) and grass silages (n=99) determined, using the washing machine method (washing with water) and the modified method (shaking with buffer solution) were compared. The different methodological approaches of both methods resulted in different values for the W, the soluble (S) and the D+U fractions of N of maize and grass silages and for the W, the insoluble washout (W-S) and the D+U fractions of starch of maize silages. The relationships between the N and starch fractions determined using both methods and the chemical composition of maize and grass silages were also investigated. The developed regression equations can be used for the prediction of the W, S and D+U fractions of N of grass silages and the D+U fraction of starch of maize silages from the chemical characteristics, without conducting extensive experiments.

The following conclusions were drawn from the research described in this thesis:

- The chemical composition significantly affects the *in situ* rumen degradation characteristics of dietary nutrients of maize and grass silages.
- The developed regression equations between chemical composition and rumen degradation characteristics of maize and grass silages provide a better understanding and, in certain cases, allow rapid estimation of rumen degradation characteristics of dietary nutrients.
- The *in situ* MNB digestibility of dietary nutrients of maize and grass silages is not only influenced by their chemical composition but also by the pre-incubation period in the rumen and the degree of degradation in the rumen and so by the rumen escape faction.
- The developed regression equations for small and large intestinal digestibility of CP can be used for the rapid estimation of intestinal CP digestibility from chemical characteristics of maize and grass silages used in practice.
- Three cows are sufficient for *in situ* nylon bag incubations of grass silages but four or more are required for maize silages.
- Different values for N and/or starch fractions of maize and grass silages were obtained due to different methodological approaches of using a washing machine method or a newly developed modified method.

As maize and grass silages are major feed materials for dairy herd, the information on nutrient bioavailability obtained in the present work can be used to meet the nutrient requirements of

dairy cows in terms of their maintenance, health and production, leading to an increased economic profitability of dairy farmers. A number of the developed regression equations presented in this thesis can be used for a rapid estimation of the ruminal and postruminal degradation characteristics of dietary nutrients of maize and grass silages, without conducting time consuming and expensive *in situ* experiments.

Samenvatting

Het toenemend gebruik van maïs- en graskuilen in het rantsoen voor melkvee in grote delen van de wereld, maakt het noodzakelijk om onze kennis over factoren die de voederwaarde van deze silages bepalen en beïnvloeden wordt uitgebreid. De voederwaarde van maïs- en graskuilen varieert sterk met de chemische samenstelling, wat weer het gevolg is van een combinatie van verschillende factoren, zoals het gebruik van verschillende cultivars, bemestingspraktijken, groeiomstandigheden, oogsttechnologie, rijpheidstadium op het moment van de oogst en inkuiltechnieken. Verschillende van deze individuele factoren zijn reeds onderzocht in *in situ* experimenten. Gegevens over de relatie tussen de chemische samenstelling van de kuilen en de *in situ* afbraak in de pens en in de darmen van koeien zijn op beperkte schaal beschreven in de wetenschappelijke literatuur. Deze informatie is echter van belang voor een nauwkeurige schatting van de voedingswaarde, ten einde de samenstelling van rantsoenen te kunnen optimaliseren voor een optimale diergezondheid en productiviteit, zonder dat het noodzakelijk is om dure en tijdrovende experimenten met dieren uit te voeren.

Het eerste doel van dit proefschrift was om de relatie tussen de chemische samenstelling van de maïs- en graskuilen en de *in situ* pensafbraak (nylonzakjestechniek) en de *in situ* verteerbaarheid in de darmen (mobiele nylonzakjestechniek) van de diverse nutriënten te onderzoeken. Het tweede doel was om te bepalen of drie koeien al dan niet voldoende zijn om de variatie tussen individuele koeien, voor wat betreft *in situ* pensafbraakkenmerken van de maïs- en graskuilen, te dekken, of dat meer dieren nodig zijn. Het derde doel van dit proefschrift was om twee fractioneringsmethoden met elkaar te vergelijken, de wasmachinemethode en een nieuwe aangepaste methode, om stikstof (N) en zetmeel in maïskuilen en N in graskuilen te scheiden in verschillende fracties. Bovendien werd de mogelijkheid om de N- en zetmeelfracties van de maïs- en graskuilen te schatten met behulp van de chemische analyses onderzocht.

In hoofdstuk 2 en 3 werd de relatie onderzocht tussen de chemische samenstelling van de maïs- en graskuilen en de *in situ* pensafbraakkarakteristieken van de droge stof (DS), organische stof (OS), ruw eiwit (RE), neutraal detergent fibre (NDF) en zetmeel. Monsters van maïs- en graskuilen werden genomen van verschillende boerderijen gelegen in verschillende delen van Nederland. Na het bepalen van de chemische samenstelling, werden 75 maïskuilmonsters en 69 graskuilmonsters geselecteerd om verder onderzoek mee te verrichten, om zodoende een brede range aan chemische samenstelling en kuilkwaliteit van de monsters te verkrijgen. De maïskuilmonsters werden geselecteerd op basis van de gehaltes aam RE, NDF en zetmeel en de graskuilmonsters werden geselecteerd op basis van de

gehaltes RE en NDF. De maïskuil- en graskuilmonsters werden in poreuze nylon zakjes geïncubeerd in de pens van drie melkkoeien, waardoor data werden verkregen onder uniforme experimentele omstandigheden (dezelfde koeien, hetzelfde incubatieprotocol en dezelfde procedures voor chemische analyses) en dus zonder variatie in de experimentele condities. De brede range in de chemische samenstelling van de maïs- en graskuilen leidde tot een grote variatie in de pens-afbreekbare fracties van DS, OS, RE, NDF en zetmeel. De verkregen gegevens werden gebruikt om regressievergelijkingen af te leiden tussen de chemische samenstelling van de maïs- en graskuilen en de afbraakkarakteristieken van DS, OS, RE, NDF en zetmeel. Enkele van deze vergelijkingen kunnen gebruikt worden voor een snelle schatting van de pensafbraakkarakteristieken onder praktijkomstandigheden.

Verschillende voederwaarderingssystemen voor herkauwers, zoals het DVE/OEB_{2010} systeem in Nederland, het PDI-systeem in Frankrijk en het Scandinavische NorFor systeem, adviseren het gebruik van drie dieren om in situ nylonzakjesincubaties mee uit te voeren. In hoofdstuk 4 werd onderzocht of het aanbevolen minimum aantal van drie koeien voldoende is om de variatie tussen de individuele koeien te dekken zodat een accurate schatter wordt bepaald. Vijftien maïskuil- en 15 graskuilmonsters werden hiervoor gebruikt. De resultaten laten zien dat er significante verschillen (P<0,05) waren tussen de individuele koeien voor de afbraakkarakteristieken van DS, OS en RE in maïskuilen, dat aangeeft dat het gebruik van slechts drie koeien niet voldoende is om in situ nylonzakjesincubaties met maïskuilen mee uit te voeren en dat vier of meer koeien noodzakelijk zijn. De verschillen tussen individuele koeien die werden gevonden voor de afbraakkarakteristieken van DS, OS, RE en NDF in graskuilen waren niet significant (P>0,05). De resultaten suggereren dat het gebruik van drie koeien voldoende is om nylonzakjesincubaties met graskuilen mee uit te voeren en ook dat het samenvoegen van pensincubatieresiduen van graskuilen is toegestaan om een representatief monster te verkrijgen. Informatie over variatie tussen individuele koeien is belangrijk voor een nauwkeurige schatting van de pensafbraakkarakteristieken.

In hoofdstuk 5 worden relaties beschreven tussen de chemische samenstelling van de maïs- en graskuilen en de afbreekbaarheid van RE, NDF en zetmeel in de dunne en dikke darm, bepaald met de mobiele nylonzakjestechniek. Voorafgaande aan de incubatie in de darmen van de koeien werden de monsters verschillende periodes geïncubeerd in de pens van koeien. Er was een grote variatie in de afbreekbaarheid van RE, NDF en zetmeel in de pens en in de darmverteerbaarheid, hetgeen mede veroorzaakt werd door de brede range in de chemische samenstelling van de maïs- en graskuilen. De darmverteerbaarheid van RE, NDF en zetmeel

werd beïnvloed door het gehalte van deze componenten in de maïs- en graskuilen en door de afbraak in de pens. De ontwikkelde regressievergelijkingen bieden aanvaardbare schattingen voor de afbraak van RE en NDF in maïs- en graskuilen in de pens en in de darmen van lacterende koeien.

Verschillende wasprocedures worden gebruikt voor het fractioneren van voermonsters in een uitwasbare fractie (W) en een niet-uitwasbare (D+U) fractie. Na incubatie van de nylon zakjes in de pens bestaat de D+U fractie uit een potentieel pensafbreekbare (D) fractie en een niet-pensafbreekbaar (U) fractie. De wasmachinemethode om de grootte van de fracties te bepalen werd (en wordt) gebruikt in veel in situ onderzoek om de W-fractie te bepalen en om de zakjes te wassen na incubatie in de pens. In veel beschreven studies worden verschillende wasmachines, wasprogramma's en wastijden gebruikt, hetgeen leidt to variatie in de grootte van de W-fractie en in de uiteindelijke in situ resultaten. Een nieuwe aangepaste methode werd onlangs geïntroduceerd, waarbij gespoeld wordt met een bufferoplossing in plaats van met water, onder gecontroleerde experimentele omstandigheden. In hoofdstuk 6 werd deze nieuwe aangepaste methode vergeleken met de traditionele wasmachine methode. De N en zetmeel fracties van maïskuilen (n=99) en de N fracties van graskuilen (n=99) werden bepaald met de wasmachinemethode (wassen met water) en met de aangepaste methode (schudden met bufferoplossing). De verschillende benaderingen van beide methoden leidde tot verschillende waarden voor de uitwasbare fractie (W), voor de oplosbare fractie (S) en voor de D+U fracties van N in maïs- en graskuilen en van de W fractie, de onoplosbare uitwasbare fractie (W-S) en de D+U fractie van zetmeel in maïskuilen. De relatie tussen de chemische samenstelling van de maïs- en graskuilen en de N-fracties en de zetmeelfracties, bepaald met beide methodes, werd ook onderzocht. De ontwikkelde regressievergelijkingen kunnen worden gebruikt voor het voorspellen van de fracties W, S en D+U van N in graskuilen en van de fracties D+U in maïskuilen uit de chemische samenstelling, zonder dat uitgebreide experimenten benodigd zijn.

De volgende conclusies kunnen worden getrokken uit het onderzoek beschreven in dit proefschrift:

- De chemische samenstelling heeft een significant effect op de *in situ* afbraak-karakteristieken van de verschillende chemische componenten van maïs- en graskuilen.
- De ontwikkelde regressievergelijkingen tussen chemische samenstelling en pensafbraak-karakteristieken van maïs- en graskuilen zorgen voor een beter begrip en, in bepaalde gevallen, voor een snelle schatting van de pensafbraakkarakteristieken van de nutriënten.

- De verteerbaarheid van nutriënten van maïs en- graskuilen in de darmen, bepaald met de mobiele nylonzakjestechniek, wordt niet alleen beïnvloed door hun chemische samenstelling maar ook door de pre-incubatieperiode in de pens en de mate van afbraak in de pens.
- De ontwikkelde regressievergelijkingen voor de vertering van ruw eiwit in de dunne en dikke darm kunnen worden gebruikt voor een snelle bepaling van de darmverteerbaarheid van ruw eiwit uit de chemische samenstelling van de maïs- en graskuilen in de praktijk.
- Voor de nylonzakjesincubaties van graskuilen zijn drie koeien, maar voor incubaties van maïskuilen in nylon zakjes in de pens zijn vier of meer dieren vereist.
- Verschillende waarden voor de fracties van N en zetmeel van maïs- en graskuilen werden verkregen met de twee beschreven methodes, de wasmachinemethode en een nieuwe aangepaste methode, die methodologisch verschilden.

Omdat maïs- en graskuilen de belangrijkste voedermiddelen zijn voor melkvee, kan de informatie over de beschikbaarheid van nutriënten, beschreven in dit proefschrift, worden gebruikt om de behoeften van melkkoeien aan nutriënten te voldoen in termen van onderhoud, gezondheid en productie wat leidt tot een verhoogde economische rentabiliteit van de melkveehouders. Sommige van de ontwikkelde regressievergelijkingen kunnen worden gebruikt voor een snelle schatting van de afbraakkarakteristieken van nutriënten van maïs- en graskuilen in de pens en in de darmen, zonder dat tijdrovende en dure *in situ* experimenten benodigd zijn.

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Curriculum vitae

About the author

Mubarak Ali was born on October 15, 1981 in Sialkot, Pakistan. He attended elementary and secondary school education in the city he was born. In 2000, he obtained the intermediate higher secondary school (Grade 12) certificate with premedical subjects from Faisalabad College of Science, Faisalabad. From 2001 to 2007 he studied at the University of Agriculture, Faisalabad, Pakistan and obtained B.Sc. (Hons.) degree in Animal Husbandry and M.Sc. (Hons.) degree in Animal Nutrition. His M.Sc. thesis was about the apparent metabolizable energy of local feed ingredients in broilers using total collection method and ileal digestibility assay. In July, 2007 he became a farm production advisor in Pakistan Dairy Development Company and in 2009, he was promoted as the team manager of Northern Punjab for his hard work for the dairy sector of Northern Punjab, Pakistan. He was awarded an overseas scholarship by the Higher Education Commission (HEC) Pakistan to pursue doctorate studies in the Netherlands. In August 2009, he started his PhD in the Animal Nutrition Group of Wageningen University and Wageningen UR Livestock Research Centre, Lelystad, the Netherlands. During his PhD, he worked on rumen fermentation profile and intestinal digestibility of maize and grass silages. Moreover, he compared the different fractionation methods for nitrogen and starch of maize silages and nitrogen of grass silages.

Publications

Refereed scientific publications

Ali M, Weisbjerg MR, Cone JW, van Duinkerken G, Blok MC, Bruinenberg M and Hendriks WH 2012. Postruminal degradation of crude protein, neutral detergent fibre and starch of maize and grass silages in dairy cows. Animal Feed Science and Technology 177, 172-179.

Habib G, Khan NA, **Ali M** and Bezabih, M 2013. *In situ* ruminal crude protein degradability of cereal grains, oilseeds and animal by-product based protein feedstuffs. Livestock Science 153, 81-87.

Habib G, **Ali M**, Bezabih M and Khan NA 2013. *In situ* assessment of ruminal dry matter degradation kinetics and effective rumen degradability of feedstuffs originated from agro-industrial by-products. Pakistan Veterinary Journal (in press).

Ali M, Cone JW, van Duinkerken G, Klop A, Kruisdijk J, Blok MC, Bruinenberg M and Hendriks WH. Relationship between chemical composition and *in situ* rumen degradation characteristics of grass silages in dairy cows. Netherlands Journal of Agriculture Science (revison submitted).

Ali M, Cone JW, van Duinkerken G, Klop A, Blok MC, Bruinenberg M and Hendriks WH. Variation between individual cows in *in situ* rumen degradation characteristics of maize and grass silages. Animal (revision submitted).

Ali M, van Duinkerken G, Cone JW, Klop A, Blok MC, Spek JW, Bruinenberg M and Hendriks WH. Relationship between chemical composition and *in situ* rumen degradation characteristics of maize silages in dairy cows. Animal (revison submitted).

Ali M, de Jonge LH, Cone JW, van Duinkerken G, Blok MC, Bruinenberg M and Hendriks WH. Comparison of fractionation methods and prediction of nitrogen and starch from chemical composition of maize and grass silages. Animal (submitted).

Ali A, Khan S, Akhter S, Inam M, Khan NA, Ahmad I and **Ali M**. Effect of different levels of organic acids supplementation on feed intake, milk yield and milk composition during thermal stress. Tropical Animal Health and Production (submitted).

Khan NA, **Ali M**, Cone JW and Hendriks WH. Invited review; Nutritional value of maize silage and effect on milk production and composition. Journal of the Science of Food and Agriculture (in preparation).

Contributions to conferences and symposia

Ali M, Cone JW, van Duinkerken G, Blok MC, Klop A and Hendriks WH. 2010. Rumen fermentation and intestinal digestibility of grass and maize silages. In: Proceedings of the 35th Animal Nutrition Research Forum, Lelystad, The Netherlands. Pages 24-26.

Ali M, Cone JW, van Duinkerken G, Weisbjerg MR and Hendriks WH. 2012. Postruminal degradation of crude protein, neutral detergent fiber and starch of maize and grass silages. In: Proceedings of the 37th Animal Nutrition Research Forum, Wageningen, The Netherlands. Pages 57-58.

Ali M, Weisbjerg MR, Cone JW, van Duinkerken G, Blok MC, Bruinenberg M and Hendriks WH. 2012. Postruminal degradation of crude protein, neutral detergent fiber and starch of maize and grass silages in Holstein Friesian dairy cows. In: Proceedings of the BSAS annual conference on Healthy Food from Healthy Animals, Nottingham, England. Page 91.

Ali M, van Duinkerken G, Weisbjerg MR, Cone JW and Hendriks WH. 2012. Postruminal digestibility of crude protein of maize and grass silages in dairy cows. In: Proceedings of the 63rd EAAP Annual Meeting, Bratislava, Slovakia. Page 56.

Ali M, Cone JW, van Duinkerken G and Hendriks WH. 2012. Relationship between chemical composition and *in situ* rumen degradation characteristics of grass silages. In: Proceedings of the 63rd EAAP Annual Meeting, Bratislava, Slovakia. Page 57.

Ali M, Cone JW, van Duinkerken G and Hendriks WH. 2013. Variation between individual cows in rumen degradation characteristics of maize and grass silages. In: Proceedings of the 64th EAAP Annual Meeting, Nantes, France (accepted).

Ali M, van Duinkerken G, Cone JW and Hendriks WH. 2013. Relationship between chemical composition and rumen degradation characteristics of maize silages. In: Proceedings of the 64th EAAP Annual Meeting, Nantes, France (accepted).

Training and Supervision Plan

Name	**Mubarak Ali**
Group	Animal Nutrition Group
Daily supervisors	Dr. John W. Cone, Dr. ir. Gert van Duinkerken
Supervisor	Prof. dr. ir. Wouter H. Hendriks

The Basic Package	**Year**	**Credits ***
WIAS Introduction Course	2010	1.5
Ethics and Philosophy of Life Sciences	2011	1.5
International conferences		
BSAS Annual Conference, Nottingham, England	2012	0.6
63rd EAAP Annual Conference, Bratislava, Slovakia	2012	1.5
64th EAAP Annual Conference, Nantes, France	2013	1.5
Seminars and workshops		
Role of plant cell wall in dairy cow nutrition; Symposium, Wageningen, The Netherlands	2009	0.15
35th ANR Forum, Lelystad, The Netherlands	2010	0.3
Scientific research in Animal Welfare; Do we make a difference? Seminar	2011	0.15
Nutrition and Fat Metabolism in Dairy Cattle, Wageningen, The Netherlands	2011	0.3
WIAS Science Days (3x)	2011-2013	0.9
37th ANR Forum, Wageningen, The Netherlands	2012	0.3
100 Doctors of Philosophy in Animal Nutrition; Which questions can possibly remain?	2012	0.3
Presentations		
35th ANR Forum, Lelystad, The Netherlands (Oral presentation)	2010	1.0
37th ANR Forum, Wageningen, The Netherlands (Oral presentation)	2012	1.0
BSAS Annual Conference, Nottingham, England (Oral presentation)	2012	1.0
63rd EAAP Annual Conference, Bratislava, Slovakia (2 oral presentations)	2012	2.0
64th EAAP Annual Conference, Nantes, France (1 oral and 1 potser)	2013	2.0
Disciplinary and interdisciplinary courses		
Epigenetics and Epigenesis	2011	0.8
Food fermentation	2012	1.1
Advanced statistics courses		
Statistics for Life Sciences	2011	2.0
Design of Experiment	2010	1.0
MSc level course		
Analytical work and possibilities within Animal Nutrition Sciences	2010	1.0
Professional Skills Support Courses		
Techniques for Writing and Presenting Scientific Papers	2011	1.2
Project and Time Management	2011	1.5
Information Literacy, including Introduction to EndNote	2009	0.6
Working with Endnote X2	2009	0.3
Scientific Publishing	2011	0.3
Competence Assessment	2011	0.3
Voice matters: Voice and Presentation Skills Training	2012	0.3
High Impact Writing in Science	2012	1.3
Research Skills Training		
Preparing own PhD research proposal	2009	6.0
Research work in Denmark (1 December, 2009 - 11 February, 2010)	2009/2010	2.0
Supervising practicals and excursions		
Review the RMC research proposals	2011	0.5
Practical Demonstration: Principles of Animal Nutrition course	2013	1.0
Total		**37.2**

* One ECTS credit equals a study load of approximately 28 hours.

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