Protein evaluation for ruminants:
THE DVE/OEB 2007 SYSTEM

S. Tamminga[1)], G.G. Brandsma[2)], J. Dijkstra[1)],
G. van Duinkerken[3)], A.M. van Vuuren[3)] and M.C. Blok[2)]

[1)] Group Animal Nutrition, Wageningen University, Wageningen
[2)] CVB, Product Board Animal Feed, Lelystad
[3)] Animal Sciences Group, Wageningen UR, Lelystad

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CVB
Product Board Animal Feed
P.O.Box 29739
NL 2502 LS The Hague
Telephone number 070 – 3708 708
Telefax number 070 – 3708 290
E-mail address cvb@pdv.nl
Internet www.cvb.pdv.nl

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[1)] Group Animal Nutrition, Wageningen University, Wageningen
[2)] CVB, Product Board Animal Feed, Lelystad
[3)] Animal Sciences Group, Wageningen UR, Lelystad

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CVB
Product Board Animal Feed
P.O.Box 23176
NL 8203 AD Lelystad
Telephone number 0320 – 29 35 34
Telefax number 0320 – 29 35 38
E-mail address cvb@pdv.nl
Internet www.pdv.nl

Preface

In 1990 CVB introduced a preliminary new protein system for ruminants, the DVE system, to replace the existing system based on faecal protein digestibility, the VRE system. The new DVE system was put into use in the Netherlands in 1991.

Since 1991 several scientific developments have occurred. The knowledge on physiological processes in the rumen, and on the behaviour of feedstuffs in the rumen (e.g. during nylon bags incubation) has increased considerably.

Also, different organisations (premix manufacturers and the animal feed industry) have developed their own systems, based on the 1991 DVE system.
Ten years ago, synchronizing the energy supply and the N supply at the rumen level was a key issue.
In the DVE system of 1991, the amount of rumen fermentable organic matter (FOM) was calculated based on the amount of faecal digestible organic matter. Various feed companies have adapted this and calculate the amount of rumen fermentable organic matter based on the truly rumen degradable fractions (effective degradation). Also, the fixed efficiency factor for the conversion of FOM into microbial (crude) protein was replaced by an efficiency depending on the substrate to be degraded.
Next to these national developments, new systems for dairy cattle have been developed worldwide (CNCPS in the USA and FiM in the UK).

The research project Rumen Synchronisation (Animal Sciences Group, Lelystad, the Netherlands; financed by the Ministry of Agriculture and by the Product Board for Dairy) has lead to strong impulses to renew the protein evaluation system for dairy cattle.
Eventually, the new protein evaluation system, DVE/OEB2007, has been completed under responsibility of the CVB. Dr ir S. Tamminga (former professor Animal Nutrition, Wageningen University) headed a project group that formulated the DVE/OEB2007 system.

The updated system was discussed and approved by a sector wide response group “Improvement Protein Evaluation System Ruminants”. The concept system was also discussed with specialists of several feed companies.

The evaluation of individual feedstuffs can be found in a separate CVB publication.

The new DVE/OEB2007 system has been introduced at a meeting on March 2nd 2007. It should be put into use by October 2007.

On behalf of the Product Board Animal Feed, I hereby thank all contributors to the completion of this new protein evaluation system for ruminants.

Lelystad, March 2007,
Dr M.C. Blok
Product Board Animal Feed

Members of the project group

Dr. M.C. Blok	CVB, Product Board Animal Feed, Lelystad/Den Haag (Chairman)
Ir. G.G. Brandsma	CVB, Product Board Animal Feed, Lelystad/Den Haag
Dr. Ir. J. Dijkstra	Group Animal Nutrition, Wageningen University, Wageningen
Ir. G. van Duinkerken	Animal Sciences Group, Lelystad
Prof. Dr. Ir. S. Tamminga	Group Animal Nutrition, Wageningen University, Wageningen (Project leader)
Dr. A.M. van Vuuren	Animal Sciences Group, Lelystad

Members of the response group

Dr. M.C. Blok	CVB, Product Board Animal Feed, Lelystad/Den Haag (Chairman)
Drs. A. Bannink	Animal Sciences Group, Lelystad
Ir. G.G. Brandsma	CVB, Product Board Animal Feed, Lelystad/Den Haag
Mrs. Dr. Ir. M. Bruinenberg	Blgg, Oosterbeek
Dr. Ir. J. Dijkstra	Group Animal Nutrition, Wageningen University, Wageningen
Ir. G. van Duinkerken	Animal Sciences Group, Lelystad
Ing. M. van den Ende	Overleggroep Producenten Natte Veevoeders (OPNV)
Dr. Ir. J. Goelema	Nederlandse Vereniging Diervoederindustrie (NEVEDI)
Ing. M. Hoogenboom	Nederlandse Vereniging van Handelaren in Stro, Foerages en Aanverwante Producten (HISFA)
J. de Kleijne	LTO Nederland
Dr. Ing. J. Th. Schonewille	Department Nutrition, Faculty of Veterinary Science, Utrecht University, Utrecht
Prof. Dr. Ir. S. Tamminga	Group Animal Nutrition, Wageningen University, Wageningen
Dr A.M. van Vuuren	Animal Sciences Group, Lelystad

Contents

List of Abbreviations

Abbreviation	Unit	Description
AA		Amino acids
AADI		Intestinal digestible amino acids
ADICP		Acid Detergent Insoluble CP
ADIN	kg	Acid detergent insoluble nitrogen
ADP		Adenosinediphosphate
ATP		Adenosinetriphoshate
BW	kg	Body weight
$BW^{0.75}$	kg	Metabolic body weight
CASH	g/kg	Crude ash
%dASH		Apparent faecal digestibility of (crude) ash
CF		Crude fibre
CFAT	g/kg	Crude fat
CHO		Carbohydrates
CNCPS		Cornell Net Carbohydrate and Protein System (USA)
COMP	g/kg DM	Content of component in feedstuff
CP	g/kg	Crude protein
D		Non-washout but potential degradable fraction in nylon bag incubations
DAPA		Di amino pimelinic acid (marker for bacterial protein)
DASH		Digestible (crude) ash
DM		Dry matter
DMCLYS		(Intestinal) digestible lysine from rumen undegradable protein
DMCMET		(Intestinal) digestible methionine from microbial protein
DMCP		(intestinal) degradable Microbial Crude Protein
DMFP		Endogenous protein
DMFLYS		Digestible lysine in endogenous protein
DMFMET		Digestible methionine in endogenous protein
DMI	kg	Dry matter intake
DOM		Digestible organic matter
dNDF		Apparent faecal digestibility of the NDF fraction
DNDF		Potential rumen degradable fraction of NDF
dRNSP		Apparent faecal digestibility of the RNSP fraction
DRULYS		(Intestinal) digestible lysine from rumen undegradable protein
DRUMET		(Intestinal) digestible methionine from rumen undegradable protein
DRUP		(intestinal) degradable Rumen Undegradable Protein
%DRUP		Intestinal digestibility of rumen undegraded protein
DVE	g/kg	Darm Verteerbaar Eiwit (intestinal digestible protein)
ED		Effective degradation
ED(W)		Effectively degraded fraction W
FA		Fatty acids
FCOMP	g/kg DM	Amount of component effectively degraded in the rumen
FDM		Fermentable dry matter
FiM		Feed into Milk (UK)
FOMr		Fermentable organic matter in the rumen
FP		Fermentation products
GOS	g/kg	Glucose Oligo Saccharides
HIS		Histidine

Abbreviation	Unit	Description
Kd		Fractional degradation rate (constant)
Kg		Kilogram
Kp		Fractional passage rate (constant)
Kpl		Fractional passage rate of liquid
Kpf		Fractional passage rate of forages
Kpc		Fractional passage rate of concentrates
LAB		Liquid associated bacteria
LYS		Lysine
LYSDI		Intestinal digestible lysine
MCP		Microbial Crude Protein
MCPN		Microbial CP to be synthesized from rumen available N
MCPE		Microbial CP to be synthesized from rumen available Energy
MET		Methionine
METDI		Intestinal digestible methionine
NAAN		Non amino acid nitrogen
NDF	g/kg	Neutral detergent fibre
NDICP		Neutral Detergent Insoluble CP
NPN		Non Protein Nitrogen
NSP	g/kg	Non Starch Polysacharides
NSP	g/kg	Non Starch Polysacharides
NW		Non washable
OEB	g/kg	Onbestendig Eiwit Balans (= Rumen degraded protein balance)
OM		Organic matter
PAB		Particle associated bacteria
PUFA		Poly unsaturated fatty acids
RDP		Rumen degradable protein
RDPB		Rumen degraded protein balance
RNSP	g/kg	Residual non-starch polysaccharides
%RUP		Percentage rumen undegraded protein
%RUSTA		Percentage rumen undegraded starch
R_t		Residue at time t
R_0		Residue at time zero
S		Soluble fraction in nylon bag incubations
SC		Soluble carbohydrates
SR		Synchrony Ratio
STA	g/kg	Starch
SU	g/kg	Sugars
T		Time
U		Undegradable fraction
UADF		ADF not available for degradation in the rumen
UNDF		NDF not available for degradation in the rumen
VFA		Volatile fatty acids
W		Washout fraction in nylon bag incubations
Y_{ATP}		Microbial growth yield, expressed as g microbial cells mol^{-1} ATP

1 Introduction

In 1991 the DVE/OEB system for protein evaluation in dairy cows, hereafter referred to as DVE/OEB1991, was introduced in The Netherlands (CVB, 1991) and published for the international community a few years later (Tamminga et al., 1994). This system has been used quite successfully by advisers and in the feed manufacturing industry. In the meantime developments have continued. In the Dutch feed manufacturing industry this has led to the further development of the concepts on which the DVE/OEB1991 system is based. Further, feed evaluation is moving towards mechanistic models that take into account the dynamics and mechanisms of the biochemical and physiological processes of feed utilisation, notably events occurring in the rumen (Dijkstra, 1993). In the past decade these new insights have led to plans for the development of dynamic mechanistic feed evaluation systems (Tamminga et al., 1999; Gerrits et al., 2000). These systems could not only replace the DVE/OEB1991 system, but also the net energy system that is used in The Netherlands since 1977 (VEM) to describe energy utilisation in dairy cows (Van Es, 1978). Because of the complicated nature of the subject, progress in this area is slower than anticipated. It was therefore decided to formulate an update of the DVE/OEB1991 system. In this update, hereafter referred to as DVE/OEB2007 also international developments in the field of feed evaluation like the Cornell Net Carbohydrate and Protein System (CNCPS) in the USA (Fox et al., 2004) and the Feed into Milk (FiM) system in the UK (Offer et al., 2002; Thomas, 2004) have been taken into account.

In this report the outline of the new Dutch protein evaluation system for dairy cows (DVE/OEB2007) is described. The structure of this report is as follows:

- Chapter 2 describes the degradation of feed components in the rumen that are relevant for the calculation of the protein value of a feed;
- Chapter 3 deals with the various fractions that determine the protein value of a feed;
- Chapter 4 describes the rumen degradable protein balance (OEB or RDPB) and focuses on aspects dealing with synchronisation of N- and energy supply in the rumen;
- Chapter 5 describes the evaluation of feedstuffs in DVE/OEB2007
- Chapter 6 outlines the protein requirements of dairy cows;
- Chapter 7 gives a description of the intestinal availability of amino acids from DVE and gives a preliminary statement on the amino acid requirements of dairy cows;
- Chapter 8 lists the literature that is referred to in the previous chapters.

In the Annexes added, more detailed information is provided on calculations, performed in connection with the development of the new system.

2 Degradation of feed components in the rumen

2.1 Feed components and fractions

The organic matter (OM) in ruminant feeds can be separated in the following components: crude protein (CP, always including NH_3), starch (STA), sugars (SU), glucose oligosaccharides (GOS), crude fat (CFAT), cell walls or neutral detergent fibre (NDF), the fermentation products (FP) lactic acid (LA) and volatile fatty acids (VFA) and residual non-starch polysaccharides (RNSP).
RNSP is a calculated fraction, defined as
RNSP = OM – (CP + STA + SU + GOS + CFAT + NDF + 0.92*LA + 0.5* VFA),

Where:

GOS = glucose oligosaccharides, fragments of incomplete starch degradation that may be present in some high moisture by-products;
LA = lactic acid;
VFA = sum of volatile fatty acids (acetic, Ac, propionic, Pr, and butyric, Bu[1)] acid)

The main contributors to FP in feeds are LA and VFA. Both are determined before drying, but, depending on component and drying conditions, the proportion that is lost in the drying process, varies. In a study of Porter and Murray (2001), alcohols (ALC) and ammonia (NH_3) evaporated almost completely; of the VFA 55-90% and of LA 10-40% evaporates, respectively. These figures are in agreement with the practical approach of the CVB, assuming that drying results in the vaporisation of 8% of LA, 50% of the VFA, and 100% of ALC.
In some cases information on individual FP is lacking, but an estimate of the total FP in the (dried) feed is available (e.g., table values in the CVB Feed Table). For silages the equation of the CVB/OEB1991 system (see paragraph 3.3.4, Table 9) can be used to estimate FP. In such situations the part '0.92*LA + 0.5*VFA' in the equation given above can be replaced by 'FP'. When no information on the level of FP is available it is assumed that the feed does not contain FP.

It should be noted that the nature of the component RNSP is not well-defined, but is believed to be composed to a large extent of non-starch polysaccharides (NSP) such as pectins, arabans, xylans and beta-glucans. In some feedstuffs organic acids (for instance oxalic acid in sugar beets) may also contribute to RNSP.

In general, the degradative behaviour of feed components in the rumen is estimated with the *in situ* (sometimes referred to as *in sacco)* technique, in which feeds are incubated in nylon or dacron bags in the rumen for various lengths of time. This approach assumes that each component can be separated into four fractions: a soluble fraction (S), a washout fraction (W), a non-washout but potentially degradable fraction (D) and a non-washout but undegradable fraction (U). These four fractions are expressed as g/g DM. The size of the fraction U is determined as the residue remaining in nylon bags after prolonged rumen incubation (336 h). The size of W is determined as the fraction that is washed out of a nylon bag with a pore size of 35-45 microns in a washing machine. The soluble fraction (S) is considered to be part of the washout fraction (W), but is determined separately through centrifugation (CVB, 2004). The (W-S) fraction is the washout fraction (W) minus the soluble fraction (S), and consists of particles smaller than the pore size of the nylon bag. The size of fraction D is calculated as 1.0 - W - U. Procedures are as described in the protocol for *in situ* incubations (CVB, 2004).

[1] The amount of other volatile fatty acids, e.g. branched chain fatty acids, mostly are so low that they can be neglected.

Degradation of fractions D, (W-S) and S, as well as passage behaviour of each fraction is assumed to follow first order kinetics described by the equation:

$$R_t = R_0 . e^{-kt} \quad \text{[eq. 1]}$$

Where:

R_t = residue at time t (g/g)
R_0 = residue at time zero (g/g)
k = fractional rate constant either of degradation (kd), or passage (kp) (h^{-1})
t = time (h^{-1})

2.2 Effective ruminal degradation of feed components

The amount of a feed component (COMP) that is effectively degraded in the rumen is calculated from the combination of fractional degradation and passage rates, as the summation of the different fractions:

$$FCOMP = COMP*\{S*kd_S/(kd_S+kp_S) + (W-S)*kd_{(W-S)}/(kd_{(W-S)}+kp_{(W-S)}) + D*kd_D/(kd_D+kp_D)\} \quad \text{[eq. 2]}$$

Where:

FCOMP	=	Amount of component ($g.kg^{-1}$ DM) effectively degraded in the rumen
COMP	=	Content ($g.kg^{-1}$ DM) of the relevant component in feedstuff
S	=	The water soluble fraction ($g.g^{-1}$) after centrifugation
kd_S	=	The fractional rate (h^{-1}) of degradation of fraction S
kp_S	=	The fractional rate (h^{-1}) of passage out of the rumen of fraction S
W	=	The fraction ($g.g^{-1}$) that can be washed out of nylon bags
(W-S)	=	The washout fraction (W) minus the soluble fraction (S) ($g.g^{-1}$)
$kd_{(W-S)}$	=	The fractional rate (h^{-1}) of degradation of fraction (W-S)
$kp_{(W-S)}$	=	The fractional rate (h^{-1}) of passage out of the rumen of fraction (W-S)
D	=	The non-washout, but potentially degradable fraction ($g.g^{-1}$)
kd_D	=	The fractional rate (h^{-1}) of degradation of fraction D
kp_D	=	The fractional rate (h^{-1}) of passage out of the rumen of fraction D

2.3 Fractional degradation rates

Fractional degradation rates of D (kd_D) of the different components are determined by nylon bag incubations in the rumen, following the procedure of Ørskov and McDonald (1979), as adapted by CVB (2004). It is further assumed that fraction S is always degraded at a fixed fractional rate (kd_S) of 2.0 h^{-1}, and (W-S) at a fractional rate equal to that of D ($kd_{(W-S)} = kd_D$), except for starch as is discussed later (paragraph 2.3.2).

2.3.1 The degradation of the S fraction

The degradation rate of 2.0 h^{-1} for S is based on the assumption that 5% of the fraction S of protein and carbohydrates escapes degradation in the rumen, leading to a $kp_S/(kp_S+kd_S)$ ratio of 0.05. Assuming a fractional passage rate of rumen fluid (kp_S) of 0.11 h^{-1} (see paragraph 2.4), results in an average fractional degradation rate of 2.0 h^{-1}, derived from $0.05=0.11/(0.11+kd_S)$. This agrees with values reported in literature. In the CNCPS, fractional degradation rates for soluble true protein and soluble carbohydrates in concentrate ingredients were assumed to vary between 1.0 and 4.0 h^{-1} (Sniffen et al., 1992). For soluble N fractions in forages, Volden et al. (2002) also observed around 5% to escape.
For the degradation (rate) of the W fraction of starch, a different approach was needed as will be discussed in the next paragraph.

2.3.2 The degradation of the W fraction of starch

2.3.2.1 Comparison of *in vivo* and *in situ* data on rumen degradation of starch

Starch is not or hardly soluble in water (Azarfar, 2007). The S fraction of starch is therefore (almost) zero. Starch washed out of nylon bags *in situ* is therefore considered to consist fully of small particles (< 35-45 micron). This means that (W-S) equals W. Recently, Offner et al. (2003) reviewed the literature and published a database of 302 observations from 48 experiments on *in situ* starch degradation in the rumen (Annex 1). Next to differences between feedstuffs, they identified the laboratory, the mean particle size, and various ways of processing as important factors affecting the *in situ* starch degradability. Reducing the mean particle size increased the effective degradation (ED in $g.g^{-1}$) by almost 0.16 per mm reduction, most likely because of a shift between the fractions D and W. Therefore, the degradative behaviour of W is of critical importance, as already indicated by Nocek and Tamminga (1991). In a comparison of starch degradation obtained *in situ* and *in vivo*, these authors concluded that 10% of the starch in the W fraction escaped degradation in the rumen, without indicating what caused such an escape. In a more recent paper, based on a much larger database, Offner and Sauvant (2004) derived regression equations to predict the *in vivo* starch degradation from *in situ* results, without the proposed correction of 10% for the W fraction. All equations showed an underestimation *in vivo* at low *in situ* ED values and an overestimation at high *in situ* ED values.

From the data base of Offner et al. (2003), we eliminated feedstuffs that are assumed not to contain starch (soy products, beet pulp, sunflower meal, alfalfa) and feedstuffs for which no W fraction was specified. For the remaining 40 feedstuffs, the *in vivo* rumen starch degradation was estimated using the regression equation (*in vivo* ED = 0.263 + 0.63 *in situ* ED) of Offner and Sauvant (2004). This equation was based on 84 experiments and 179 observations in which both *in vivo* and *in situ* measurements had been performed. To calculate rumen degradation of starch, a fractional outflow rate of 0.06 h^{-1} was used. Our estimated *in vivo* ED was subsequently separated in a W and a D (1-W) fraction. First the *in vivo* ED for the D fraction was calculated assuming a fractional outflow rate (kp_D) of 0.06 h^{-1}, and the fractional degradation rate of D obtained *in situ* (kd_D). The ED of the W fraction (ED(W)) could now be calculated as the difference between the *in vivo* ED and the ED of the D fraction. The *in vivo* ED of W was then regressed on the *in situ* ED of W. The results are shown in figure 1. This resulted in the equation:

$$\textit{in vivo}\ \mathrm{ED(W)} = 0.781 * \textit{in situ}\ \mathrm{ED(W)} + 0.0627\ (R^2 = 0.926),$$

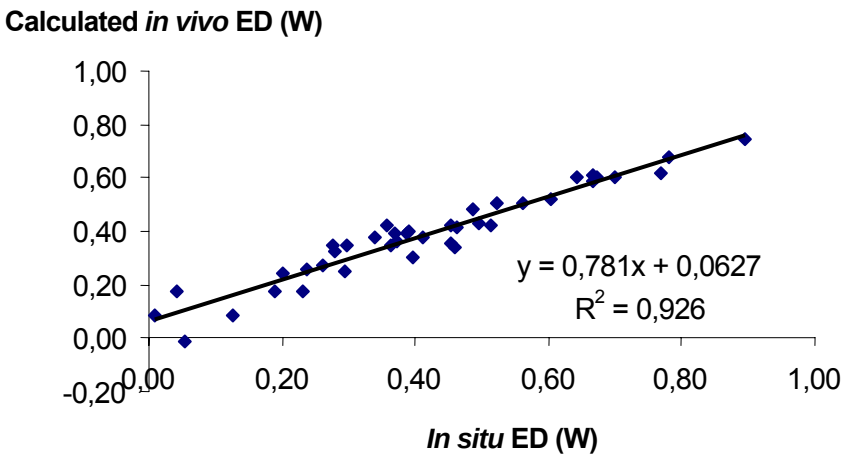

Figure 1. Relationship between calculated *in vivo* W and *in situ* W

In the DVE/OEB2007 system it is assumed that the fractional outflow rate of the W fraction is 0.08 h^{-1}, and that the ED of W *in vivo* results from kd/(kd+kp)*W *in situ*. Hence, the average fractional degradation rate of the W fraction (kd_W) of starch *in vivo* can be calculated from $0.781=kd_W/(0.08+kd_W)$. This results in an average kd_W value *in vivo* of 0.285 h^{-1}. Forcing the regression line through the origin increased the slope of the line to 0.902, with a concomitant increase of the calculated kd_W to 0.736.

2.3.2.2 In vitro degradation of starch

From a direct measurement of starch disappearance *in vitro* (Cone and Van Gelder, 2005) on isolated W and D fractions from a limited number (4) of feedstuffs (Annex 2) the kd_W appeared to be 1.7 to 2.6 times as high as the kd_D.

2.3.2.3 Degradation rate of the W fraction of starch in the DVE/OEB2007 system

From all these observations there remains little doubt that the kd_W of starch is considerably higher than the kd_D. It was also felt that a dependency must exist of kd_W on kd_D, but the data are inconclusive on the type of relationship.
The two extremes are that kd_W is a multiple of kd_D, or that kd_W differs from kd_D with a constant value. Both options alone and mixes of the two extremes were simulated over a range of values and compared with the data of Offner and Sauvant (2004). The kd_W was varied as follows: In the first exercise kd_W was set at 0.25, 0.50, 0.75 and 1.0 h^{-1}; in the second exercise kd_W was set at 1.0, 2.0, 3.0, 4.0 and 5.0 times the value of kd_D; in the third exercise kd_W was set at kd_D + 0.25, kd_D + 0.50, 1.5 x kd_D + 0.25, 2 x kd_D + 0.25, 2 x kd_D + 0.375, and 2 x kd_D + 0.50. In the last set of simulations, the fit of the equation $kd_W = 2 \times kd_D + 0.375$ on the data of Offner and Sauvant (2004), was considered satisfactory (R^2=0.95) and this equation was chosen (Annex 3). For the dataset used in Figure 1, this yielded an average rumen escape of 11.5 % (s.d. 2.94), close to the earlier proposed rumen escape of the W fraction of 10% (Nocek and Tamminga, 1991). Hence:

For starch, $kd_W = 2 \times kd_D + 0.375$ [eq. 3]

In feeds where starch is not playing a role as a storage carbohydrate, and starch is analytically determined at < 50 g kg^{-1} DM, it is assumed that this starch is degraded rapidly at a fractional rate of 0.75 h^{-1}.

2.3.2.4 Effect of processing on rumen degradation of starch

A widely used processing method for dairy concentrates is pelleting. It was demonstrated that pelleting increases the degradation of starch in the rumen.; in the DVE/OEB1991 system, the percentage of rumen undegraded starch (%RUSTA) was therefore first corrected by 25%, and later by 12.5%. Research reveals that pelleting decreases the size of the D fraction by 15.6%, on average, and increases the kd of D by 9.4% (Annex 4). For reasons of simplicity, both effects of pelleting have been covered by one parameter: pelleting decreases the D fraction of feeds with 25% ($D\text{-}STA_{pelleting}$ = 0.75*D); because for starch: W + D = 100, this means that de W fraction increases ($W\text{-}STA_{pelleting}$ = W + (D - $D\text{-}STA_{pelleting}$) = W + 0.25*D).

2.3.3 The degradation of residual non starch polysaccharides (RNSP)

Residual Non Starch Polysaccharides (RNSP) is a reservoir containing not only (the non-NDF) NSP, but also all errors of the analytical procedures of all other feed fractions. As mentioned. This fraction is not analytically determined, but calculated:

RNSP = OM - (CP + STA + SU + GOS + CFAT + NDF + 0.92*LA + 0.5*VFA).

Executing this calculation not only for the original material, but also for the residues of the nylon bag incubations, with a correction for CFAT (see paragraph 2.5.1), it is possible to ap-

ply the Ørskov and McDonald (1979) model and calculate the fractions W, U, D and the kd of RNSP.
These calculations were made for a selected number of 21 feed ingredients (Annex 8) with an NDF content > 100 g kg^{-1} DM and an RNSP/NDF ratio >1. In the *in situ* experiments it was found that W-NDF had a mean value of 0. Sometimes a small positive W-fraction was found, in other cases the value was negative. This was ascribed to inaccuracies in the NDF analysis and the *in situ* procedure. It was decided that W-NDF=0. This implies that W-RNSP always exceeds the size of W-NDF. Further it was found that the size of the U fraction in NDF always exceeded the size of U in RNSP (11.0 vs 1.7, respectively) and the degradation rate of the D fraction (kd_D) of RNSP always exceeded that of D in NDF (0.095 vs 0.051 h^{-1}, respectively). Similar findings emerged for the forages grass silage and maize silage. In a number of feed ingredients, the size of W of RNSP was negative, because the total mass balance has to add up to 100%, and because all errors accumulate in the W of RNSP.
A negative value of W is set at zero, while the mass balance is maintained by an equal reduction of the size of the D fraction of RNSP. For kd_D of RNSP the values calculated from the Ørskov and McDonald (1979) model are used.
Similar to the assumptions made for starch, a certain dependency is expected of the kd_W on the kd_D. For the degradation rate of W in RNSP we agreed upon the following equation:

For RNSP: $kd_W = 2.5 \times kd_D$ [eq. 4]

In the Dutch protocol for *in situ* incubations in the rumen it is stated that the degradation characteristics (W, U, D and kd) of NDF have to be determined only when the ratio RNSP/NDF $\geq$ 0.5 and NDF > 100 g/kg DM. In all other cases it is assumed that the degradation characteristics of NDF and RNSP are identical and can be calculated from the disappearance of NSP (= NDF + RNSP). It is then further assumed that

W-NDF = 0 W-RNSP = W-NSP
D-NDF = NDF/NSP * D-NSP D-RNSP = RNSP/NSP * D-NSP
U-NDF = NDF/NSP * U-NSP U-RNSP = RNSP/NSP * U-NSP
Kd_D –D-NDF = kd_D –D-RNSP = kd_D –D-NSP

2.4 Fractional passage rates

2.4.1 Fractional passage rates of crude protein and starch

Fractional passage rates (kp_X) of feed particles are equally important to describe the behaviour of feed components in the rumen of cattle as are the fractional degradation rates. Passage affects the site of digestion and therefore the intestinal supply of protein and starch (= the amount escaping degradation in the rumen), but also the amount of fermentable organic matter (FOMr) available for microbial growth in the rumen. Besides, passage rate is a major determinant of the efficiency of microbial growth (Dijkstra et al., 2002), as is further outlined in paragraph 3.3.2.
Fractional passage rates are usually estimated for liquid and solids, the latter often separated in small particles (concentrates) and large particles (forage). Frequently used markers for liquid and solids are Co-EDTA and Cr mordanted NDF (Cr-NDF or more correctly Cr-NDR), respectively.
In the DVE/OEB1991 system, passage rates of 0.045 and 0.060 h^{-1} were adopted for crude protein in forages and concentrates, respectively. In the DVE/OEB2007 system these values are adopted for the D-fraction of crude protein in forages and concentrates, respectively. Starch in forages is limited to maize silage and GPS, and is assumed to behave more like concentrate rather than like forage. Therefore, we assume that the passage rate of the D fraction of starch is 0.060 h^{-1}, for forages and concentrates. In the DVE/OEB2007 system, the following fractional passage rates were chosen for the S and (W-S) fractions:

- The fractional passage rate of the S fraction (kp_S) is equal to that of the liquid phase, which is set at 0.11 h^{-1}

- For the (W-S) the fractional passage rate was set at 0.08 h^{-1}.

The value of 0.11 h^{-1} is based on Van der Honing et al. (2004) who estimated in their review that the rate of passage of liquid was 2.5 times higher than the rate passage of forage particles, and 1.8 times higher than the passage rate of concentrate particles. The fractional passage rate of the fraction W-S of 0.08 h^{-1} was chosen in between that of fluid (0.11) and that of particles of the D fraction of concentrates (0.06).

2.4.2 Fractional passage of NDF

2.4.2.1 General considerations

Recent literature studies (Van Straalen, 1995; Van der Honing et al., 2004; Pellikaan, 2004; Dijkstra et al., 2005) have shown that not only forages and concentrates differ in their fractional passage rate, but that the contributing components (protein, starch, cell walls) also have different passage rates. This is notably important for cell walls (NDF), because their structure is rather loose and their functional specific gravity during fermentation is very much dependent on adhering fermentation gases (Hooper and Welch, 1985).
Dairy diets usually contain between 350 and 500 g NDF kg^{-1} DM, the majority of which is present in long forage particles. Thus rumen behaviour in terms of fractional passage and degradation rates of NDF to a significant extent determines the amount of OM that becomes available for microbial protein synthesis (FOMr) in the rumen. Various conditions influence the NDF content in forage. NDF in grasses increases with age (Bosch, 1991), decreases with increased level of N fertilisation in early (6 to 8 weeks) regrowth (Peyraud and Astigarraga, 1998), varies due to grass variety (Taweel, 2004) and appears higher after oven drying than after freeze-drying (Cone et al., 1996; Valk et al., 1996). The increase in NDF content due to age of the crop is caused mainly by the concomitant decrease in CP content. The decrease of NDF with higher N fertilisation is predominantly due to an increase in CP content. Genetic variation is mainly related to variation in water soluble carbohydrates (WSC). Finally, oven drying causes an increase of the NDIN component of NDF to increase, probably due to Maillard reactions (Van Soest, 1994). This phenomenon therefore occurs more frequently in feedstuffs than in faeces or in residues in nylon bags after rumen incubation.

For measuring the clearance of NDF by passage, lignin has often been used as an internal marker. However, according to Van Soest (1994), 14% of the lignin is apparently digested, or at least not recovered in the faeces. This could be due to analytical problems or to the growth stage of the forage. Over the last 15 years, a variety of experiments have been performed with dairy cows in The Netherlands, with lignin and other internal markers (lignin, IADF), and with different measuring techniques (duodenal flow, rumen evacuation). From the results, at an average DMI of 17.8 (s.d = 3.64) kg DM d^{-1}, an average fractional passage rate of 0.0278 h^{-1} (s.d. 0.0088) emerged (Annex 5).

2.4.2.2 Research using stable isotopes

Recent research (Pellikaan, 2004; Dijkstra et al., 2005) has used ^{13}C as an internal marker. Results (Table 1) indicate that ^{13}C in forages has a lower fractional passage rate than Cr-NDR, and that ^{13}CNDR has a lower fractional passage rate than ^{13}CDM. This difference between ^{13}CNDR and ^{13}CDM was not observed for concentrates.
The results in table 1 show that cell wall components (NDF) in forage have a fractional passage rate that is on average 40% lower than that of other carbohydrates and protein. Based on the use of ^{13}C as an internal marker, Dijkstra et al. (2005) recommend fractional passage rates of 0.025 and 0.020 h^{-1} for NDF in grass silage and maize silage, respectively. DMI levels in the experiments reported by Pellikaan (2004) and Dijkstra et al. (2005) were lower (on average 15.7 kg of DM d^{-1}) than what is considered normal under more practical dairy husbandry conditions (DMI of >21 kg d^{-1}), which would allow somewhat higher fractional passage rates. The value of 0.0278 h^{-1} for long forages (Annex 5) seems therefore more appropriate. Like in DVE/OEB1991, a ratio of 0.75 is maintained between the fractional passage rates of forage and concentrate particles, a value close to the ratio of 0.72 found by Van der

Honing et al. (2004), resulting in a fractional passage rate of 0.0371 h^{-1} for concentrates. However, applying fractional passage rates of 0.0278 for NDF in forages, and 0.0371 h^{-1} for NDF in concentrates to experiments in dairy cows in which the partial digestion of NDF in the rumen had been measured *in vivo*, resulted in underestimations of the amount of NDF fermented in the rumen (A. Bannink, pers. comm.). Therefore another approach was chosen.

Table 1. Ruminal fractional passage rates (h^{-1}) determined with different markers

^{13}C source	Treatment	DMI (kg d^{-1})	Fractional ruminal passage rate				
			CoEDTA (h^{-1})	CrNDF (h^{-1})	^{13}CDM (h^{-1})	^{13}CNDR (h^{-1})	^{13}CSta (h^{-1})
Grass		16.8	0.140	0.045	0.025	0.022	
GS	HI	12.5	0.130	0.069	0.033	0.029	
	LI	7.6	0.111	0.047	0.026	0.019	
	HDig	16.5	0.132	0.053	0.043	0.020	
	LDig	15.2	0.106	0.053	0.034	0.017	
MS	HDig	19.4		0.059	0.045	0.018	
	LDig	19.6		0.056	0.040	0.016	
% Conc	High	17.5		0.037	0.054	0.059	
	Low	16.4		0.040	0.057	0.064	
Starch	Maize	15.3	0.082	0.049			
	Potato	15.1	0.093	0.048			0.072

GS = Grass Silage; MS = Maize Silage; Conc = concentrate; H = High; L = Low; I = Intake; Dig = Digestibility; DMI = Dry matter intake; ^{13}CDM = ^{13}C in DM; ^{13}CNDR = ^{13}C in NDR; ^{13}CNDS = ^{13}C in Neutral Detergent Solubles (NDS); ^{13}CSTA = ^{13}C in Starch (STA)

NDF comprises a fraction that is available for degradation (DNDF) in the rumen, and a fraction that is not available (UNDF). As it is assumed that UNDF is also indigestible in the hindgut, this fraction is only subject to passage and the ingested amount will be quantitatively excreted in the faeces. Of the DNDF, the main part is fermented in the rumen, a much smaller proportion is digested in the hindgut, and also a certain percentage will be excreted in the faeces. Which proportion of the DNDF is fermented in the rumen depends on the ratio between kp and kd. How much of the DNDF is excreted in the faeces depends on the amount of NDF that escapes rumen fermentation and the proportion thereof that is eventually degraded in the hindgut.
A reliable estimate of FOMr from DNDF requires information on:

1. The proportions of UNDF and DNDF in NDF ((NDF – UNDF)/NDF).
2. The contribution of hindgut fermentation to the apparent digestibility of NDF or DNDF.
3. The proportion of DNDF that is actually digested in the rumen
4. The ratio between rates of ruminal degradation (kd) and passage (kp) of NDF.

2.4.2.3 The UNDF/NDF ratio

In stall fed fresh grass, subjected to different levels of N fertilisation (150 to 450 kg $ha^{-1}.yr^{-1}$), and harvested at a yield of between 1500 and 2500 kg DM ha^{-1}, the UNDF/NDF ratio in oven dried samples ranged between 0.064 and 0.128 (Valk et al., 1996), with lower NDF levels at higher N fertilisation and no significant influence of season (spring vs. autumn). In stall fed fresh grass of six varieties of perennial ryegrass (*Lolium perenne*), harvested at around 2000 kg DM ha^{-1} (Taweel, 2004), the range in UNDF/NDF ratio in freeze-dried samples was between 0.125 and 0.145 with little relation to the NDF content (range 0.414 – 0.436 $g.kg^{-1}$ DM). In grass silage, harvested at different stages of growth (Bosch, 1991), the UNDF/NDF ratio in oven dried samples ranged between 0.106 and 0.297, and increased with the NDF content (range 446 – 673 $g.kg^{-1}$ DM). Because of the different causes of variation in NDF content, the UNDF/NDF ratio can not reliably be predicted from regression equations.

2.4.2.4 Hindgut fermentation.

It has been shown that in sheep fed a variety of chopped forages, the contribution of hindgut fermentation to whole tract digestion of NDF varies between 0 and 30% (Ulyatt et al., 1975). At that time similar data for dairy cows were lacking. In a more recent publication, Robinson et al. (1987) concluded that in dairy cows on average 15% of the duodenal fibre flow is digested post-ruminally. However, this conclusion was based on a limited number of observations (n=18) on crude fibre (CF) rather than on NDF. A few years later, Tamminga (1993) concluded that the contribution of hind gut fermentation of NDF to total tract digestion in dairy cows fed diets of long forage and pelleted concentrates appears to be somewhat lower (between 0 and 20%) than in sheep fed forages only (Tamminga, 1993). Both in sheep and cattle the importance of hindgut fermentation increases with a decreasing total tract digestibility. As an average value for dairy cows fed good quality diets, 10% seems an appropriate figure.

2.4.2.5 Site and extent of DNDF digestion.

From a limited number of data from experiments with dairy cows fed on grass silage (Bosch, 1991) and fresh grass (Valk, 2002), the proportion of DNDF that is actually digested could be calculated. The pattern that emerges from these data is that on average 0.82 (s.d. = 0.0314) of the DNDF is digested (Annex 6). The database is too small to arrive to firm conclusions, but in the research of Bosch (1991), a higher proportion of concentrates and a higher NDF content in the silage reduced this figure, and in the research of Valk (2002) a higher level of N fertilisation resulted in a higher proportion of DNDF to be digested post-ruminally.
The proportion of DNDF that is fermented in the rumen results from the ratio kp/(kd+kp). A certain dependence of kp on kd is to be expected, because the probability to escape the rumen increases with time in the course of the digestion process due to an increase in the "functional" specific weight of feed particles when digestion proceeds (Hooper and Welch, 1985). Indications for such a relationship were also obtained by Pellikaan (2004) using stable isotopes as markers.
Assuming that 10% of the digested DNDF (0.82 of the ingested DNDF) is digested in the hindgut, means that a proportion of 0.738 (0.9 x 0.82) of the DNDF is digested in the rumen. To reach this value, a ratio between kp and kd of 0.355 [(1 – 0.738)/0.738] is required.

2.4.2.6 Degradation and passage.

Although mathematically they can be separated, feed particles contain both UNDF and DNDF. UNDF is cleared from the rumen by passage (kp) only and DNDF is cleared either by passage (kp) or by fermentation (kd). In practice, UNDF and DNDF are components of the same feed particles.
Due to buoyancy, caused by fermentation gases adhered to them, feed particles with a high DNDF/UNDF ratio are selectively retained in the rumen and as a result UNDF is cleared from the rumen at a faster fractional rate. Tamminga et al. (1989) have estimated rumen outflow rates of DNDF and UNDF based on intake, rumen pool sizes and faecal output. Some of their results are presented in table 2.

Table 2. Fractional passage rate (kp, % h^{-1}) of cell wall components from the rumen, and fractional degradation rate (kd, % h^{-1}) of cell wall components in the rumen (Tamminga et al., 1989)

Rate	Experiment	DNDF	UNDF	DADF	UADF
kp	1	1.62±0.208	3.52±0.175	1.38±0.129	4.55±0.238
	2	2.17±0.417	4.17±0.358	2.02±0.321	4.02±0.275
kd	1	5.67±0.629		5.86±0.533	
	2	4.44±0.263		5.15±0.238	

Assuming the kp of DNDF to be half of that proposed earlier (paragraph 2.4.2.2) for (U)NDF (0.0278 and 0.0371 h^{-1} for forages and concentrates, respectively) would result in more realistic values for partial digestion of NDF in the rumen. A somewhat pragmatic solution is to take the best of two worlds and accept the average kp of the two approaches outlined above in paragraphs

2.4.2.5 and 2.4.2.6 respectively. This results in equations describing the fractional passage rate (kp) out of the rumen for NDF in forages (kpf) and concentrates (kpc) as follows:

kpf = 0.0139 + 0.1775*kd [eq. 5]

in which 0.0139 is half the value of 0.0278, and 0.1775 is half the value of 0.355, the ratio required between kd and kp.

kpc = 0.01855 + 0.1775*kd [eq. 6]

in which 0.01855 is half the value of 0.0371 and 0.1775 is half the value of 0.355, the ratio required between kd and kp.

The results were verified on a dataset in which apparent digestibilities had been measured *in vivo* and in which fractional degradation rates had been determined (Annex 7). Unfortunately, in many experiments the kd of concentrates had not been measured and a 'default value' of 0.045 h^{-1} was adopted. Regression analysis using the model dNDF-calculated = a*dNDF-calculated showed a fair agreement (Y=1.005X; R^2=0.34). Removing the data of Klop et al (1997) from the dataset increased the fit considerably (Y=0.981X; R^2=0.52).

2.4.3 Fractional passage of RNSP

As for the W fractions of other feed components, it is assumed that W of RNSP is cleared from the rumen at a fractional rate of 0.08 h^{-1}. Although the kd_D of RNSP is on average almost twice as high as that of NDF, it was felt appropriate to follow the same rules as were developed for NDF. Hence, the kp_D for RNSP in concentrate ingredients was set at 0.01855 + 0.1775*kd_D, with a maximum of 0.06, and for RNSP in forages at 0.0139 + 0.1775*kd_D, with a maximum of 0.045. Accepting a hind gut fermentation of 10%, as was also assumed for NDF, results in apparent (faecal) digestibilities of RNSP ranging between 54.9 and 94.3 % (Annex 8). Because *in vivo* faecal RNSP output may have been contaminated by endogenous secretions and residues of post-ruminal digestion of other components, the significance of this range of values could not be verified in vivo.

2.4.4 Fractional passage of the U-fraction

The components CP, NDF and RNSP contain an undigestible (U) fraction, which is only subject to passage. For CP it is assumed that $kp_U = kp_D$. As outlined in paragraph 2.4.2.6, for NDF and RNSP $kp_U \approx 2 * kp_D$.

2.5 Additional aspects

2.5.1 The behaviour of fats and long chain fatty acids in nylon bag incubations

In the DVE/OEB1991 system it was assumed that fat is an inert substance that is not degraded in the rumen, and is rapidly and completely washed out of the nylon bags. However, fat rich products like oil seeds may block the pores of nylon bags and thus impair the degradation of the other fractions. It is therefore recommended that ingredients in which CFAT exceeds 100g.kg^{-1} DM, be gently extracted prior to rumen incubations. The normally applied robust extraction procedure (boiling under reflux) may destroy the matrix of carbohydrates and proteins. Therefore a mild extraction at room temperature is recommended, but this often results in an incomplete removal of CFAT.
In recent years several papers in literature have dealt with the fate of fats and fatty acids (FA) in the rumen during nylon bag incubations of raw and treated full fat oilseeds, like soybeans (Perrier et al., 1992; Chouinard et al., 1997), canola seed (Enjalbert et al., 2003) and sun-

flower seed (Mustafa et al., 2003; Sarrazin et al., 2003). Some of the results (Chouinard et al., 1997; Enjalbert et al., 2003) showed that on average between 27 and 46% of the fatty acids is immediately washed out. The remaining FA disappeared from nylon bags at a 2 to 4 times faster rate than dry matter. Polyunsaturated FA (PUFA) disappeared faster than saturated FA, not only because PUFA leave the bags with feed particles, but also because they are biohydrogenated into more saturated FA. Fractional rates of disappearance of FA varied between 0.10 and 0.25 h^{-1}, and processing (extrusion, roasting, moist heat treatment) slowed down the fractional disappearance. Assuming the W and U fractions for CFAT to be 0.35 and 0 respectively, and the average fractional disappearance rate for the D-CFAT to be 0.15 h^{-1}, enables us to correct the W and D fractions of NSP (NSP = OM–(CP+STA+SU+GOS+CFAT+FP). At a disappearance rate of 0.15 h^{-1}, and taking into account the initial washout of 35%, CFAT decreases at 3, 6 ad 12 h to 40, 17 and 3 % of its original value, respectively. Corrections of the D fraction of NSP can therefore be restricted to 3, 6 and 12 hours with 40, 17 and 3 % of the fat fraction, respectively.

2.5.2 The protein value of NPN in fermented feeds

In CNCPS and FiM, a correction is made for the presence of ammonia (NH_3) in the S fraction of CP in fermented feeds. In fermented feeds like silages, part of the rumen degradable protein (RDP) is present as non protein nitrogen (NPN) in the S-fraction. It was shown (Givens and Rulquin, 2004; Gierus et al., 2005; Hedqvist and Uden, 2006), that between 210 and 439 g kg^{-1} of silage CP is true protein. Of the remaining CP a large proportion (250-459 g kg^{-1} CP) is present as amino acids and peptides. This leaves some 300 g kg^{-1} CP (233 to 370 g kg^{-1}CP) as N in N containing components other than amino acids, like NH_3 and nucleic acids.
Following the proposed rules for CP as outlined earlier in paragraph 2.3.1, 5% of this 300 g kg^{-1} CP in non amino acid N (NAAN), i.e. 15 g kg^{-1} CP, would escape rumen degradation and (wrongly) contribute 13.5 (15*0.9) g kg^{-1} CP to the DVE as DRUP. The remaining 95%, i.e. 285 g kg^{-1} CP, would also (wrongly) contribute to the DVE through microbial protein synthesis with soluble protein as substrate, yielding 18.0 g DVE kg^{-1} CP (based on 99 g MCP kg^{-1} CP, with an AA/CP of 0.75 and an intestinal digestibility of 0.85, see later). Total yield would thus be overestimated by 31.5 g DVE kg^{-1} CP.
The alternative is to correct the S-CP for NAAN. Since CP is calculated as N*6.25, this CP would consist of 16% N and 84% N-free residue. When N present in NAAN, is expressed as CP, other feed components (most likely soluble carbohydrates belonging to the NSP of the RNSP fraction) are wrongly included in the CP fraction. Of this (wrongly in the CP included) soluble carbohydrate fraction, also 95% would be available as substrate for microbial crude protein (MCP) synthesis in the rumen, contributing 26.6 g DVE (based on 174 g MCP kg^{-1} CHO; AA/CP of 0.75 and digestibility of 0.85, see later). At maximum the two approaches result in a difference in DVE originating from NAAN of less than 5 g kg^{-1} CP in NAAN, well within the (in)accuracy of the nylon bag method. Contrary to the approach used in CNCPS and FiM, it was decided therefore to omit a correction for NAAN or NH_3.

2.5.3 Sugars (SU) and glucose oligosaccharides (GOS)

Sugars (determined according to Luff Schoorl) are assumed to be part of the S fraction. In some feedstuffs (with a high moisture content), GOS is distinguished as a chemical parameter. These starch fragments contain ≤ 10 glucose units, and are soluble in 40% ethanol. In CVB feeding tables, the GOS content is expressed as starch equivalents. GOS is also assumed to be fully incorporated in the S fraction. Also, it is assumed that GOS is fermented similar to SU. Therefore, the DVE system uses SU+GOS, after transforming GOS into glucose equivalents (= GOS content / 0.90).

Assumptions in the DVE/OEB2007 systems are summarized as follows (Table 3):

Table 3. Overview of parameter values for different feed components

Parameter	CP	SU+GOS[a]	STARCH[b]	NDF	RNSP[c]
F total (FCOMP)	eq. 2	eq. 2	eq. 2	eq. 2	eq. 2
S, fraction	value[d]	1[e]	0	0	0[e]
W-S, fraction	value[d]	0	value[d]	value[d]	value[d]
D, fraction	value[d]	0	value[d]	value[d]	value[d]
U, fraction	value[d]	0	0	value[d]	value[d]
Kd_S, h^{-1}	2.0[f]	2.0[f]	n.a.	n.a.	n.a.
Kp_S, h^{-1}	0.11[g]	0.11[g]	n.a.	n.a.	n.a.
$Kd_{(W-S)}$, h^{-1}	= kd_D	n.a.	$2k_D$+0.375	= kd_D	=2.5kd_D
$Kp_{(W-S)}$, h^{-1}	0.08	n.a.	0.08	0.08	0.08
Kd_D, h^{-1}	value[d]	n.a.	value[d]	value[d]	value[d]
Kp_D, h^{-1} (forage)	0.045	n.a.	0.045	eq. 5	eq. 5
Kp_D, h^{-1} (conc.)	0.060	n.a.	0.060	eq. 6	eq. 6

[a] Sugars (according to Luff Schoorl) + Glucose Oligosaccharides (GOS) soluble in 40% ethanol.
[b] To account for the effect of pelleting effective degradation of starch in concentrates is increased by reducing the size of D with 25%, with a concomitant increase of fraction W.
[c] For RNSP the size of W, U and D is calculated as OM–(CP+CFAT+SU+GOS+STA+NDF+FP) for each incubation time by using equation 2. For time points others than t = 0 (zero) for SU, GOS and FP the value is 0 (zero). Of the CFAT fraction in the feed, 35% is washed out, so the fat free D fraction of NSP can be calculated by subtracting 65% of the initial CFAT content, the fat free D fractions of NSP at 3, 6 and 12 h are reduced by 40, 17 and 3 % of the initial CFAT content.
[d] "value" means analysed or derived from feed tables. When S>W, then W=S
[e] Part of the W fraction may be soluble, but this can not be measured because of "contamination" with soluble ash.
[f] For products of which the S fraction contains amino acids (in protein, peptides or free) or soluble sugars, a fractional degradation rate of 2.0 h^{-1} is used according to Volden et al (2002) for protein and Van Straalen (1995) based on Sniffen et al. (1992) for sugars. The ratio $kd_1/(kd_1+kp_1)$ equals 2.0/(2.0+0.11) or 0.95.
[g] Assumptions based on data of Van Vuuren (1993), Van Straalen (1995), Van der Honing et al. 2004), Pellikaan (2004) and Dijkstra et al. (2005).

2.6 Comparison of degradation and passage between protein evaluation systems

2.6.1 Comparison of DVE/OEB1991 and DVE/OEB2007

Table 4 gives a comparison between DVE/OEB 1991 and DVE/OEB 2007.

Table 4. Overview of components in DVE/OEB1991 and DVE/OEB2007

		DVE/OEB1991				DVE/OEB2007			
		Calculation	**kd**	**kpf**	**kpc**	**Calculation**	**kd**	**kpf**	**kpc**
OM	COMP	DM-ASH				DM-ASH			
	FOM	DOM-CFAT-ECP-EST				∑ kd/(kd+kp)*COMP			
CFAT	COMP	EE				EE			
	W					0,35*EE	∞		
	D					0,65*EE	0,15	0,045	0,060
	U			.		0			
CP	COMP	N x 6,25				N x 6.25			
	S	0				SCP	2,00	0,110	0,110
	W-S	WCP	∞			WCP-SCP	Table	0,080	0,080
	D	100-W-U	Table	0,045	0,060	100-W-U	Table	0,045	0,060
	U	T = 336 h				T = 336h			
CHO	COMP	100-ASH-CP-EE				100-ASH-CP-EE			
NDF	COMP	NDF				NDF			
	W					WNDF	Table	0,080	0,080
	D	100-U				100-W-U	Table	$0,0139+0,1775kd_D$	$0,01855+0,1775kd_D$
	U	T = 336 h				T = 336h			
RNSP	COMP					1000-ASH-CP-CFAT-STA-CF_Di*SU-0.92*LA-0.5*VFA			
	W					WRNSP (calc.)	$2,5kd_D$	0,080	0,080
	D					100-W-U (calc.)	Table	$0,0139+0,1775kd_D$	$0,01855+0,1775kd_D$
	U					T = 336 h (calc.)	Table		
NSC[1)]	COMP	STA + SU				STA + SU			
	S					SSU	2,00	0,110	0,110
	W	STA (W)	1,35	0,150	0,150	STA (W)	$2kd_3+0,375$	0,080	0,080
	D	STA (100-W)	Table	0,045	0,060	STA (100-W)	Table	0,060	0,060
	U	0				0			

[1)] NSC = non structural carbohydrates.

2.6.2 Comparison with other systems

In alternative but comparable models of feed evaluation like the CNCPS in the US (Fox et al., 2004) and the FiM in the UK (Thomas, 2004), equations are used for the fractional passage rates (kp). These fractional passages rates determine the efficiency of microbial protein synthesis (Y_{ATP}) in the rumen (paragraph 3.3.2). One distinguishes kp for liquid (kpl), kp for forages (kpf) and kp for concentrates (kpc). Equations are shown below:

CNCPS

$kpl = 0.0441 + 1.91 * kg\ DMI/kg\ BW$

$kpf = 0.0038 + 0.22 * kg\ DMI/kg\ BW^{.75} + 0.02 * pForage^2$

$kpc = -0.00424 + 1.45 * kpf$

FiM

$kpl = 0.0245 + 0.25 * kg\ DMI/kg\ BW^{.75} + 0.04 * pForage^2$

$kpf = 0.0035 + 0.22 * kg\ DMI/kg\ BW^{.75} + 0.02 * pForage^2$

$kpc = 0.0025 + 1.25 * kpf$

where pForage is the fraction of forage DM in total diet DM.
It should be noted that in both cases feed intake (either per kg BW or per kg $BW^{.75}$) and the proportion of forage (raised to the power 2), play an important role. High producing dairy cows are usually fed at or close to *ad libitum*. In the recently introduced Dutch feed intake prediction system (Zom et al., 2002), variation in feed intake capacity through an entire lactation period was estimated. The difference between the highest and the lowest feed intake capacity appeared to be less than 15%. Besides, in the course of a lactation period, the ratio between forage and concentrates follows the milk production level. In the FiM system this effect was simulated for various levels of milk production (Table 5). Assuming a contribution of the liquid fraction of 20% in all diets, Y_{ATP} (as a measure of potential microbial protein synthesis), as calculated in FiM, showed only small variation.

Table 5. The effect of a varying forage/concentrate ratio on fractional passage rates (h^{-1})

Milk yield (kg/d)	**20**	**30**	**40**	**50**	**Mean**
F/C ratio	**78/22**	**54/46**	**46/54**	**36/64**	
kpl	0.081	0.075	0.077	0.079	0.078
kpf	0.044	0.043	0.047	0.049	0.045
kpc	0.058	0.056	0.061	0.064	0.060
Y_{ATP}	11.7	11.5	12.0	12.1	11.8

Source: Feed into Milk (Thomas, 2004)

When the underlying data are lacking, the FiM system suggests default values for kp of 0.08, 0.045 and 0.06 h^{-1}, for liquid (kpl), forage (kpf) and concentrates (kpc), respectively. For a high producing dairy cow of 650 kg with an intake of 21 kg DM d^{-1} and a proportion of forage of 0.50, as is nowadays common in the Netherlands, CNCPS would calculate values for kp of 0.106, 0.045 and 0.061 h^{-1}, for liquid (kpl), forages (kpf) and concentrates (kpc), respectively.

To enable a comparison between components of the DVE/OEB2007 system with those in systems of other councils or groups, table 6 gives an overview of parameter values as defined and calculated in the CNCPS system (Fox et al., 2004), the FiM system (Thomas, 2004) and the DVE/OEB2007 system.

Table 6. Overview of components in CNCPS, FiM and DVE/OEB2007 feed evaluation systems

		CNCPS			FiM			DVE/OEB2007			
		Calculation	**kd**	**kp**	**Calculation**	**kd**	**kp**	**Calculation**	**kd**	**kpf**	**kpc**
DM	Forage			eq.[1)]			eq.[1)]				
	Conc.			eq.[1)]			eq.[1)]				
DM	COMP	DM			DM			DM			
	S	n.a.			S	0.90	0.080	n.a		0.110	0.110
	W	n.a			A	Table[2)]	0.080	n.a.		0.080	0.080
	D	n.a			B	Table[2)]	eq.[1)]	n.a.			
	U	n.a			100-A-B			n.a.			
CP	COMP	N x 6.25			N x 6.25			N x 6.25			
	S1	SNPN x 6.25	∞		n.a.			n.a.			
	S2	SCP – S1	3.00	eq.[1)]	SCP	0.90	0.080	SCP	2.0	0.110	0.110
	W-S				WCP–SCP	Table[2)]	0.080	WCP-SCP	$kd_{(W-S)}=kd_D$	0.080	0.080
	D	NDICP-ADICP	Table[2)]		B	Table[2)]		100-W-U	Table[2)]	0.045	0.060
	U	ADICP	Table[2)]		n.a.	n.a.	n.a.	T = 336h	0	0.045	0.060
CHO	COMP	100-ASH-CP-EE			n.a.	n.a.	n.a.	100-ASH-CP-EE			
NDF	COMP							NDF	Table[2)]	eq 5	eq. 6
	S	n.a.			n.a	n.a	n.a	0	0		
	W	n.a.			n.a.	n.a.	n.a.	0			
	D	CB2=NDF-NDICP-U	Table[2)]	eq. [1)]	n.a.	n.a.	n.a.	100-W-U	Table		
	U	Lignin x 2.4	Table[2)]	eq. [1)]	n.a.	n.a.	n.a.	T = 336h			
RNSP	COMP							RNSP[3)]			
	W							WRNSP (calc.)	$2.5kd_D$	0.08	0.08
	D							100-W-U (calc.)	Table[2)]	eq.5	eq. 6
	U							T = 336 h (calc.)	0		
NSC	COMP	CHO-SCD-SCU						STA + SU			
	S	CA=SSU+ACIDS	3.00	eq.[1)]	n.a	n.a	n.a	SSU	2.0	0.110	0.110
	W	CB1=STA+SNSP	Table[2)]	eq [1)].	n.a.	n.a.	n.a.	STA (W)	$2kd_D+0.375$	0.080	0.08
	D				n.a.	n.a.	n.a.	STA (100-W)	Table[2)]	0.060	0.060
	U	0			n.a.	n.a.	n.a.	0	0		

[1)]: eq. = equation given in the system; [2)]:Table = tabulated value; [3)] For calculation see Table 4.

3 Description of the protein value of feeds

3.1 Introduction

For each feed the DVE/OEB2007 system calculates two values: protein digested in the intestine (DVE) and the rumen degraded protein balance (RDPB or OEB). Of these, DVE represents the protein value of a feed, while OEB is the difference between the potential microbial protein synthesis based on available rumen degradable protein (RDP) and that based on available rumen degradable energy.
DVE (protein that enters and is digestible in the small intestine) can be separated in three fractions:

- Feed protein not degraded in the rumen, but digested in the small intestine (DRUP)
- Microbial protein synthesised in the rumen and digested in the small intestine (DMCP)
- Endogenous protein or DMFP.

The main part of endogenous protein exists of digestive enzymes, desquamated epithelial cells and mucus. This protein originates from the animal itself and is not part of the dietary protein, nor of the microbial protein. Part of the endogenous protein is not digested, but is lost in the faeces and is in fact a real protein loss to the animal. To compensate for this inevitable loss, not only the lost protein itself has to be compensated, but also some additional protein, required for the synthesis of the lost protein. Because the animal does not benefit from it, the DMFP is subtracted from the DVE supply of a feed. Hence, the DVE value of a feed can be represented as:

DVE = DRUP + DMCP - DMFP [eq. 7]

In the French PDI (Vérité and Peyraud, 1989) and the British FiM system (Thomas, 2004), the microbial protein used in the calculation of the protein value is the lowest of what could be produced, either based on the available RDP, or on the available rumen degradable energy in the feed (PDI), or in the diet (FiM). In the DVE system each feed has only one protein value (DMCP) that is based on rumen degradable energy. The inclusion of grass products in the dairy diets used in The Netherlands usually causes a surplus of RDP. The difference between the microbial protein synthesized on the basis of rumen available RDP (MPN) and on the basis of rumen available energy (MPE) is presented as OEB (RDPB = Rumen Degradable Protein Balance). This parameter gives an immediate indication of the degree of protein loss from the rumen. To avoid RDP to become limiting for microbial protein synthesis, it is recommended that the RDPB should not become negative.

In the following paragraphs an outline is presented of how the different fractions of the DVE system should be calculated.

3.2 DVE derived from rumen undegraded protein (DRUP)

3.2.1 Rumen undegraded protein (RUP)

The amount of intestinal digestible rumen undegraded feed protein (DRUP) results from the crude protein (CP) in the feed, multiplied by the percentage rumen undegraded feed protein (%RUP), the percentage amino acids (AA) in RUP and the true absorption coefficient of AA absorbed from the intestine.

The %RUP is based on the results of nylon bag incubations in the rumen as outlined in equation 2 and table 1. In the DVE/OEB1991 system (Tamminga et al., 1994), RUP was corrected with a factor 1.11, derived from the PDI system (Verité et al., 1987). Although significantly different from 1, this correction factor was based on a database derived from experiments with cattle (dairy and beef) and sheep. When only the data for dairy cattle were used, no such factor could be established (Van Straalen, unpublished).
In the DVE/OEB2007 system the fraction W has been separated in the fractions S and (W-S). Of the S fraction 5% will escape degradation in the rumen and also from the W-S fraction a significant proportion will escape. These two are assumed to compensate for the 1.11 factor, reason why in the new approach this factor was abandoned.

3.2.2 Intestinal digestion of rumen undegraded feed protein

Intestinal digestion of RUP is derived from the results of the mobile nylon bag technique, as described by Van Straalen (1995). If no results of the mobile nylon bag technique are available, intestinal digestion of RUP can be calculated as (RUP –U)/RUP.
Like in the DVE/OEB1991 system, it is assumed that RUP consists of 100% amino acids. Although this may not entirely be correct, it is known that amino acid N has a higher intestinal digestibility than non amino acid N (Oldham and Tamminga, 1980). Hence, the amount of DRUP equals the amount of intestinal digested AA.
Values for %DRUP can be found in a CVB feed table (see also Chapter 5). One could argue that the 5% of the S fraction escaping degradation in the rumen should have an intestinal digestibility of 100%. Because of the small size of this fraction and the usually high value for DRUP (usually > 0.80), this difference was ignored.

In formula: DRUP = CP * %RUP/100 * % DRUP/100 [eq. 8]

In the FiM system (Thomas, 2004), it is assumed that CP which is part of the AD fraction (ADIN) is not digestible, and that the digestibility coefficient of the remainder of the protein is 0.9. In the CNCPS system (Fox et al., 2004), it is also assumed that ADIN is not available, and that feed protein in the fractions B1, B2 and B3 have an intestinal digestibility of 100, 100 and 80%, respectively.

3.3 DVE derived from microbial growth and protein synthesis (DMCP)

3.3.1 Introduction

Microbial growth in the rumen requires nutrients (precursors) for the synthesis of macromolecules (protein, nucleic acids, carbohydrates, lipids), and for the supply of energy (ATP).
Because they are essential components of proteins and nucleic acids (together assumed to account for 62.5 % of the microbial OM), also minimum requirements exist for nitrogen (N), sulphur (S) and phosphorus (P). Precursors as well as energy are released from the anaerobic fermentation of feed components, notably carbohydrates and sources of N, S, and P. In the DVE/OEB1991 system (CVB, 1991; Tamminga et al., 1994) it was assumed that per kg of FOMr in feed, a fixed amount of 150 g of microbial crude protein (MCP) was produced. However, it has now become apparent that the amount of ATP that can be extracted from the feed differs between components, and that the amount of microbial biomass that is produced differs between bacterial strains and their growing conditions (Russell and Strobel, 2005).

3.3.2 Fermentable organic matter in the rumen (FOMr)

In the DVE/OEB1991 system (CVB, 1991; Tamminga et al., 1994) fermentable organic matter (FOM) was calculated as follows:

FOM = DOM – CFAT – CP*(%RUP/100) – STA*(%RUSTA/100) – 0.50*FP [eq. 9]

In which

DOM = (faecal) Digestible Organic Matter (g kg^{-1} OM), derived from digestibility trials with sheep and published in the CVB Feeding Tables (CVB, 2007a)

CFAT = Crude fat (g kg^{-1}), assumed not to be fermented in the rumen

CP = Crude protein (g kg^{-1})

%RUP = Rumen undegraded protein (% of CP), derived from in situ measurements

STA = Starch (g kg^{-1})

%RUSTA = Rumen undegraded starch (% of STA), derived from *in situ* measurements, and corrected in case of pelleted concentrate ingredients.

FP = Fermentation products (g kg^{-1}) in ensiled feeds. It is assumed that FP, the majority of which are lactic acid and ethanol, still contain 50% of their original energy supplying capacity. Note that the FiM system (Thomas, 2004) assumes no energy (ATP) to be derived from FP, whereas CNCPS also assumes that 50% of the original energy supplying capacity is still present in FP (Fox et al., 2004).

In the DVE/OEB2007 system, an alternative approach is used. For all dietary ingredients equation 2 can be applied to each of the components of the OM (NDF, RNSP, CP, STARCH, SUGARS), and the FOMr can be calculated as the sum of FCOMP. Fermentation products in ensiled feeds (FP) are treated the same way as in the DVE/OEB1991 system as is discussed later (paragraph 3.3.3).

The new approach requires information on the distribution of FOMr in the different fractions. Table 7 gives an overview of the FOMr distribution in fresh grass, grass silage, maize silage and mixed concentrates of a number of forage and concentrate samples recently analysed in studies of Van Duinkerken et al. (2007).

Table 7. Distribution of FOMr in various feeds for dairy cattle (Van Duinkerken et al., 2007)

	Fraction	**Grass**	**Grass Silage**	**Maize Silage**	**Mixed Conc.**
n		**3**	**8**	**6**	**7**
FOMr (g/kg DM)	Total	517-523	381-499	327-394	406-567
NSP (g/kg FOMr)	W	47-61	0-75	27-81	50-170
NSP (g/kg FOMr)	D	511-547	488-656	347-551	296-406
SUGARS (g/kg FOMr)	S	176-255	24-280	0	153-259
STARCH (g/kg FOMr)	W	0	0	129-262	64-139
STARCH (g/kg FOMr)	D	0	0	40-367	36-158
CP (g/kg FOMr)	S	0-80	73-301	0-86	15-51
CP (g/kg FOMr)	W-S	0-4	3-11	0-9	16-128
CP (g/kg FOMr)	D	144-189	22-120	1-58	68-223

3.3.3 Efficiency of microbial growth and protein synthesis

Microbial growth in the rumen means essentially the formation of the macromolecules protein (41.7%), nucleic acids (20.8%), carbohydrates (20%) and lipids (17.5%). Requirements for microbial growth fall apart in a requirement for precursors, an energy (ATP) requirement for mainte-

nance, and an energy (ATP) requirement to link the precursors together in polymers. Precursors and ATP are derived from the microbial degradation of feed substrate in the rumen.
The yield of ATP varies between 1.5 and 4.4 mmol ATP $mmol^{-1}$ substrate (Russell and Strobel, 2005). The highest yields are derived from fermented polysaccharides, containing 6.2 moles of hexose equivalents per kg, hence yielding 27.3 moles ATP per kg. In our approach, substrates are distinguished in structural polysaccharides (NSP=NDF+RNSP), non structural polysaccharides (starch), sugars (mono- and disaccharides), oligosaccharides, and protein, with assumed ATP yields of 27.3, 27,3, 23.9, 23,9 and 13.7 moles per kg of substrate, respectively.
The value of 27.3 equals that in the FiM system (Thomas, 2004), and represents a yield of 4.4 mol ATP mol^{-1} polysaccharides, regardless whether they are structural (the D-fraction of NDF and RNSP) or non structural (STA). Fermentation of protein yields considerably less ATP than that of carbohydrates (Russell and Strobel, 2005) and was set at half the value attributed to polysaccharides. The FiM system (Thomas, 2004) follows a similar approach, where 24.8 mol ATP kg^{-1} CP is subtracted. Mono- and disaccharides (S fraction of SU) are degraded rapidly and because of their shorter chain length, contain fewer molecules per unit of weight. Due to their fast rate of degradation their degradative pathways may also yield somewhat less ATP. Hence their yield was set at 23.9 moles of ATP kg^{-1} monosaccharides (the gross SU content is in CVB feed tables always referred to as the amount of glucose equivalents).
For the W fraction of RNSP, also a yield of 23,9 moles of ATP kg^{-1} is assumed, because this fraction is ill defined and accumulated all analytical errors. That is why the ATP yield of this fraction is estimated cautiously.
Microbial growth yield is usually expressed as Y_{ATP} or g microbial cells mol^{-1} ATP; its maximum is assumed to be 32 (Russell and Strobel, 2005). Because of the energy requirement for maintenance, this maximum is not reached in practice; the actual microbial growth yield can be described with the equation of Pirt (1965):

$$1/Y = M/GR + 1/Y_{max}$$

or:

$$Y = Y_{max}/(M/GR.Y_{max} + 1$$

Where:

Y	=	Yield of microbial dry matter (in g per mole of ATP)
M	=	Maintenance requirement of the microbes (mole of ATP * h^{-1} per g microbial material)
GR	=	Fractional growth rate (h^{-1})
Y_{max}	=	Maximum microbial growth yield without losses in maintenance (g per mole of ATP)

The microbial population of the rumen contains at least three rather distinct sub-populations. These are cell wall degrading bacteria, starch degrading bacteria and protozoa. The role of protozoa in the rumen is mainly to predate on bacteria and to engulf starch particles, thus preventing a too rapid conversion of starch into VFA which would be the cause of a rapid drop in rumen pH. The protozoa are assumed to be selectively retained in the rumen as a separate fraction, and not to contribute significantly to the flow of microbial protein to the small intestine. Analytical methods for bacteria are based on markers (^{15}N, DAPA, or nucleic acids) that will also contaminate protozoa. Therefore, taking into account the protozoa as a separate fraction would lead to a double counting in the intestine. For reasons of simplicity it is assumed that the D fraction is fermented by Particle Associated Bacteria (PAB), and that the S and W fractions are degraded by Liquid Associated Bacteria (LAB). The PAB and LAB are assumed to have maintenance requirements of 0.05 and 0.15 g of carbohydrates g^{-1} bacteria h^{-1} (Fox et al., 2004), equivalent to 1.365 and 4.095 mmoles of ATP per g bacteria h^{-1}, respectively. Note that the amount of data originally used to derive these values is limited to some five bacterial species, each related to substrate preference rather than to being free or attached (Russell and Baldwin, 1979).

From the equation of Pirt (1965) it also becomes clear that the fractional growth rate (GR) of microbes is mainly determined by the fractional rumen outflow rate. This implies that the rumen outflow rate determines the proportion of the available ATP that is lost in maintenance. Precursors for the formation of macromolecules in microbial mass are supposed to become available from the pool of intermediates from feed degradation. Accepting this approach means that variation in protein yield is determined by variation in type of substrate (ATP yield), variation in outflow rate (maintenance) and the distinction between PAB and LAB (maintenance).
Table 8 shows the degradation and outflow rates of feed components in FOMr (soluble (S), washable (W) and non washable (D) fractions) on the one hand, and between particle associated (PAB) and liquid associated (LAB) bacteria on the other. In the table FOMr is separated in the contributing fractions which are either allocated to LAB or to PAB. The actual Y_{ATP} is calculated by taking into account the ATP yield of each component and the fractional passage rate (that determines the bacterial maintenance requirement), assuming a maximum yield (Y_{max}) of 0.032 g dry bacterial biomass per mmol of ATP. From this, the yield of microbial biomass (g dry bacterial biomass per kg of substrate) per component and per fraction is calculated. Bacterial biomass is assumed to contain 62.5% bacterial crude protein (MCP). Finally, like in the CNCPS system (Fox et al., 2004), a correction factor of 0.20 is applied, to account for predation by protozoa.
Protozoa grow slowly and predate on bacteria. As a result, the net production of bacteria is reduced. One may assume that protozoa have a preference for LAB, but, because PAB stay much longer in the rumen the net result will probably be the predation of equal amounts of LAB and PAB.
The calculation of efficiency in the present approach is fundamentally different from that in the CNCPS, as this system assumes that efficiency is related to the fractional degradation rate, i.e., CNCPS has kd rather than kp in the Pirt equation. Values at the extreme upper and lower range of kd values would give rise to biological impossible results, as demonstrated before (Dijkstra et al. 2002). Soluble substrates and denser particles have a higher probability to escape from the rumen and the density or specific weight of a particle increases more rapidly with a higher fractional degradation rate. Therefore, a positive relationship may be expected between the fractional rates of degradation and outflow (Pellikaan, 2004).

Table 8. Distribution of feed components in FOMr over soluble (S), washable (W) and non washable (D) fractions and between Particle associated (PAB) and liquid associated (LAB) bacteria.

	COMP	Type	ATP maint	Out-flow	ATP yield	Y_{ATP}	Main-tenance	g bact	MCP	MCP per kg FOMr
			mmol g^{-1} bact h^{-1}	h^{-1}	mol. kg^{-1}	g. mol^{-1}	% ATP	g kg^{-1} substrate		
			a)	b)	c)	d)	e)	f)	g)	h)
Forage NDF	D	PAB	1.365	0.020	27.3	10.1	68.5	275	172	138
Concentrate NDF	D	PAB	1.365	0.027	27.3	12.3	61.4	337	211	168
Forage RNSP	W	LAB	4.095	0.080	23.9	12.1	62.1	290	181	145
	D	PAB	1.365	0.027	27.3	12.3	61.6	335	210	168
Conc. RNSP	W	LAB	4.095	0.080	23.9	12.1	62.1	290	181	145
	D	PAB	1.365	0.029	27.3	12.8	59.9	350	219	175
Forage SU+GOS	S	LAB	4.095	0.110	23.9	14.6	54.4	349	218	174
Conc.SU+GOS	S	LAB	4.095	0.110	23.9	14.6	54.4	349	218	174
Ferm. Products	S	LAB	4.095	0.110	11.9	14.6	54.4	174	109	87
Forage starch	W	LAB	4.095	0.080	27.3	12.1	62.1	331	207	166
	D	PAB	1.365	0.045	27.3	16.2	49.3	443	277	222
Conc. Starch	W	LAB	4.095	0.080	27.3	12.1	62.1	331	207	166
	D	PAB	1.365	0.060	27.3	18.5	42.1	506	316	253
Forage protein	S	LAB	4.095	0.110	13.6	14.6	54.4	198	124	99
Foage protein	W-S	LAB	4.095	0.080	13.6	12.1	62.1	165	103	82
Forage protein	D	PAB	1.365	0.045	13.6	16.2	49.3	221	138	110
Conc. Protein	S	LAB	4.095	0.110	13.6	14.6	54.4	198	124	99
Conc. Protein	W-S	LAB	4.095	0.080	13.6	12.1	62.1	165	103	82
Conc. Protein	D	PAB	1.365	0.060	13.6	18.5	42.1	251	157	126

Explanation per column: a): see text (par. 3.3.3); b): see Table 3; c): see text (p. 3.3.3); d): calculated with formula of Pirt with Y_{max} = 0.032 g $mmol^{-1}$ ATP; e): ($Y_{max} – Y_{ATP}/Y_{max}$*100 (with Y_{max} = 32 g mol^{-1} ATP); f): c*d (ATPyield * Y_{ATP}); g): f * 0.625 (g bact/kg *0.625);hj): g*0.8 (0.8 = correction for predation)

Hence, relating efficiency of microbial growth to fractional degradation rate may work in practice in conjunction with the assumed difference in microbial maintenance requirement between PAB and LAB, be it, as indicated (Dijkstra et al., 2002), that this approach is not based on sound biological principles and experimental *in vivo* evidence exists to indicate otherwise (Oba and Allen, 2003). The FiM system (Thomas, 2004) does relate efficiency to fractional passage, but assumes a linear relationship between these characteristics whereas the Pirt equation gives curvilinear results.
Only limited information is available on the effect of the source of carbohydrates on the efficiency of microbial growth and protein synthesis (EMPS). The CNCPS assumes EMPS to be influenced by rate of degradation and type of carbohydrates and to vary between 170 and 230 g MP per kg FOMr. According to a review of Archimède et al. (1997), EMPS varies in mixed diets between less than 90 and more than 200 g MP per kg of FOMr. The nature of the carbohydrates in the diet had a substantial effect on this figure with highest values for starch-rich diets. The variation in EMPS can at least partly be explained from the different ATP yield of different carbohydrates, and by the rate of degradation. For instance, when starch is degraded rapidly it will be degraded via the so-called “acrylate pathway”, with a lower ATP yield than the “succinate pathway”. In a recently published *in vivo* experiment, Oba and Allen (2003) compared the effect of carbohydrates varying in rumen fermentability and rate of fermentation. The efficiency decreased significantly with an increasing fractional rate of starch degradation and increased significantly with an increased rate of starch passage, contrasting the assumptions on efficiency in the CNCPS.

In the DVE/OEB2007 system the variation in outflow rate is assumed to depend more on the physical characteristics of the substrate fractions (S, W-S and D with fractional outflow rates of 0.11 for S, 0.08 h^{-1} for W-S and, depending on the component, varying between 0.020 and 0.060 h^{-1} for D), than on differences in dry matter intake (DMI). Besides, if the nutrient supply varies with the level of intake it becomes impossible to draw up feed tables. Hence, although unattractive from a biological perspective, such influences are better incorporated in the requirements, like is for instance the case for the VEM (Van Es, 1978) and the DVE/OEB1991 system (Subnel et al., 1994). Therefore, the DVE/OEB2007 system does not discriminate between fractional rates of outflow on the basis of DMI, like in the FiM system (Thomas, 2004), but on the basis of type of substrate. This seems a workable approach, which does take into account differences in fractional outflow rate between fractions. For practical reasons the differences in efficiency of protein yield are attributed to substrates rather than to fractional outflow rates.

3.3.4 Fermentation products in ensiled feeds

In a number of feedstuffs, (e.g., silages and high moisture by-products) part of the carbohydrates have already been degraded to fermentation products (FP) by bacteria before the ingredient is consumed by the animal. The presence of FP is important, because lactic acid and ethanol contain rumen extractable energy, and because for a correct calculation of the RNSP fraction (see paragraph 3.3.4.1), FP have to be subtracted.

3.3.4.1 Energy from fermentation products

The most important fermentation products (FP), especially in ensiled feeds, are lactic acid (LA) and ethanol (ALC), which are assumed to contain still 50% of the rumen extractable energy (FOMr) of their precursor carbohydrates. The energetic contribution of VFA to FOMr may be neglected.

The best way to take into account the contribution of FP to FOMr is to analyse the amount in the feed sample before drying, and (for a correct calculation of the RNSP fraction) in the dried sample, too. However, this is both too laborious and too expensive. Therefore table values for the amount of LA and ALC are used for high moisture ingredients like pressed

beet pulp, potato peelings etc. The CVB Feed Table gives information about the content of (individual) FP determined in the non-dried feedstuff, but expressed as the level in the dried material assuming that no evaporation occurs.
In the DVE/OEB1991 system (CVB, 1991) the equations given in table 9 were presented to estimate the level of FP in dried silage samples.

Table 9. Equations to estimate fermentation products (FP)

Forage	Equation (g/kg DM)	Minimum value
Grass silage	FP = -0.3 x DM + 2 x NH_3-fraction +170	15
Maize silage	FP = -0.4 x DM + 210	n.a.
Lucerne silage	FP = -0.3 x DM + 190	15
Field bean silage	FP = -0.3 x DM + 190	15

Because no new information has become available since 1991, these equations are maintained in the DVE/OEB2007 system.

To calculate the contribution of LA and ALC to FOMr correctly, one should use the amount present in an ingredient as fed, i.e. before drying. In case of ingredients for which table values are used, these values can be taken directly from the product sheets.
When only table values of FP in the dried material are available, or when FP in the dried material is estimated with the equations of table 9, it is assumed that FP is the sum of all fermentation products present after drying, of which part does not contribute to FOMr. Therefore, and because of the inaccuracy in the estimations, the FP content as such has to be used.

3.3.4.2 Fermentation products and calculation of RNSP

To calculate the RNSP fraction in dried samples of high moisture by-products and silages correctly, the amount of FP present after drying has to be subtracted. When information on the level of FP in the non-dried material is available, this, in combination with information on the degree of evaporation, can be used to calculate RNSP. When the amount of FP in the dried sample is available (table values of equations in Table 9) this information can be used.
Details about the calculation of RNSP is given in paragraph 2.1.

A new approach to determine FP is by using Near Infrared Reflectance Spectroscopy (NIRS). Using as a calibration set a dataset of samples in which LA has been determined in the fresh samples, it appeared possible to calibrate the NIRS apparatus and (due to its low volatility, especially when drying at moderate temperatures), to determine LA in dried samples of silages. Further it appeared that for the majority of silages there is a good relation between the LA content and the (total) FP content in the fresh samples. This implies that, after estimating the LA content in the fresh samples with NIRS, also the FP content can be estimated.

3.3.5 Amino acids in rumen microbial protein

Like in the DVE/OEB1991 system (CVB, 1991; Tamminga et al., 1994), it is assumed that 75% of the microbial crude protein is present as amino acids, which are assumed to be absorbed from the intestine with an efficiency of 85%. These values are equal to the ones used in the FiM system (Thomas, 2004), but slightly deviate from those in the PDI system that uses 80% for both (Vérité and Peyraud, 1989).

3.4 DVE lost in endogenous faecal protein (DMFP)

The digestive process is associated with endogenous CP losses. These losses include digestive enzymes, bile, desquamated epithelial cells and mucus. Despite the fact that these losses originate from the animal, they are thought to be caused more by the characteristics of the feed than of the animal. In the DVE/OEB1991 system it was assumed that each kg of DM excreted in the faeces caused a protein loss of 8 g N per kg, the equivalent of 50 g of (crude) protein. This approach was extensively investigated (Van Gestel et al., 2004) and it was concluded that there is no reason to change this approach. It is further assumed that the re-synthesis of endogenously excreted protein occurs with an efficiency of 67%. Hence the replacement of endogenous protein excreted in the faeces requires 75 g of DVE per kg of faecal DM. It was also thought appropriate to charge this loss on the feed ingredient itself rather than to include it in the requirements. Hence this requires an estimate of the dry matter lost in faeces per feed.

FDM = DM – DOM – DASH

FDM can be separated in indigestible OM and indigestible inorganic mater. An estimate of organic matter (OM) excreted in faeces can be derived from the digested organic compounds. The digestibility of the ash is subsequently calculated from

dASH = % dASH/100 x Crude Ash (CASH) [eq. 10]

A comparison of the ash content calculated on the basis of the sum of the oxides and the determined ash content showed good agreement for a wide variety of feedstuffs. It was subsequently assumed that Na, K and Cl had digestibilities of 100% and Ca, Mg and P of 50%. On the basis of the composition of their ash fraction, feedstuffs were allocated to 3 groups with digestibilities for crude ash (CASH) of 35, 50 or 65% with for each feed a maximum based on:

$dASH_{max}$ = % dASH/100 x ($CASH_{mean}$ + 10) [eq. 11]

where $CASH_{mean}$ is the mean crude ash content of an ingredient, as published in feed tables.

Now it follows that (with all values in g. kg^{-1}):

DMFP = 0.075 X (DM – DOM – dASH), [eq 12]

3.5 Comparison of DVE/OEB1991 and DVE/OEB2007

DVE/OEB1991 and DVE/OEB2007 were compared on a dataset containing 56 concentrate ingredients, of which *in situ* data were available. Datasets containing fresh grass (n=120), grass silage (n=102), grass hay (n=14), and maiza silage (n=78) were also used to compare the systems.
From the available data on forages, regression formulas were derived for each forage, to estimate the fractions W and U, and the kd for CP, STA (only for maize silage), NDF, and RNSP.
Based on Blgg (Oosterbeek) datasets containing 500 grass silages and 500 maize silages, and an ASG (Lelystad) dataset containing 65 samples of fresh grass, the regression formulas were used to estimate the parameters needed for the calculation of DVE. FOMr, MCP (g/kg FOMr), RUSTA, DRUP, and DVE were calculated using the formulas from DVE/OEB1991 and DVE/OEB2007.
Results are shown in table 10.

The DVE of concentrate ingredients on average hardly changes in the new system, compared to the 1991 system. Although FOMr decreases by 9%, this is compensated by an increase in MCP. The DVE of maize silage also does not change in the 2007 system. The slight decrease in DRUP is compensated by a slightly higher MCP. The DVE of fresh grass is on average 11% lower in the new system, compared to 1991; this is mainly caused by a lower FOMr, and a lower MCP. The DVE of grass silage decreases by almost 15%, caused by a decrease in DRUP and a decrease in DMCP.

Table 10. Comparison between DVE/OEB 1991 and DVE/OEB 2007.

	Concentrate ingredients (n=56)		Maize silage (n=500)	
	DVE/OEB1991	DVE/OEB2007	DVE/OEB1991	DVE/OEB2007
FOMr (g/kg DM)[1]	611	559	508	533
RUSTA (g/kg DM)	85.3	86.5	114.3	92.9
DRUP (g/kg DM)	89.0	88.3	19.2	16.9
MCP (g/kg FOMr)	150	168	150	151
DMCP (g/kg DM)	58.4	59.7	48.7	51.2
DVE (g/kg DM)	132	133	48.1	48.4
RDPB	28.2	23.4	-30.6	-31.2
	Fresh grass (n=65)		Grass silage (n=500)	
	DVE/OEB1991	DVE/OEB2007	DVE/OEB1991	DVE/OEB2007
FOMr (g/kg DM)[1]	592	538	592	567
RUSTA (g/kg DM)	n.a.	n.a.	n.a.	n.a.
DRUP (g/kg DM)	52.0	49.4	37.2	34.8
MCP (g/kg FOMr)	150	143	150	
DMCP (g/kg DM)	56.6	49.2	56.6	47.9
DVE (g/kg DM)	90.5	80.5	75.3	64.4
RDPB	27.2	40.6	27.6	43.6

4 The rumen degradable protein balance and synchronisation of rumen fermentation

4.1 Rumen degradable protein balance

The rumen degradable protein balance (RDPB or OEB) is defined as the difference between the amount of microbial protein based on rumen available energy (MCPE) and the amount of microbial protein based on rumen available nitrogen (MCPN). In the DVE/OEB1991 system (CVB, 1991; Tamminga et al., 1994) it was stated that the RDPB (or OEB) of a dairy diet should not fall below zero. Later research (Meijer et al., 1996) suggested that because of differences in feed intake and feed intake pattern between cows, in practice this might lead to an N deficiency at rumen level for individual cows under certain conditions and a safety margin of at least 150 g rumen degradable protein was recommended. The results of a study on the effect of RDP on feed intake or milk yield by Van Vuuren and Tamminga (2001) indicated that there is no need for a minimum requirement of a positive OEB. They calculated that in a mature cow, the recycling of urea provides between 175 and 280 g RDP per day, which was considered sufficient as a safety margin. They do however stress that as an additional safety margin, farmers should in practice try to avoid a negative RDP balance at any time. This advice is maintained.

4.2 Synchronisation of rumen fermentation

Balancing diets for a synchronised rumen fermentation can serve different purposes. On a daily basis, calculation of the balance of supply of protein and energy sources for the rumen microbes will indicate potential shortages of protein, leading to reduced microbial growth, or potential oversupply of protein, giving rise to production of ammonia subsequently excreted as urea in the urine. A synchronised supply indicates the balance on a shorter time frame (e.g. hourly basis), and hence instantaneous imbalances between protein and energy supply. However, the most important purpose of synchronisation is probably that it prevents the pH in the rumen to drop below a level where microbial activity and feed intake is impaired (Dijkstra et al., 2002; Russell and Strobel, 2005). An additional advantage could be that the nutrients required by the microbes are available at the right moment, which may prevent energy spillage. A balanced rumen fermentation can be achieved by feeding a totally mixed ration (TMR), the frequent feeding of concentrates, using a computer controlled concentrate dispenser or balancing feed on the basis of the degradative and passage behaviour (Cone et al., 2003).
For each of the components in a diet as presented in table 5, the cumulative amount available in the rumen ($FOMr_t$) can be calculated for each time span, using the equation 5:

$$FOMr_t = kd/(kp+kd) * COMP* (1-e^{-(kp+kd)*t}) \quad \text{[eq. 13]}$$

A synchronisation ratio can then be calculated as the ratio between rumen degradable protein and rumen degradable non protein components (RDP/RDCHO). This approach was applied on the data in table 8 and the results are shown in table 11.
The results in table 11 enabled us to calculate some indexes and ratio's. First of all a Rate index (% of FOMr per defined time frame) provides information on the amount of substrate that is available in defined periods after ingestion of the feed as a % of the chosen endpoint. Synchronisation ratio's can also be calculated by expressing the amount of RDP as proportion of the amount of RDCHO at periods varying in length. Finally the Rumen Degradable Protein Balance (RDPB) can be calcu-

lated as the difference between degraded protein and microbial protein synthesis in a defined time frame.

Table 11. Synchronisation ratio's of a standard diet (Tamminga et al., 2004)

Component		**Fraction**	**Kd**	**Kp**	**0-1**	**0-3**	**0-6**	**0-12**	**0-24**	**0-∞**
Forage NDF	W	0.010	0.035	0.080	0.000	0.001	0.002	0.002	0.003	0.003
Forage NDF	D	0.320	0.035	0.020	0.011	0.031	0.057	0.098	0.149	0.203
Concentrate NDF	W	0.015	0.050	0.080	0.001	0.002	0.003	0.005	0.006.	0.006.
Concentrate NDF	D	0.060	0.050	0.027	0.002	0.007	0.012	0.020	0.027	0.032
Forage RNSP	W	0.030	0.188	0.080	0.005	0.012	0.017	0.020	0.021	0.021
Forage RNSP	D	0.075	0.075	0.027	0.005	0.015	0.025	0.039	0.050	0.055
Concentrate RNSP	W	0.005	0.150	0.080	0.001	0.002	0.002	0.003	0.003	0.003
Concentrate RNSP	D	0.020	0.060	0.029	0.001	0.003	0.006	0.009	0.012	0.013
Forage sugars	S	0.070	2.000	0.110	0,058	0,066	0,066	0,066	0,066	0,066
Concentrates sugars	S	0.030	2.000	0.110	0,025	0,028	0,028	0,028	0,028	0,028
Fermentation products	FP	0.030	2.000	0.110	0.025	0.028	0.028	0.028	0.028	0.028
Forage starch	W	0.050	0.525	0.080	0.020	0.036	0.042	0.043	0.043	0.043
Forage starch	D	0.010	0.075	0.045	0.001	0.002	0.003	0.005	0.006	0.006
Concentrate starch	W	0.050	0.695	0.080	0.024	0.040	0.044	0.045	0.045	0.045
Concentrate starch	D	0.030	0.160	0.060	0.004	0.011	0.016	0.020	0.022	0.022
Forage protein	S	0.020	2.000	0.110	0.017	0.019	0.019	0.019	0.019	0.019
Forage protein	W-S	0.040	0.050	0.080	0.002	0.005	0.008	0.012	0.015	0.015
Forage protein	D	0.035	0.050	0.045	0.002	0.005	0.008	0.013	0.017	0.018
Concentrate protein	S	0.025	2.000	0.110	0.021	0.024	0.024	0.024	0.024	0.024
Concentrate protein	W-S	0.035	0.050	0.080	0.002	0.004	0.007	0.011	0.013	0.013
Concentrate protein	D	0.050	0.050	0.060	0.002	0.006	0.011	0.017	0.021	0.023
Total FOMr		1,000			0.229	0.346	0.430	0.527	0.618	0.689
	Time frame				0-1	0-3	0-6	0-12	0-24	0-∞
Rate index (% of t=∞)					33,2	50,3	62,4	76,4	89,7	100,0
Synchronisation ratio 1			RDP/RDCHO		0.245	0.222	0.219	0.219	0.211	0.195
	Time frame				0-1	2-3	3-6	6-12	12-24	24-∞
Synchronisation ratio 2			RDP/RDCHO		0.245	0.178	0.208	0.219	0.170	0.072

Based on the average figures extracted from those presented in table 7 and 8, different diets were composed. Diet 1 is the average Dutch dairy diet estimated for the Dutch manure legislation (Tamminga et al., 2004) that is fed as TMR and contains fresh grass (22.3%), grass silage (33.3%), maize silage (18.5%) and concentrates (25.9%). Diet 2 contains fresh grass only and the diets 3 and 4 are winter diets that contain 50% concentrates and either grass silage (GS) or maize silage (MS) as the only forage. Rate indexes and synchronisation ratios as well as RDPB are presented in table 12.

According to table 13, the microbial protein production for various raions varies between 142.6 and 154.9 g/kg FOMr. To obtain a RDPB=0, the RDP/RDCHO ratio should vary between 0.166 and 0.183 (MCP/kg FOMr/(1000-MCP/kg FOMr)). The results show that, for all diets, the recommended ratio is exceeded, notably shortly after feed intake. The results also show that during the first 12 hours after ingestion, the RDPB does usually not become negative. It should be noted that the SR does not take into account any buffering mechanisms in

the rumen, including the supply of N due to urea recycling or the reduced starch degradation rate when engulfed and temporarily stored by protozoa. Furthermore, the SR is calculated for the feed that enters the rumen during a meal assuming that there is no feed present from previous meals that can change the ratio. Finally, SR values after a time frame of some 6-12 h have little practical relevance, as dairy cows usually have a new meal well within that time-frame. The cumulative RPDB (calculated over a time period from zero to infinity) is comparable to the current OEB value and indicates per feed the degradable protein balance given the assumptions as described in tables 7 and 8.

Table 12. Degradation (Rate), Synchronisation Ratio's (SR) and Rumen Degradable Protein Balance (RDPB) of different diets for dairy cows.

Diet	**Characteristic**	**Time frame (h)**						
		1	**2**	**3**	**6**	**12**	**24**	**∞**
TMR table 8	Rate	33.2	43.9	50.3	62.4	76.4	89.7	100
	SR-1	0.245	0.229	0.222	0.219	0.219	0.211	0.195
	SR-2	0.245	0.180	0.178	0.208	0.219	0.170	0.072
	RDPB-1[a]	11.3	11.2	10.9	12.4	15.3	15.2	9.6
Fresh grass Only	Rate	27.7	36.8	43.0	57.2	74.9	90.6	100
	SR-1	0.360	0.386	0.402	0.419	0.408	0.371	0.335
	SR-2	0.360	0.470	0.486	0.473	0.371	0.220	0.067
	RDPB-1[a]	22.8	34.5	43.5	62.4	79.4	83.7	78.1
Grass silage + 50 % conc.	Rate	27.8	38.6	45.8	60.5	76.7	90.2	100
	SR-1	0.261	0.280	0.294	0.312	0.306	0.276	0.246
	SR-2	0.261	0.332	0.349	0.372	0.281	0.088	0.002
	RDPB-1[a]	11.9	19.4	25.6	38.1	46.0	42.8	34.0
Maize silage + 50 % conc.	Rate	37.3	50.6	58.2	71.1	83.0	92.3	100
	SR-1	0.182	0.189	0.199	0.220	0.229	0.218	0.200
	SR-2	0.182	0.212	0.231	0.323	0.288	0.082	0,001
	RDPB-1[a]	1.3	2.9	5.6	13.5	19.0	16.2	8.7

[a]: RDPB-1: Time frame from t = 0 till the hour indicated in the heading

The ratio's or indices presented in table 12 can be summarised for certain time periods considered critical, for instance the first 2 hours after feeding. Such data are presented in table 13.

Table 13. Synchronisation characteristics of different diets.

Ration	**FOMr**	**Efficiency Kg^{-1}FOMr**	**Synchronisation ratio (SR-1) (RDP/RDCHO)**			**Rumen Degradable Protein Balance**		
			Total	0 - 2 h				Total
TMR (Table 8)	654	149.4	0.195	0.229	0.171	9.6	11.2	-1.5
Grass only	676	142.6	0.335	0.386	0.307	78.1	34.5	43.5
50% Grass silage + 50% conc.	676	149.6	0.246	0.280	0.226	34.0	19.4	14.6
50% Maize silage + 50% conc.	697	154.9	0.200	0.189	0.211	8.7	2.9	5.8

To provide insight in the short term availability of N from RDP on the one hand, and energy from fermented organic components on the other hand, the CVB feed tables will contain the parameter RDPB-2, in which '2' stands for t0-t2 hour period.

As indicated in paragraph 4.1, equation 13 may be used to calculate the degree of fermentation of a component at any given moment in time.To clarify the rate of fermentation of OM, the CVB feed tables will contain the parameter FOMr-2. This parameter indicates the amount of FOMr that is degraded from all fermentable fractions during the first two hours in the rumen.

5 Evaluation of feedstuffs in the DVE/OEB2007 system

Individual feedstuffs should be evaluated according to the principles of the new system, to obtain a system that is applicable in practice.
Table 3 (Chapter 2) gives an overview of feed components and parameter values in the DVE/OEB2007 system. For some parameter values, results from nylon bag incubations are required.

To obtain an optimal feed evaluation, the variation between *in situ* incubations of different samples from one feedstuff (or group of similar feedstuffs) should be related to (variations in) chemical and (possibly) physical characteristics of the feedstuffs . This proved only possible in a limited number of cases.
For some (for dairy cattle quantitatively less important) feedstuffs, the evaluation had to be based upon estimation by experts, by comparing the feedstuff to related feedstuffs.

The evaluations of individual feedstuffs are not included in this publication. For concentrate ingredients, high moisture ingredients and roughages they will first be published in a separate publication as a provisional evaluation. In this publication (CVB, 2007a), the following parameters will be given for the average composition of a feedstuff, or certain qualities of a feedstuff: FOMr, $FOMr_{t0\text{-}2}$, the ratio $FOMr_{t0\text{-}2}$/FOMr, RDPB, $RDPB_{t0\text{-}2}$, DVE, DVMET, and DVLYS.
In September 2007 these provisional evaluations will be eventually adjusted and subsequently be published in definitive publications (namely a bilingual feed table for ruminants, the CVB Feeding Table, edition 2007 and the Handleiding Voederwaardeberekening Ruwvoeders, edition 2007). In addition to the parameters mentioned above, in he last two tables also DRUP, DMCP, DMFP, and RUSTA will be given, as relevant intermediate values.

CVB will also include the parameter values of the DVE/OEB2007 system for the individual feedstuffs in the first edition of a “On line feed evaluation calculator”, to be published in April 2007. This calculator can be used to recalculate the feeding value (and therefore also the parameters of the DVE/OEB2007 system) of feedstuffs based on own analytical results.
Later on, a “plus-version” will be introduced, from which a larger number of intermediate values (e.g., the individual fractions that contribute to the FOMr or DMCP) may be calculated and reported.
The parameter values of individual feedstuffs will also be made available by CVB through the CD “Factors and coefficients derived nutrients calculation”.

6 Protein requirements of dairy cows

The DVE/OEB2007 system distinguishes in requirements for maintenance (paragraph 6.1), milk (protein) production (paragraph 6.2), body protein mobilisation and deposition (paragraph 6.3) and fetal growth (paragraph 6.4).

6.1 Maintenance

An important proportion of inevitable protein losses in faeces are not used to maintain the animals organs and tissues, but result from endogenous losses thought to be more related to the undigested feed residues than to the animal. As discussed earlier, for these endogenous losses a correction was attributed to the supply rather than keep them as a component of the maintenance requirement. The resulting requirements for maintenance were restricted to those necessary to compensate for losses in urine and in hair and skin. Both are related to the animals BW and are calculated (just as in DVE/.OEB1991) from the equation:

$DVE_{maintenance}$ (g DVE/d) = $(2.75*BW^{0.5} + 0.2* BW^{0.6})/0.67$ [eq. 14]

The FiM (Thomas, 2004) system uses an equation derived from NRC (2001):

$MP_m^{FiM} = 4.1*BW^{0.5} +0.3*BW^{0.6} + 30*TDMI – 0.5*((DMTP)/0.8) - DMTP) + 2.34*DMI$

(in which TDMI = total dry matter intake, and DMTP = digestible microbial true protein).

The components related to BW are the same as in the DVE system, the other components are related to dry matter intake (like in NRC, 2001), but corrected for indigestible rumen-synthesized microbial protein that is degraded and absorbed (as ammonia) from the hind gut. The CNCPS (Fox et al., 2004) assumes that protein requirements for maintenance are the sum of scurf protein, urinary protein and metabolic feed protein. Scurf and urinary protein are related to BW and calculated the same way as in DVE and FiM. Metabolic fecal protein is calculated as 9% of indigestible DM.

6.2 Milk yield

$DVE_{milk\ yield}$ = (kg milk * milk protein content)/efficiency.

Initially, the DVE system (CVB, 1991) assumed a constant efficiency of 0.64. More recent research (Hof et al., 1994; Subnel et al., 1994) has shown that this efficiency is variable and influenced by the DVE/NEL ratio as well as by the FPCM production level. According to Subnel et al (1994), this efficiency could adequately be described by the equation

Efficiency = 117.6 – 3.044 * DVE/NEL – 0.23 * FPCM [eq. 15]

Where:
Efficiency = milk protein/DVE milk (%)
DVE/NEL = ratio between DVE and Net Energy (g/MJ)
FPCM = fat and protein corrected milk (kg/d).

The effect of milk production is at least partly the result of the way the NEL (VEM) system is used to formulate energy requirements (Van Es, 1978). This system also gives a decreasing efficiency of energy utilisation with increasing milk production. This decrease is primarily thought to be the result of a decreasing digestion. The equation to describe the protein requirements then becomes (Subnel et al, 1994):

$$DVE = 1{,}396 * E + 0.000195 * E^2 \quad \text{[eq. 16]}$$

Where:
DVE = g DVE required per g milk protein (E)
E = milk protein production (g/day)
The FiM system and the CNCPS use a constant efficiency of utilisation. FiM uses 0.68, the CNCPS uses 0.65, but corrects crude milk protein to true milk protein with the factor 0.93, which reduces the conversion of E to crude milk protein to 0.60.

The DVE/OEB1991 system has been validated by Van Straalen et al. (1994).
The DVE/OEB2007 system has been validated by the Animal Sciences Group, Lelystad, using 11 specially selected feeding trials (in total ... treatments). The results of this validation study will be published in a separate Documentation report (CVB, 2007b, in preparation).
For this validation, it is assumed that the maintenance requirement of dairy cows (see paragraph 6.1), as well as the additional DVE allowances for pregnancy (see paragraph 6.4), are correct.
At the moment this report was published, the validation study was not completed. However, the preliminary results show only relative small differences in utilisation of DVE for milk protein synthesis between the new and the old system. Therefore, for the time being there is no need to adapt the DVE requirement for milk protein synthesis described by equation 16.
On the other hand, the preliminary results show a distinct difference in RDPB, calculated according to the DVE/OEB 2007 system, compared to the RDPB, calculated according to the DVE/OEB 1991 system. Generally the difference can be described as: (RDPB-new system) = 1.25*(RDPB-old system).

6.3 Body protein mobilisation and deposition

In the DVE/OEB1991 system it was assumed that energy mobilised from the body yields 45 g of DVE per 1000 VEM (127 g DVE/kg BW loss) and that the re-deposition of energy in the body requires 57 g DVE per 1000 VEM (200 g DVE/ kg BW gain). However, more recent research (Tamminga et al., 1997; Van Knegsel et al., 2007) has revealed that protein balance and energy balance do not follow the same pattern. A negative protein balance turns into a positive protein balance already after 2 to 3 weeks, while the energy balance remains negative up to 8 to 12 weeks after calving. It is assumed that protein mobilised from the body is primarily used as a source of energy to which no protein value is attributed.
The re-deposition of protein in 75 kg body weight gain would require 15 kg DVE. At the same time the production in protein in 8000 kg of milk requires a minimum of 425 kg of DVE. The requirement for re-deposition therefore equals less than 3.5% of the requirement for milk protein production, the majority of which is deposited during the second half of the lactation period and for this no extra requirement is allocated.

6.4 Pregnancy

The DVE/OEB1991 system (CVB, 1991; Tamminga et al., 1994) recommends an extra protein (DVE) allowance varying between 35 (month 5) and 105 (month 7) g DVE/day during the last 5 months of pregnancy. These requirements were recently updated (Van den Top et al.,

2000) for a cow of 650 kg and a calf birth weight of 44 kg. Also an allowance for twins was included (Table 13). It is assumed that the diet for normal milk production in late lactation can accommodate for this requirement. During the last 2 months, when the animal is dry between 170 and 270 g DVE/day is required.

Table 14. Extra protein allowance for dairy cows during pregnancy (in g DVE/day)

	Single	**Twins**
Maintenance	119	
Extra allowance		
6 months (161-190 days)	62	112
7 months (191-220 days)	107	193
8 months (221-250 days)	177	319
9 months (251-280 days)	278	500

7 Amino acids (AADI) vs. Protein (DVE)

7.1 Rumen degradative behaviour of amino acids in undegraded feed protein

Rumen undegraded protein (RUP) is in fact undegraded N*6.25 and is assumed to be composed of amino acids (AA), linked together in true protein. A crucial question is whether the degradative behaviour in the rumen of the total AA or individual AA differs from that of protein in the rumen. This question was addressed by Van Duinkerken and Blok (1998) and restricted to Lysine (LYS) and Methionine (MET) . Their conclusion was that total AA in concentrate ingredients and LYS and MET follow the same pattern of degradation as protein. For forages they concluded that the rumen degradation of individual AA may significantly deviate from that of protein, but that the data base that led to this conclusion was too small and inadequate to derive reliable correction equations to estimate rumen degradation for individual AA in forages. It was also assumed that AA in forages follow the same pattern as protein.
This question of agreement between intestinal AA flow calculated on the basis of the three contributing fractions and measured intestinal AA flow was also addressed by Rulquin et al (1998). They used a different approach to estimate the supply of intestinal undegraded feed protein (PIA = CP * 1.1 * (1-DT)), microbial protein (0.145 * FOMr * 0.8) and endogenous protein (PIendo = 33 * NDOM * 0.5). After eliminating the effect of the research group, their calculated values overestimated LYS by 5% and underestimated MET by 12%.

7.2 Digestive behaviour of rumen undegraded amino acids in the intestine

The next question to be addressed was if the digestive behaviour in the intestine of individual AA differs from that of protein. On the basis of regression analysis, van Duinkerken and Blok (1998) concluded that the intestinal digestion of LYS was equal to that of protein, but that the digestion of MET was underestimated by 4%. Applying this correction partly compensates for the difference in supply as mentioned in the previous paragraph.

7.3 LYS and MET in microbial (DMCP) and endogenous protein (DMFP)

For microbial protein (DMCP) an average AA pattern was calculated (Van Duinkerken and Blok, 1998) and from that it appeared that the contribution of LYS and MET were 77 and 25 g kg^{-1} total AA. Similarly the contribution of LYS and MET to DMFP was estimated at 57 and 15 g kg^{-1} total AA respectively. As pointed out earlier, it was also estimated that the efficiency of absorption of LYS did not differ significantly of that of DVE, but that the absorption of METt was slightly (4.2%) higher.
Hence:

DRULYS = 0.010 * LYS * DRUP
DMCLYS = 0.077 * DMCP
DMFLYS = 0.057 * DMFP
DRUMET = 0.01042 * MET * DRUP
DMCMET = 0.025 * DMCP
DMFMET = 0.015 * DMFP

Note that the FiM system (Thomas, 2004) uses for LYS and MET the same intestinal absorption coefficients as for total rumen undegraded protein (RUP) and the contribution to microbial protein digested in the intestine (DMCP) was estimated at 77.9 g kg^{-1} total AA for LYS and 24.3 g kg^{-1} total AA for MET.

7.4 Amino acid requirements

Like all other mammals, ruminants have a requirement for essential amino acids. Of these, Lysine (LYS) and Methionine (MET) are usually considered as first limiting. For grass silage based diets, Histidine (HIS) has also been nominated as limiting (Huthanen et al., 2002). Based on dose-response relationships, Rulquin et al. (1993) observed that an optimum milk protein production was obtained when the PDI contained 7.3% LYS and 2.5% MET. NRC (2001) recommends levels of 7.2 and 2.4 for LYS and MET respectively. Based on practical and economical considerations, later on (Rulquin et al.,1998; 2001) critical lower levels were established and set at 6.8 and 2.1% for LYS and MET respectively. The latter values were also adopted by FiM. Requirements for other AA are less well established, but Rulquin et al. (2001) recommend between 2.5 and 3.2% for His and at least 8.8% for Leucine (Leu).

One of the goals of the actualisation of the DVE/OEB 1991 system was, in line with the systems mentioned above, to set standard for the first two limiting amino acids (MET and LYS).
As CVB does not have at its disposal a database with doses response experiments, as executed by e.g. Rulquin and collaborators, we tried to derive these standards in the framework od the DVE/OEB 2007 system using the standards published by Rulquin et al (1998 and 2001) as s starting point.
To derive standards, expressed as percentage AADI per unit DVE, the supply METDI and LYSDI (in g/day) according to both the DVE/OEB 2007 system and the French PDI system was calculated in the experiments of the validation study (see paragraph 6.2). After calculation of the %METDI and %LYSDI per unit DVE and unit PDIE, respectively, a comparison could be made of the supplied proportion of %METDI and %LYSDI in DVE on the one hand, and of %METDI and &LYSDI in PDIE on the other hand.
Our aim was to adapt the standards of Rulquin et al by multiplying their standards by the ratio (AADI in DVE)/(AADI in PDIE) and to use the result as the standards for METDI and LYSDI in the DVE/OEB 2007 system.
However, is appeared that the standards, calculated as described above, had numerical values that had to be considered as incorrect. The reason laying behind this probably is that the way the supply of AADI in both systems was calculated differed in such a way that no direct comparison is allowed. This may be due to the use of different amino acid pattern for feedstuffs (especially for grass and grass silage) and the proportional contribution of DRUP and DMCP in DVE. A second reason probably is the difference in the calculation of the supply of AADI. In the DVE/OEB 2007 system the calculation of Van Duinkerken and Blok (1998) is followed, implying that RUP has the same amino acid pattern as the original feedstuff. Further, for LYS in RUP it is assumed that the intestinal digestibility is identical to that of RUP, whereas the intestinal digestibility of MET is 0.96 that of RUP. In the French system the supply of AADI from rumen undegradable feed protein and microbial protein is calculated and subsequently corrected with (amino acid specific) regression formulas (Rulquin et al, 1998 and 2001).

From these calculations it has been concluded that a correct derivation of standards or intestinal digestible amino acids within the DVE/OEB 2007 system has to be based on calculations using a database with detailed information (especially of the feed ingredients used) of doses response studies.

8 Literature

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Annex 1. Database derived from Offner et al (2003) and Offner and Sauvant (2004)

Feed	Treatment	W situ	Kd (1-W)	ED situ	ED vivo	E(1-W) vivo	EW vivo	Regression
Corn	Untreated	0,236	0,059	0,597	0,639	0,379	0,260	0,247
	ground	0,338	0,055	0,679	0,691	0,317	0,374	0,327
	Cracked	0,200	0,057	0,584	0,631	0,390	0,241	0,219
	steam-rolled	0,041	0,027	0,338	0,476	0,298	0,178	0,095
	steam-flaked	0,127	0,216	0,803	0,769	0,683	0,086	0,162
	Expanded	0,487	0,064	0,773	0,750	0,265	0,485	0,443
	Extruded	0,522	0,087	0,828	0,785	0,283	0,502	0,470
	Pelleted	0,387	0,059	0,685	0,695	0,304	0,391	0,365
	f. treated	0,296	0,038	0,569	0,621	0,273	0,348	0,294
Sorghum	Untreated	0,277	0,042	0,603	0,643	0,298	0,345	0,279
	Ground		0,044	0,756	0,739	0,423		
	Epanded		0,035	0,798	0,766	0,368		
	Extruded	0,363	0,097	0,757	0,740	0,394	0,346	0,346
	f. treated	0,357	0,024	0,541	0,604	0,184	0,420	0,342
Barley	Untreated	0,515	0,350	0,913	0,838	0,414	0,424	0,465
	Ground	0,460	0,387	0,859	0,804	0,468	0,337	0,422
	Cracked	0,010	0,060	0,503	0,580	0,495	0,085	0,071
	steam-rolled	0,295	0,109	0,706	0,708	0,455	0,253	0,293
	steam-flake	0,053	0,344	0,839	0,792	0,806	-0,015	0,104
	Expanded		0,219	0,690	0,698	0,785		
	Extruded		0,292	0,683	0,693	0,830		
	f. treated	0,397	0,264	0,844	0,795	0,491	0,303	0,373
Wheat	Untreated	0,604	0,329	0,939	0,855	0,335	0,520	0,534
	by products	0,782	0,238	0,937	0,853	0,174	0,679	0,673
	Ground		0,213					
	steam flaked		0,061					
	Expanded		0,129	0,669	0,684	0,683		
	Extrude		0,350	0,841	0,793	0,854		
	f. treated	0,644	0,076	0,855	0,802	0,199	0,603	0,566
Oats		0,668	0,189	0,918	0,841	0,252	0,589	0,584
brewers grains		0,770	0,174	0,833	0,788	0,171	0,617	0,664
Hominy feed		0,390	0,053	0,673	0,687	0,286	0,401	0,367
corn gluten meal		0,230	0,286	0,866	0,809	0,636	0,172	0,242
corn gluten feed		0,562	0,119	0,841	0,793	0,291	0,502	0,502
Formol treated CGF		0,673	0,130	0,890	0,824	0,224	0,600	0,588
Triticale		0,453	0,583	0,934	0,851	0,496	0,355	0,416
Rice		0,260	0,076	0,674	0,688	0,414	0,274	0,266
Ricebran		0,188	0,127	0,734	0,725	0,551	0,174	0,210
Peas		0,462	0,116	0,802	0,768	0,355	0,414	0,424
Ground peas		0,494	0,192	0,878	0,816	0,386	0,431	0,449
cracked peas		0,411	0,153	0,848	0,797	0,423	0,374	0,384
Toasted peas		0,278	0,045	0,587	0,633	0,309	0,323	0,280
Extruded peas		0,699	0,344	0,951	0,862	0,256	0,606	0,609
Beans		0,371	0,079	0,729	0,722	0,357	0,365	0,352
Toasted beans		0,369	0,043	0,628	0,659	0,263	0,395	0,351
Potato		0,452	0,091	0,779	0,754	0,330	0,424	0,416
Manioc		0,895	0,139	0,880	0,817	0,073	0,744	0,762
corn silage		0,668	0,087	0,865	0,808	0,196	0,611	0,584

Annex 2. Degradation of starch in vitro (Cone and Van Gelder, 2005)

Feed	Time	Starch residue		kd	
		W	D	W	D
Maize meal	0	100	100		
	6	62.1	88.7		
	8	32.9	65.3		
	10	31.4	50.5	0.113	0.055
Wheat	0	100	100		
	6	36.9	78.1		
	8	19.0	46.6		
	10	12.2	30.0	0.190	0.091
Potato starch	0	100	100		
	6	82.2	91.5		
	8	42.6	75.1		
	10	26.2	66.2	0.095	0.037
Tapioca pellets	0	100	100		
	6	36.4	67.2		
	8	17.3	33.8		
	10	10.9	20.9	0.197	0.119

[1)]. Calculated from Genstat (NLIN)

Annex 3. Regression of rate of degradation of fraction W (exercises 1 to 3) on fit of in vivo starch degradation

	Model	**Y = aX**			**Y = aX + C**		
	kd(W)	**Y**	**R^2**		**Y**	**C**	**R^2**
Exercise 1	0,25	1.0956	0,6062		0,7033	0,2713	0,8928
	0,50	1.0281	0,6486		0,6613	0,2706	0,9508
	0,75	1.0031	0,6397		0,6393	0,2753	0,9619
	1,00	0.9901	0,6300		0,6269	0,2785	0,9757
Exercise 2	1 x kd(D)	1.1753	1,2145		0,4524	0,4692	0,6979
	2 x kd(D)	1.0860	0,4427		0,4931	0,4165	0,8283
	3 x kd(D)	1.0482	0,1275		0,5135	0,3890	0,8794
	4 x kd(D)	1.0270	0,0429		0,5262	0,3716	0,9058
	5 x kd(D)	1.0134	0,1489		0,5351	0,3595	0,9215
Exercise 3	kd(D) + 0.25	1.0441	0,4730		0,6197	0,3088	0,9225
	kd(D) + 0.50	1.0096	0,5781		0,6246	0,2896	0,9537
	1,5 x kd(D) + 0,25	1.0312	0,4510		0,6053	0,3138	0,9295
	2 x kd(D) + 0,25	1.0217	0,4402		0,5965	0,3164	0,9347
	2 x kd(D) = 0,375	1.0083	0,5116		0,6053	0,3037	0,9484
	2 x kd(D) + 0,50	0.9992	0,5462		0,6084	0,2972	0,9557

Annex 4. The effect of pelleting on starch fractions and rumen degradation (Tamminga and Goelema, 2004, unpublished)

Feed	Starch	Meal			Pellet		
		D	kd	RSTA	D	kd	RSTA
Maize (M)[1)]	358	87	0.050	49.2	79	0.052	45.2
Barley (B)[1)]	370	39	0.203	17.3	34	0.239	15.9
Tapioca (T)[1)]	360	25	0.141	17.8	19	0.158	16.4
Miz M,B,T[1)]	373	49	0.077	28.5	41	0.093	24.2
A-standard[2)]	76	70	0.120	27.5	56	0.124	24.3
A-select[2)]	55	65	0.126	25.8	44	0.106	23.6
DVE-high[2)]	307	56	0.125	24.2	39	0.114	21.9
DVE-low[2)]	294	58	0.120	25.1	40	0.178	18.4
Mix (maize)[3)]	203	84	0.065	42.5	75	0.055	42.6
Mix (maize)[3)]	200	75	0.056	42.2	74	0.056	41.9
Mix (maize[3)]	200	74	0.084	24.3	74	0.099	31.5
Average		62	0.106	30.4	52	0.116	27.8

Source: [1] Tamminga et al., 1989; [2] Houtmans, Kemp, Van der Velden, Hof, unpublished; [3] Perdok, Smink, Veen, 1991, unpublished; [4] Perdok, Groot & Veen, 1992, unpublished; [5] Pelleted with steam conditioning and pre-compression (Mixcompres)

RSTA = resistant starch.

Annex 5. Rumen clearance by passage based on lignin or IADF

Type of forage	cows	DMI	kg conc	NDF/DM	kp	kp'	Method	Marker	Reference
Grass silage	dry	10,3		442	2,82	2,82	rumen evacuation	IADF	Bosch, 1991
Grass silage	dry	11,1		515	3,18	3,18	rumen evacuation	IADF	Bosch, 1991
Grass	lactating	13,3	1,0	352	3,10	2,59	duodenal flow	Lignin	vVuuren et al., 1992
Grass	lactating	16,8	1,0	374	3,10	2,59	duodenal flow	Lignin	vVuuren et al., 1992
Grass	lactating	13,0	1,0	381	2,90	2,43	duodenal flow	Lignin	vVuuren et al., 1992
Grass	lactating	15,2	1,0	317	2,40	2,01	duodenal flow	Lignin	vVuuren et al., 1992
Grass	lactating	16,3	1,7	406	3,50	2,93	duodenal flow	Lignin	vVuuren, 1993
Grass	lactating	16,3	7,0	352	4,70	3,93	duodenal flow	Lignin	vVuuren, 1993
Grass	lactating	16,5	7,2	407	4,60	3,85	duodenal flow	Lignin	vVuuren, 1993
TMR (barley)	lactating	24,0	12,0	367	4,76	4,76	rumen evacuation	IADF	de Visser et al., 1992
TMR (maize)	lactating	23,1	11,6	345	4,74	4,74	rumen evacuation	IADF	de Visser et al., 1992
TMR (beetpulp)	lactating	22,9	11,5	438	4,25	4,25	rumen evacuation	IADF	de Visser et al., 1992
TMR (maize bran)	lactating	24,2	12,1	448	4,81	4,81	rumen evacuation	IADF	de Visser et al., 1992
Grass silage (young)	lactating	21,0	10,0	360	1,60	1,34	duodenal flow	Lignin	Klop et al., 1997
Grass silage (young)	lactating	20,0	9,0	360	1,60	1,34	duodenal flow	Lignin	Klop et al., 1997
Grass silage (young)	lactating	19,0	9,0	360	1,60	1,34	duodenal flow	Lignin	Klop et al., 1997
Grass silage (old)	lactating	17,0	8,0	420	2,10	1,76	duodenal flow	Lignin	Klop et al., 1997
Grass silage (old)	lactating	18,0	8,0	420	2,10	1,76	duodenal flow	Lignin	Klop et al., 1997
Grass silage (old)	lactating	16,0	7,0	420	2,10	1,76	duodenal flow	Lignin	Klop et al., 1997
TMR (WGS)	lactating	24,4	17,1	483	4,00	3,35	rumen evacuation	Lignin	de Visser, 1993
TMR (MGS)	lactating	23,7	16,6	459	3,70	3,10	rumen evacuation	Lignin	de Visser, 1993
TMR (PGS)	lactating	23,5	16,5	464	3,80	3,18	rumen evacuation	Lignin	de Visser, 1993
TMR (WW)	lactating	23,8	16,7	483	4,00	3,35	rumen evacuation	Lignin	de Visser, 1993

Type of forage	cows	DMI	kg conc	NDF/DM	kp	kp'	Method	Marker	Reference
Grass silage (100IM)	lactating	18,5	4,5	470	2,70	2,70	rumen evacuation	IADF	Bruinenberg, 2003
Grass silage (20SPP)	lactating	18,1	4,5	481	2,30	2,30	rumen evacuation	IADF	Bruinenberg, 2003
Grass silage (60SPP)	lactating	16,7	4,5	497	2,50	2,50	rumen evacuation	IADF	Bruinenberg, 2003
Grass silage 60SPR)	lactating	17,5	4,5	486	2,80	2,80	rumen evacuation	IADF	Bruinenberg, 2003
Grass	lactating	16,2	4,6	415	2,70	2,26	rumen evacuation	Lignin	Taweel, 2004
Grass	lactating	16,5	4,6	430	3,50	2,93	rumen evacuation	Lignin	Taweel, 2004
Grass	lactating	16,6	4,6	426	3,00	2,51	rumen evacuation	Lignin	Taweel, 2004
Grass	lactating	16,2	4,6	414	2,80	2,34	rumen evacuation	Lignin	Taweel, 2004
Grass	lactating	17,3	4,6	422	2,40	2,01	rumen evacuation	Lignin	Taweel, 2004
Grass	lactating	16,8	4,6	436	3,10	2,59	rumen evacuation	Lignin	Taweel, 2004
Grass	lactating	15,7	2,8	422	3,60	3,01	rumen evacuation	Lignin	Taweel, 2004
Grass	lactating	16,0	2,8	442	3,10	2,59	rumen evacuation	Lignin	Taweel, 2004
Grass	lactating	15,0	2,8	465	3,40	2,85	rumen evacuation	Lignin	Taweel, 2004
Grass	lactating	15,2	2,8	462	3,00	2,51	rumen evacuation	Lignin	Taweel, 2004
Grass	lactating	15,2	2,8	456	2,90	2,43	rumen evacuation	Lignin	Taweel, 2004
Grass	lactating	15,7	2,8	443	3,40	2,85	rumen evacuation	Lignin	Taweel, 2004
Average		17,76				2,78			
S.D..		3,64				0,88			

Annex 6. Proportion of DNDF that is actually digested

Experiments H. Valk (2002)

Concentrates	%	NDF/DM	UNDF/DM	NDF	UNDF
beet bulp	20	400	24	80	5
citrus pulp	20	200	15	40	3
maize gluten feed	22,5	350	20	79	5
palm kernel expeller	15	600	150	90	23
soyabean hulls	8	650	25	52	2
other					
Total	100			341	37

	%	NDF/DM	UNDF/DM	NDF	UNDF
beet pulp	15	400	24	60	4
maize gluten feed	20	350	20	70	4
palm kernel expeller	17,5	600	150	105	26
soy bean hulls	14	650	25	91	4
linseed expeller	8	200	50	16	4
coconut expeller	8	430	80	34,4	6
wheat	7,5	120	16	9	1
Others	10				
Total	100			385	49

Treatment		NDF Intake			UNDF intake		DNDF intake				Faecal output		DNDF		
		Total	Conc	Forage	Conc.	Forage	Forage	Conc	Total	dNDF	NDF	UNDF	Faecal	Max	Real
		(kg)	(kg)	(kg)	(kg)	(% NDF)	(kg)	(kg)	(kg)	(%)	(kg)	(kg)	(kg)	(%)	(%)
S91	450	7,80	0,73	7,07	0,086	0,597	6,48	0,64	7,12	77,3	1,77	0,683	1,088	91,2	84,7
	300	8,00	0,73	7,27	0,086	0,645	6,63	0,64	7,27	75,1	1,99	0,731	1,261	90,9	82,7
	150	8,00	0,73	7,27	0,086	0,701	6,57	0,64	7,21	70,1	2,39	0,787	1,605	90,2	77,7
S92	450	8,90	0,73	8,17	0,086	0,899	7,27	0,64	7,91	75,3	2,20	0,985	1,213	88,9	84,7
	300	8,70	0,73	7,97	0,086	0,853	7,12	0,64	7,76	72,9	2,36	0,939	1,419	89,2	81,7
	150	8,80	0,73	8,07	0,086	0,953	7,12	0,64	7,76	71,5	2,51	1,039	1,469	88,2	81,1
A92	450	8,60	1,07	7,53	0,123	0,697	6,84	0,94	7,78	75,5	2,11	0,820	1,287	90,5	83,5
	300	9,20	1,07	8,13	0,123	0,842	7,29	0,94	8,23	74,2	2,37	0,965	1,409	89,5	82,9
	150	8,70	1,07	7,63	0,123	0,977	6,66	0,94	7,60	70,5	2,57	1,100	1,467	87,4	80,7
A93	450	8,60	1,07	7,53	0,123	0,478	7,05	0,94	7,99	76,1	2,06	0,601	1,455	93,0	81,8
	300	8,60	1,07	7,53	0,123	0,505	7,03	0,94	7,97	73,1	2,31	0,628	1,686	92,7	78,9
	150	8,10	1,07	7,03	0,123	0,619	6,41	0,94	7,35	70,5	2,39	0,742	1,648	90,8	77,6

Experiments M. Bosch et al. (1992)

Concentrates		%	NDF/DM	NDF	UNDF/DM	UNDF
beet pulp		20	400	80	24	5
citrus pulp		20	200	40	15	3
maize gluten feed		25	350	88	20	5
soyabean meal		10,7	100	11	3,5	0
Linseed		3,8	133	5	30	1
wheat middlings		13,8	422	58	90	12
Total				281		27

	Conc.	NDF Intake			UNDF intake		DNDF intake				Faecal output		DNDF		
	DM	Total	Conc	Forage	Conc.	Forage	Forage	Conc	Total	dNDF	NDF	UNDF	Faecal	Max	Real
	(kg)	(kg)	(kg)	(kg)	(kg)	(% NDF)	(kg)	(kg)	(kg)	(%)	(kg)	(kg)	(kg)	(%)	(%)
G1L	0,875	5,65	0,25	5,40	0,023	0,572	4,83	0,22	5,05	78,0	1,24	0,596	0,646	89,4	87,2
G2L	0,875	6,45	0,25	6,20	0,023	1,029	5,17	0,22	5,39	70,9	1,88	1,053	0,823	83,7	84,7
G3L	0,875	8,05	0,25	7,80	0,023	1,248	6,55	0,22	6,77	73,8	2,11	1,271	0,837	84,2	87,6
G4L	0,875	7,15	0,25	6,90	0,023	1,684	5,22	0,22	5,44	62,9	2,65	1,707	0,944	76,1	82,6
G1H	6,125	6,82	1,72	5,10	0,164	0,541	4,56	1,56	6,12	74,7	1,73	0,704	1,022	89,7	83,3
G2H	6,125	7,42	1,72	5,70	0,164	0,946	4,75	1,56	6,31	66,4	2,49	1,110	1,385	85,0	78,1
G3H	6,125	8,42	1,72	6,70	0,164	1,072	5,63	1,56	7,19	71,4	2,41	1,236	1,174	85,3	83,7
G5H	6,125	8,32	1,72	6,60	0,164	1,960	4,64	1,56	6,20	56,8	3,60	2,124	1,472	74,5	76,3
Mean															82,07
Standard deviation															3,14

Annex 7. Calculated and measured NDF digestibilities

		Kdf	Kdc	kpf	kpc	kg NDFf	kg NDFc	VCNDF	VCNDF	
		Determined		Calculated		Determined		Calc. (%)	Det. (%)	
grass silage	G1L	5,91	4,50	2,44	2,65	5,40	0,25	0,783	0,780	Bosch et al., 1992
grass silage	G2L	3,75	4,50	2,06	2,65	6,20	0,25	0,717	0,709	
grass silage	G3L	4,60	4,50	2,21	2,65	7,80	0,25	0,749	0,738	
grass silage	G4L	3,90	4,50	2,08	2,65	6,90	0,25	0,723	0,629	
grass silage	G1H	6,35	4,50	2,52	2,65	5,10	1,75	0,771	0,747	
grass silage	G2H	3,99	4,50	2,10	2,65	5,70	1,75	0,721	0,664	
grass silage	G3H	3,79	4,50	2,06	2,65	6,70	1,75	0,715	0,714	
grass silage	G5H	2,71	4,50	1,87	2,65	6,60	1,75	0,666	0,568	
fresh grass	S91 450	5,51	4,50	2,37	2,65	7,11	0,61	0,771	0,773	Valk, 2002
fresh grass	S91 300	4,67	4,50	2,22	2,65	7,43	0,61	0,749	0,751	
fresh grass	S91 150	4,07	4,50	2,11	2,65	7,34	0,61	0,729	0,701	
fresh grass	S92 450	4,32	4,50	2,16	2,65	8,26	0,61	0,738	0,753	
fresh grass	S92 300	3,97	4,50	2,09	2,65	8,05	0,61	0,725	0,729	
fresh grass	S92 150	3,51	4,50	2,01	2,65	8,07	0,61	0,706	0,715	
fresh grass	A92 450	5,11	4,50	2,30	2,65	7,62	0,91	0,759	0,755	
fresh grass	A92 300	4,58	4,50	2,20	2,65	8,21	0,91	0,745	0,742	
fresh grass	A92 150	3,84	4,50	2,07	2,65	7,83	0,91	0,719	0,705	
fresh grass	A93 450	7,54	4,50	2,73	2,65	7,67	0,91	0,803	0,761	
fresh grass	A93 300	6,80	4,50	2,60	2,65	7,67	0,91	0,793	0,731	
fresh grass	A93 150	5,36	4,50	2,34	2,65	7,15	0,91	0,765	0,705	
fresh grass	none	4,73	4,70	2,23	2,69	5,99	0,64	0,750	0,787	Van Vuuren, 1993
	HS	4,73	4,50	2,23	2,65	3,84	1,63	0,738	0,745	
	HF	4,73	9,60	2,23	3,56	3,84	2,93	0,779	0,792	
fresh grass	N500	5,60	4,50	2,38	2,65	4,64	0,32	0,774	0,770	Van Vuuren et al., 1992
	N275	4,90	4,50	2,26	2,65	6,35	0,32	0,757	0,797	
	N500	4,20	4,50	2,14	2,65	4,96	0,32	0,734	0,756	
	N275	6,60	4,50	2,56	2,65	4,74	0,32	0,794	0,768	
young silage	no starch	4,00	8,34	2,10	3,34	4,65	2,96	0,754	0,844	Klop et al., 1997
	0.75 kg starch	4,00	8,34	2,10	3,34	4,47	2,85	0,754	0,826	
	1.5 kg starch	4,00	8,34	2,10	3,34	4,23	2,69	0,754	0,845	
old silage	no starch	3,50	8,32	2,01	3,33	5,01	2,18	0,732	0,776	
	0.75 kg starch	3,50	8,32	2,01	3,33	5,28	2,30	0,732	0,791	
	1.5 kg starch	3,50	8,32	2,01	3,33	4,69	2,04	0,732	0,775	

Annex 8. Feed ingredients selected to calculate rumen degradation characteristics of RNSP

Sample	NDF	**D**	U	**kd_D**	**kp_D**	FOMr	dNDF	RNSP	W	U	D	**kd_D**	**kp_D**	**FOSp**	**dRNSP**
	g/kg DS			% p.u.		g/kg NDF	%	g/kg DS		%		% p.u.		g/kg RNSP	%
Potato peelings	220	90.2	9.8	3.85	2.54	544	60.4	256	0.0	98.0	2.0	11.83	3.96	735	81.6
Potatopulp	326	82.8	17.2	6.02	2.92	558	62.0	272	38.9	61.1	0.0	32.34	7.59	849	94.3
Sugarbeet pulp, pressed	502	94.8	5.2	4.45	2.65	595	66.1	292	0.0	98.8	1.2	5.53	2.84	653	72.6
Sugarbeet pulp, pressed	542	96.0	4.0	4.21	2.60	593	65.9	267	0.0	99.1	0.9	4.32	2.62	616	68.5
Sugarbeet pulp, dehy-drated	356	93.2	6.8	6.59	3.03	639	71.0	232	13.2	86.1	0.7	8.62	3.39	715	79.4
Sugarbeet pulp, dehy,	421	94.3	5.8	8.32	3.33	673	74.8	304	0.0	100.0	0.0	9.92	3.62	733	81.4
Sugarbeet pulp, dehy	380	93.7	6.3	8.55	3.37	672	74.7	338	35.3	64.7	0.0	15.17	4.55	789	87.7
Citruspulp	195	92.6	7.4	7.57	3.20	651	72.3	424	22.1	77.9	0.0	11.88	3.96	758	84.2
Citruspulp	191	93.6	6.4	5.65	2.86	622	69.1	384	25.7	74.3	0.1	7.94	3.26	709	78.8
Citruspulp	205	92.7	7.3	6.86	3.07	640	71.1	456	18.7	80.8	0.4	11.54	3.90	751	83.4
Linseed expeller	205	65.2	34.8	5.72	2.87	434	48.2	190	0.0	91.2	8.8	9.78	3.59	667	74.1
Linseed meal solvent extracted	221	70.3	29.7	3.50	2.48	412	45.8	200	44.2	53.4	2.3	7.14	3.12	677	75.2
Lupins	292	95.1	4.9	3.50	2.48	557	61.9	234	25.1	74.9	0.0	9.83	3.60	738	82.0
Reference sample*	189	81.7	18.3	4.29	2.62	508	56.4	84	52.6	38.5	8.9	5.17	2.77	575	63.9
Reference sample*	189	82.0	18.0	3.28	2.44	470	52.3	84	21.7	77.7	0.6	7.11	3.12	690	76.7
Reference sample*	191	82.2	17.8	2.95	2.38	455	50.6	82	48.9	48.9	2.2	3.53	2.48	543	60.4
Reference sample*	191	85.5	14.5	5.33	2.80	560	62.3	82	36.4	56.9	6.8	3.01	2.39	494	54.9
Reference sample*	191	89.6	10.4	2.96	2.38	497	55.2	82	45.2	51.4	3.5	3.20	2.42	518	57.6
Soya beans, toasted	118	97.0	3.0	5.59	2.85	643	71.4	132	27.7	72.3	0.0	10.47	3.71	746	82.9
Soya beans non toasted	151	98.7	1.3	4.76	2.70	630	70.0	140	28.8	71.2	0.0	11.42	3.88	756	84.0
Soyabean meal solvent extracted	204	98.0	2.0	4.07	2.58	600	66.7	137	44.9	55.1	0.0	9.21	3.49	733	81.4
Mean	261	89.0	11.0	5.14	2.77	569	63.2	222	25.2	73.0	1.8	9.47	3.54	688	76.4
Standard deviation	116	8.9	8.9	1.70	0.30	80	8.9	117	17.7	18.3	2.8	6.19	1.10	92	76.2

*: CVB reference sample: 30% soybean meal (CP 47%), 35% corn meal with low germ content and 35% grass meal (low sugar content). This mixture contains per kg approx. 230 g CP, 220 g NDF and 220 g Starch.

**: dNDF and dRNSP: apparent faecal digestibility (calculated) of the NDF and RNSP fraction, respectively.