FACTORS AFFECTING DIET DIGESTIBILITY IN DAIRY CATTLE

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INTRODUCTION

Feces typically comprises the greatest loss of ingested energy in ruminants, and the variation in converting digestible energy to metabolizable energy is usually small. Diet digestibility is determined by intrinsic properties of inter- and intramolecular structure of plant cell walls, which establish the upper potential for the rate and extent of cell wall digestion in ruminants (Mertens, 1993). Typically digestibility for feed evaluation in ruminants is estimated with sheep fed at maintenance level of feeding. It can be considered as an intrinsic digestibility of the feedstuff, although only 80-90% of potentially digestible NDF (**pdNDF**) is digested at maintenance level of intake (Huhtanen et al., 2006). The intrinsic rate and extent of digestion of cell walls set the upper limit of digestion in ruminants, but both the animal and diet factors can have a dramatic effect on the extent to which the potential digestibility is attained. It is well-known that digestibility of dairy cow diets is reduced with increasing intake (Tyrrel and Moe, 1975), but the discounts for the feeding level effects on digestibility are variable in feed evaluation systems. The rate of decline has been shown to be related to diet digestibility at maintenance intake (NRC, 2001). The changes in digestibility observed with increased intake have been associated with reduced digesta retention time in the rumen. Further, both negative and positive associative effects between dietary components on digestion can occur, especially at high levels of feeding. The objective of this paper is to analyze the effects of some intrinsic and extrinsic factors influencing digestibility of mixed dairy cow diets, and to compare different models in predicting discounts in digestibility with increasing intake. The analysis is based on a dataset from North European feeding trials, in which diet digestibility was determined in dairy cows.

MATERIAL AND METHDODS

Data

Treatment mean data were collected from feeding trials with lactating dairy cows fed ad libitum of grass silage, or legume (mainly red clover) or whole-crop cereal (barley, wheat or corn) silages partly or completely substituted for grass silage. The silages were supplemented with concentrate feeds differing both in the amount and composition. The minimum prerequisite of an experiment to be included in the data set was that diet digestibility, production parameters (silage and total DMI, milk production), adequate silage (plant species, harvest, concentrations of DM, CP, NDF, fermentation acids, in vivo or in vitro digestibility), and concentrate characterization (ingredients and concentrations of CP, fat and NDF) were reported. Diet digestibility was determined by total fecal collection method (n=176 diets) or by using acid insoluble ash (**AIA**) determined as described by Van Keulen & Young (1970) as an internal marker (n=321).

The total data included 497 diets in 92 studies. In addition to the analysis of the whole data set, the data was divided into three subsets to estimate specifically the effects of silage digestibility (42 diets, 17 studies), the amount of concentrate supplementation (142 diets, 59 studies) and concentrate CP concentration (215 diets, 82 studies) on the digestibility of diets fed to dairy cows. Silage digestibility expressed as concentration of digestible organic matter (**DOM**) in DM was influenced by the stage of maturity at harvest and concentrate CP concentration by substituting protein supplements such as rapeseed (canola) feeds, soybean meal or fish meal for energy supplements, typically cereal grains.

Calculations

Diet OM digestibility (**OMD**) at maintenance intake (**OMD_m**) was estimated using in vivo or in vitro digestibility for the forage component of the diet. In vivo digestibility was measured using sheep at maintenance level of feeding with the total fecal collection method. In vitro digestibility was measured using rumen fluid (Tilley and Terry, 1963) or pepsin-cellulase (Nousiainen et al., 2003). When silage NDF and indigestible NDF (**iNDF**) concentrations were not reported, the estimates were derived from regression equations based on the Finnish dataset (Huhtanen et al., 2006). Digestibility (OMD_m) of the dietary concentrate component was estimated from ingredient composition, analyzed chemical composition and digestibility coefficients (MTT, 2006). If the chemical composition was not reported, default feed table values were used. Concentrate iNDF concentrations were based on either determined values or the values were derived from MTT data sets. For most common and quantitatively most important concentrate ingredients either one of these values was available. For some ingredients the values were based on analyzed lignin concentration or lignin concentration published in NRC (2001) and Fox et al. (2003) feed libraries. The concentration of iNDF (g/kg DM) was estimated as 2.4 × Lignin (Van Soest et al., 2002).

Total digestible nutrients (**TDN**) at maintenance level of feeding (TDN_m; g/kg DM) was calculated from digestible OM at maintenance (**DOM_m**) as follows: $TDN_m = DOM_m + 1.25 \times$ Fatty acids (g/kg DM) to correct for the higher energy concentration of fat compared with the other digestible nutrients. TDN at production level was calculated as $DOM_p + 1.25 \times$ Fatty acids, where DOM_p is the concentration of DOM determined in digestibility trials with cows. Fatty acid concentration was estimated from ether extract concentration with empirical equations. The TDN concentrations at production level (**TDN_p**) were calculated according to NRC (2001) using the following equation to discount for the reduced digestibility with increased feeding level: TDN_p (g/kg DM) = TDN_m - [0.18 × TDN_m - 103) × FL], where FL = feeding level multiple of maintenance - 1. The Cornell Net Carbohydrate and Protein System (**CNCPS**) predicts TDN concentration at production level separately for the forage and concentrate components of the ration using following equations:

Forage $TDN_p = 5.3 + 0.99 \times TDN_m - 0.009 \times NDF + 0.000005 \times TDN_m \times NDF + 89.6 \times FL - 0.1 \times TDN_m \times FL + 0.00005 \times TDN_m \times NDF \times FL$, where units of NDF and TDN_m are g/kg DM.

Concentrate $TDN_p = 1.01 \times TDN_m - 17.7 \times FL - 9.9$.

Statistical analysis

The data was analyzed using mixed model procedure of SAS (Littell et al., 1996). The model was $Y = B_0 + B_1X_{1ij} + b_0 + b_1X_{1ij} + B_2X_{2ij} + \ldots + B_nX_{nij} + e_{ij}$, where B_0, B_1X_{1ij}, $B_2X_{2ij} \ldots B_nX_{nij}$ are the fixed effects and b_0, b_1 and e_{ij} are the random experiment effects (intercept and slope), where i = 1…n studies and j = 1…n_i values.

In order to exclude the effects of the intrinsic digestibility characteristics on the digestibility at production level, OMD_m and the ratio of pdNDF to NDF were used as a random factor in the model. The ratio of pdNDF/NDF was computed as (NDF – iNDF) / NDF. The root mean squared errors (**RMSE**) presented are adjusted for the random study effect.

RESULTS AND DISCUSSION

Data

The data displayed a large variation in intake, production, digestibility and diet composition (Table 1). The proportion of concentrate was on average 41% ranging from 0 to 80 %. Standard deviation of NDF digestibility (**NDFD**) was almost two-fold compared with OMD. On average OMD was 38 g/kg (3.8 %-units) lower at production level in dairy cows than that estimated at the maintenance level of intake.

Table 1. Description of experimental data

	N	Mean	s.d.	Min	Max
DM intake (kg/d)	497	18.6	2.98	9.9	25.2
Milk yield (kg/d)	497	26.7	5.06	13.0	45.8
Digestibility (g/kg)					
Organic matter	497	736	38.1	621	830
Crude protein	471	691	41.1	542	782
NDF	388	627	71.8	408	830
pdNDF[1]	388	754	63.3	506	946
Diet composition (g/kg DM)					
Crude protein	497	166	20.0	111	229
NDF	497	419	47.0	283	559
Indigestible NDF	495	68	22.4	19	148
Crude fat	497	44	10.1	22	112
Starch	497	141	56.3	0	292

OMD_m[2] (g/kg)	497	774	31.5	658	846
TDN_m[3] (g/kg)	497	746	32.3	643	832

[1]pdNDF = potentially digestible NDF; [2]OMD_m = OM digestibility at maintenance intake
[3]TDN_m = total digestible nutrients at maintenance intake

The relationship between OMD estimates based on total fecal collection or AIA in four studies including 18 diets was good as indicated by a high R^2 value (0.81) despite a relatively narrow range of data. The mean OMD estimated by total fecal collection and AIA were 73.2 vs. 72.5% with MSPE of 1.7 %-units. In fifteen dairy cow studies, the mean residual standard deviation of OMD determined with AIA method was on average 1.3 %-units ranging from 0.5 to 2.0 %-units.

Forage digestibility

Higher silage digestibility associated with harvesting grass at an earlier stage of maturity affected the whole diet OMD as expected (Table 2). The effect was strongest on NDFD, but also the pdNDF digestibility increased with improved silage digestibility. Improved apparent digestibility in dairy cows can be attributed to reduced concentration and improved digestibility of NDF, whereas the metabolic fecal organic matter output (**MFOM;** fecal OM – fecal NDF per kg DMI) was not related to silage digestibility. Improved potential digestibility of pdNDF with increased silage DOM concentration may indicate a faster rate of NDF digestion.

Table 2. Effects of forage DOM concentration (g/kg DM) determined in sheep on diet digestibility in cows

Digestibility (g/kg)	N	Intercept	s.e.	Slope	s.e.	P-value	RMSE[1]
OM	44	360	44	0.575	0.057	<0.001	7.7
CP	36	443	52.1	0.363	0.068	<0.001	5.8
NDF	36	66	58.7	0.893	0.079	<0.001	7.9
pdNDF[4]	36	623	49.1	0.236	0.068	0.002	12.5

[1]RMSE = residual mean squared error (adjusted for random study effect)
[2]OM = organic matter
[3]pdNDF = potentially digestible NDF

Concentrate intake

Increasing the amount of concentrate DMI (**CDMI**) had no effect on OMD of the total diet (Figure 1). However, when OMD_m was used as a second independent variable in the model, increasing concentrate supplementation decreased OMD in cows. Because the OMD_m increased by 3.8 g/kg per kg increase in CDMI, the lack of response in OMD in dairy cow studies clearly indicates negative associative effects in digestion with increased concentrate feeding. The present data agrees with Tyrrel and Moe (1975) and Colucci et al. (1989), who reported that the effects of increased concentrate feeding on digestibility diminished as the feeding level increased. However, in their studies the diet digestibility improved with increased concentrate feeding; probably because the difference in forage and concentrate digestibility was much greater in their studies than in the present data due to differences in cell quality between grass vs. alfalfa/corn silage. Digestibility of both NDF and pdNDF decreased significantly (P<0.001) with increased concentrate supplementation. The NDF of pdNDF digestibility responses to increased concentrate did not change when either iNDF concentration or pdNDF/NDF ratio were used as a second independent variable in the model.

Negative effects of increased concentrate supplementation are at least partly related to a depression in the rate of NDF digestion. Reduced ruminal NDF digestion rates have been reported both using in situ and rumen evacuation techniques with increased concentrate feeding.

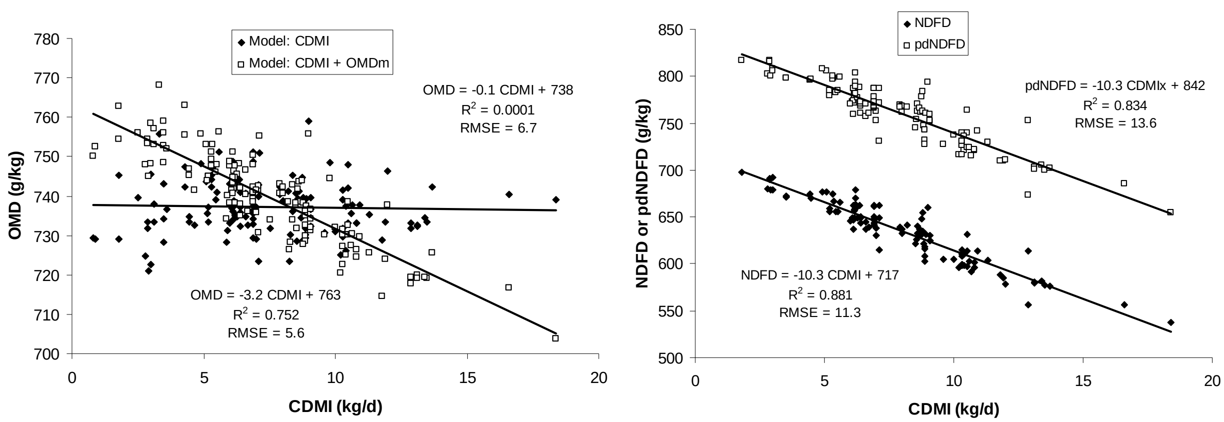

Figure 1. The effects of the amount of concentrate supplementation on digestibility of organic matter (OMD), NDF (NDFD) and potentially digestible NDF (pdNDFD). The values are adjusted for random study effect.

Greater depression in digestibility with high concentrate diets with increased intake may be related to the effects of these two factors on the retention time of feed particles in the rumen and total tract. At low intake level increased concentrate proportion increases retention time (Colucci et al., 1982; 1990), which can compensate for the effects of reduced digestion rate of NDF with high concentrate diets. However, with increased intake the effect of concentrate proportion on retention time is reduced resulting in a greater depression in digestibility of high rather than low concentrate diets with increased intake. The relationship recalculated from the data of Colucci et al. (1982; 1990) between the decrease in total particle retention time and digestibility depression was surprisingly good (Figure 2). In sheep the depression in digestibility was smaller than dairy cows, probably because starch digestibility decreases much less in sheep with increasing intake than in cattle.

The Lucas test (a regression between digestible amounts on intake of the entity) showed that NDS were completely digestible (0.99) when the amount of concentrates was increased. The output of MFOM averaged 91.4 (SE 1.42 g/kg DMI), and it was not influenced by the level of concentrate supplementation.

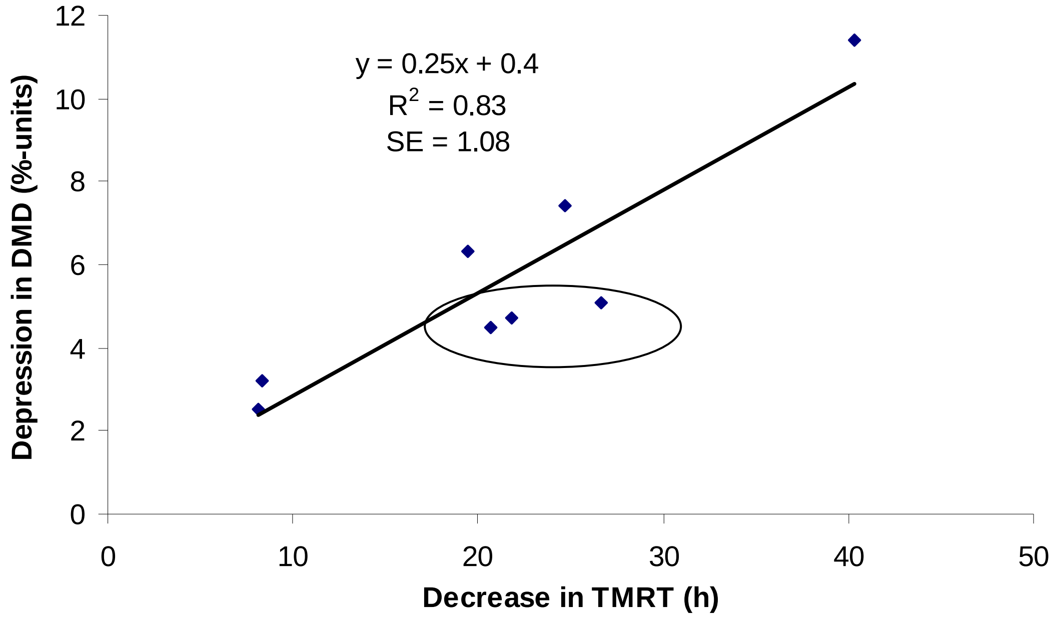

Figure 2. Relationship between depression in total tract mean retention time (TMRT; difference between low and high DM intake) and corresponding depression in DMD (recalculated from Colucci et al., 1982; 1990). Circled data points from sheep trials.

Protein supplementation

The digestibility of diets fed to dairy cows improved quadratically in response to increased dietary CP concentration (Figure 3). The ratio between linear slopes of OMD and NDFD (0.33/0.77= 0.43) was similar to NDF/OM ratio (0.46) suggesting that digestibility response to protein supplementation was entirely associated with improved NDFD (0.46 × 0.77 = 0.35 ~ 0.33). Because dietary concentration of iNDF increased with increased protein supplementation (e.g. canola meal has a high iNDF concentration compared with grain), the positive effect of protein supplementation was greater on pdNDFD than on NDFD. This also rules out that improved OMD with increasing CP concentrations was due to improved intrinsic digestibility of the diet.

The effect of protein supplementation on diet digestibility has seldom reached statistical significance in a single study, but this meta-analysis of a large dataset demonstrated the positive effect of supplementary protein on the digestibility of dairy cow diets. The diets in the present dataset were mainly based on grass silage, but similar effects could be expected in cows fed corn silage and/or alfalfa haylage. Oldham (1984) reported a slightly greater response in DM digestibility to increased dietary CP concentration in cows fed corn silage compared with grass silage based diets. Several mechanisms may be associated with improved OMD in response to replacement of energy supplements with protein supplements: overcoming deficiency of rumen degradable protein (1), higher intrinsic rate and potential extent of fiber digestion of protein supplements (2), better rumen conditions for fiber digestion due to reduced dietary starch concentration (3), and stimulation of cellulolytic bacteria by amino acids and peptides derived from supplementary protein (4).

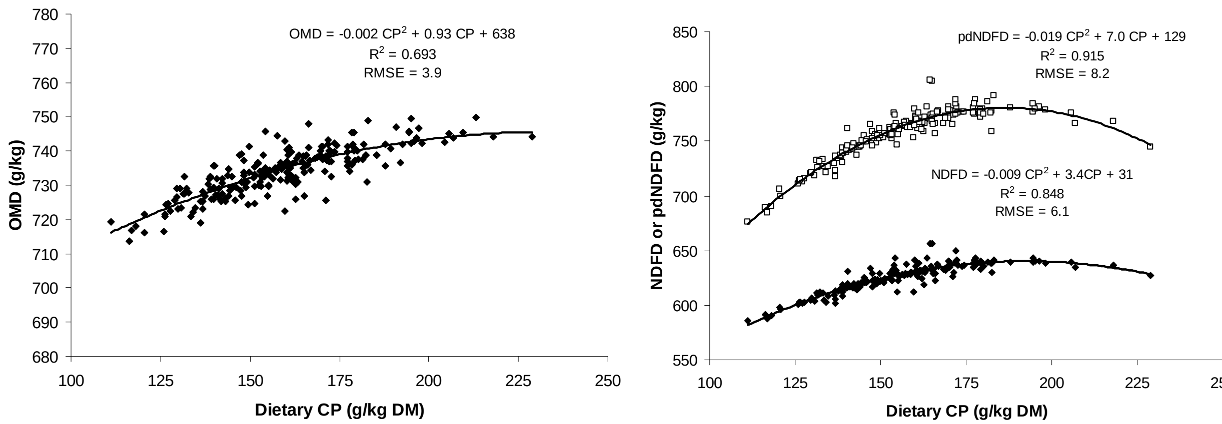

Figure 3. The effects of dietary CP concentration on digestibility of OM (OMD), NDF (NDFD) and potentially digestible NDF (pdNDFD). The values are adjusted fro random study effect

Multiple factor models

When the intrinsic digestibility (OMD determined at maintenance in sheep) is known, the digestibility can be adjusted using DMI (extrinsic factor) to predict digestibility of diets fed to dairy cows as demonstrated by the following equation:

$$OMD = 257(\pm43) + 0.685(\pm0.054) \times OMD_m \text{ (g/kg DM)} - 2.6(\pm0.44) \times DMI \text{ (kg/d)}$$

The RMSE adjusted for random study effect was 8.4 g/kg. A prediction error below 1 %-unit within a study indicates that this simple equation predicts accurately the differences in digestibility between dairy cow diets. This equation shows that discounts in intake of DOM (or TND) increase as intake and/or OMD_m increase. Basically this function has the same parameters as the NRC (2001) equation predicting the TDN discount.

It has been suggested (Vandehaar, 1998) that the depression in digestibility is not linear, but that the digestibility depression decreases at diminishing rate as feed intake increases. The present data does not support that suggestion; vice versa, depression in digestibility per unit of DMI tended to increase slightly as DMI increased. Kinetically decreasing depression in digestibility with increased intake is questionable, since rumen

NDF pool size should increase quadratically unless the rate of NDF digestion also increase with enhance DMI, which is unlikely.

A multiple mixed model regression equation including some additional independent variables is shown in Table 3. Although the effects of additional parameters were significant, the prediction error of the model decreased only from 8.4 to 7.8 g/d. Parameter values for OMD_m and DMI remained similar compared with the simple model indicating that the model is stabile. Also the protein parameters remained rather similar in the model based on the whole data set (n = 497) to those derived from protein supplementation studies. Concentrate fat intake had a negative effect on diet digestibility.

Table 3. The multiple mixed model regression equation predicting OM digestibility (g/kg) in cows (RMSE = 7.8).

Item	Unit[1]	Estimate	SE[2]	P-value
Intercept		141	52.8	0.009
OMD_m[3]	g/kg	0.66	0.05	<0.0001
DMI	kg/d	-2.6	0.46	<0.0001
CP	g/kg DM	1.4	0.36	0.000
CP^2	g/kg DM	-0.0034	0.0011	0.002
CFat[4]	kg/d	-19	5.75	0.001

[1]Unit of independent variable
[2]Standard error
[3]OM Digestibility at maintenance intake
[4]Concentrate fat intake

A multiple regression equation including both intrinsic and extrinsic factors influencing NDF digestibility in dairy cows is presented in Table 4. The proportion of pdNDF of total NDF, which expresses the potential extent of digestion, had the strongest effect (highest F-value) on NDF digestibility in cows. The regression coefficient of pdNDF/NDF was below one, i.e. pdNDF is not completely digested. The negative coefficient of DMI is related to increased passage rate as intake increases. The positive effect of the proportion of forage NDF of the total NDF is probably related to the longer retention time of forage particles compared with concentrate particles in the rumen (e.g. Colucci et al., 1990). Feeding high concentrate diets containing fibrous by-products has resulted in large decreases in digestibility with increased intake (Woods et al., 1999). The negative quadratic effect of dietary carbohydrate ratio (NFC/NDF) and concentrate fat are most likely related to inhibitory effects of rapidly digestible carbohydrates and fat on rumen cellulolytic bacteria, whereas the positive effect of dietary protein may be related to stimulatory effects on rumen bacteria or neutralization of rumen pH.

At least for grass silage-based diets, most of the variation in OMD in dairy cows is related to dietary NDF concentration and NDF digestibility. This is consistent with almost complete true digestibility of ND solubles, and that the fecal output of MFOM was not significantly related to DMI or any other dietary parameter. However, with diets containing corn silage (Gabel et al., 2003) or cracked corn (Colucci et al., 1989), fecal output of starch per kg DMI increased as feed intake increased. Therefore with diets containing less digestible starch sources such as corn and sorghum, incomplete and variable starch digestibility should be considered in predicting digestibility of diets fed to dairy cows.

OMD = 622(±11) + 0.390(±0.016) × NDFD (g/kg) – 0.332(±0.016) × NDF (g/kg/ DM); RMSE = 6.9

Table 4. The multiple mixed model regression equation predicting NDF digestibility (g/kg) in cows (RMSE = 15.1).

Item	Unit[1]	Estimate	SE[2]	P-value
Intercept		-71	91	0.44
pdNDF/NDF	g/kg NDF	0.78	0.10	<0.0001
DMI	kg/d	-5.7	1.22	<0.0001
NFC[3]/NDF		66	38	0.08
$(NFC/NDF)^2$		-124	22	<0.0001
CP	g/kg DM	0.64	0.10	<0.0001
FNDF[4]/NDF		67	21.2	0.002
CFat[5]	kg/d	-32	16.4	0.05

[1]Unit of independent variable
[2]Standard error
[3]NFC = non-fiber carbohydrates (OM – NDF – Fat – CP)
[4]FNDF = forage NDF (kg/ diet DM)
[5]Concentrate fat intake

The parameter values in the global regression model predicting OMD from dietary NDF concentration and NDFD were almost similar to those estimated by the mixed model analysis taking into account the random study effect.

OMD = 587(±10) + 0.434(±0.012) × NDFD (g/kg) – 0.314(±0.019) × NDF (g/kg/ DM); RMSE = 16.2; R^2 = 0.79

Discount factors

Both NRC and CNPCS models overestimated the total discount in TDN_p intake (Figure 4). The difference between observed TDN intake in cows (TDN_p) and TDN estimated at maintenance intake was 0.75 kg/d, whereas the NRC and CNCPS systems predicted differences of 1.26 and 1.46 kg/d, respectively. Mean square errors were 0.59 and 0.88 kg/d with the mean bias, slope and random errors accounting for proportionally 0.86, 0.06 and 0.08 of the error variance for the CNCPS system, and 0.88, 0.09 and 0.03 for the NRC system, respectively. In the CNCPS system the prediction error (observed – predicted) was significantly (P=0.001) and positively related to the proportion of concentrate in the diet. This indicates that CNCPS overestimated more the reduction in TDN concentration with increasing intake for high forage compared to high concentrate diets. The CNCPS estimates separately TDN_p concentrations with increased intake for forage and concentrate portions of the diet. In the present data, estimated decreases in TDN_p were 19.6 and 32.4 g/kg DM for average concentrates and forages (TDN_m 799 and 709 g/kg DM). Greater discount for forages in the CNCPS is in disagreement with NRC (2001) and our two-factor (OMD_m + TDMI) OMD-equation, which predicts greater depressions in digestibility for high TDN_m diets.

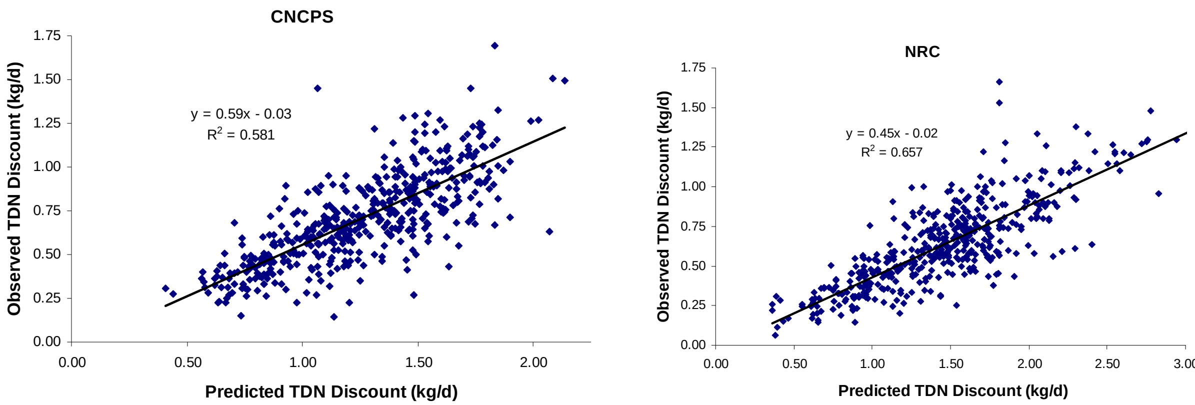

Figure 4. Relationships between predicted and observed TDN discounts (n = 470). The values are adjusted for random study effects.

Overestimation of the TDN discounts by both the CNCPS and NRC systems could partly be related to the nature of diets fed to the cows. Only small proportion of the diets included in the present dataset contained corn grain or corn silage. Digestibility of starch decreases with increased intake as discussed before, which can cause greater depression in digestibility with the typical U.S dairy diets. However, the difference between the NRC (2001) predicted and observed TDN discount increased 0.102 kg per kg increase in DMI. Assuming values of 300 g/kg DM and 90% for dietary starch concentrations and digestibility fecal starch output would increase 0.03 kg per kg DMI, i.e. much less than the difference between NRC predicted and observed TND discount.

CONCLUSIONS

Digestibility of diets fed to dairy cows is influenced by intrinsic characteristics of feeds, especially characteristics of plant cell wall fraction. Intrinsic digestibility can be described as the potential extent and rate of digestion in ideal conditions when only substrate characteristics limit digestion. In practice, digestibility determined at maintenance intake can be considered as intrinsic digestibility of the feedstuff, although potential the NDF digestibility is not totally reached. When the feeds are fed to dairy cows at high intake, different extrinsic factors influencing both digestion and passage rates have a strong influence on digestibility. As a result of increased passage rate digestibility is depressed with increased intake with the responses being linear or slightly increasing with intake. When non-fiber carbohydrates replace fiber in the diet, microbial populations could shift away from those responsible for optimal fiber digestion. Similarly, increased concentrate fat intake is inhibitory to cellulolytic bacteria, whereas supplementary protein stimulates fiber digestion. The reasons for improved fiber digestion can be variable and dependent on dietary circumstances. The two-variable model indicated that the depression in digestibility with increased intake become greater as the digestibility determined at maintenance intake increases. Validation of the NRC and CNCPS models estimating digestibility discounts suggested overestimation of the depression in digestibility as the intake is increased. This may partly be because the majority of the diets in our dataset did not contain corn silage or corn grain, of which starch digestibility decreases as intake increases. Most of the variation in digestibility of grass silage based diets is related to variation in dietary NDF concentration and digestibility, since the true digestibility of ND solubles was almost complete.

REFERENCES

Colucci, P. E., G. K. MacLeod, W. L. Grovum, L. W., Cahill, and I. McMillan. 1989. Comparative digestion in sheep and cattle fed different forage to concentrate ratios at high and low intakes. J. Dairy Sci. 72:1774-1785.

Colucci, P. E., L. E Chase, and P. J. Van Soest. 1982. Feed intake, apparent diet digestibility, and rate of particulate passage in dairy cattle. J. Dairy Sci. 65:1445-1456.

Colucci, P. E., G. K. MacLeod, W. L. Grovum, I. McMillan and D. J. Barney. 1990. Digesta kinetics in sheep and cattle fed diets with different forage to concentrate ratios at high and low intakes. J. Dairy Sci. 73:2143-2156.

Gabel, M., B. Pieper, K. Friedel, M. Radke, A. Hagemann, J. Voigt,and S. Kuhla. 2003. Influence of nutrition level on digestibility in high yielding cows and effects on energy evaluation systems J. Dairy Sci. 86:3992–3998.

Fox, G.G., P. Tylutki, L.O. Tedeschi, M.E. Van Amburg, L.E. Chase, A.N. Pell, T.R. Overton,and J.B. Russel. 2003. The net carbohydrate and protein system for evaluating herd nutrition and nutrient excretion. Version 5.0. Animal Science Department Mimeo 213. Cornell University, Ithaca, NY, USA.

Huhtanen, P., J. Nousiainen, and M. Rinne. 2006. Recent developments in forage evaluation with special reference to practical applications. Agric. Food Sci. 15:293-323.

Littel, R. C., G. A. Milliken, W. W. Stroup, and R. D. Wolfinger. 1996. SAS® System for Mixed Models. SAS Institute Inc., Cary, NC, USA.

Mertens, D.R. 1993. Kinetics of cell wall digestion and passage in ruminants. In: Jung, H.G. et al. (eds.). Forage cell wall structure and digestibility. American Society of Agronomy. p. 535–570.

MTT 2006. *Rehutaulukot ja ruokintasuositukset* (Feed tables and feeding recommendations). Jokioinen: MTT Agrifood Research Finland. Updated 14 Feb 2006.. Available on the Internet: http://www.mtt.fi/rehutaulukot/. URN:NBN:fi-fe20041449.

Nousiainen, J., M. Rinne, M. Hellämäki, and P. Huhtanen. 2003. Prediction of the digestibility of the primary growth of grass silages harvested at different stages of maturity from chemical composition and pepsin cellulase solubility. Anim. Feed Sci. Technol. 103:97-111.

NRC, 2001. National Research Council. Nutrient Requirements of Dairy Cattle. 7th revised Edition. National Academy Press, Washington, DC. pp. 381.

Oldham, J. D. 1984. Protein energy relationships in dairy cows. J. Dairy Sci. 67:1090-1114.

Tilley, J., and R. Terry. 1963. A two-stage technique for the in vitro digestion of forage crops. J. Br. Grassl. Soc.18:104-111.

Tyrrel, H. F., and P. W. Moe. 1975. Effect of intake on digestive efficiency. J. Dairy Sci. 58:1151-1163.

Van Keulen, J., and B.A. Young. 1977. Acid insoluble ash as marker for digestibility studies. J. Anim. Sci. 44:282-287.

Van Soest, P.J., M.E. Van Amburgh, J.B. Robertson, and W.F. Knaus. 2005. Validation of the 2.4 times lignin factor for ultimate extent of NDF digestion, and curve peeling rate of fermentation curves into pools. In: Proceedings Cornell Nutrition Conference for Feed Manufacturers. 24–26 Oct 2005. East Syracuse, NY. p. 139–149.

Vandehaar, M.J. 1998. Efficiency of nutrient use and relationship to profitability of dairy farms. J. Dairy Sci., 81:272-282.

Woods, V. B., A. P. Moloney, F. J. Mulligan, M. J. Kenny, and F. P. O’Mara. 1999. The effect of animal species (cattle or sheep) and level of intake on in vivo digestibility of concentrate ingredients. Anim. Feed. Sci. Technol. 80:135-150.