A meta-analysis of feed digestion in dairy cows. 1. The effects of forage and concentrate factors on total diet digestibility

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ABSTRACT

A meta-analysis based on published experiments with lactating dairy cows was conducted to study the effects of dietary forage and concentrate factors on apparent total diet digestibility. A data set was collected that included a total of 497 dietary treatment means from 92 studies. The diets were based on grass silage or on legume or whole-crop cereal silages partly or completely substituted for grass silage. The silages were supplemented with concentrates given at a flat rate within a dietary comparison. For the statistical evaluation, the data were divided into 5 subsets to quantify silage (digestibility, 42 diets in 17 studies; fermentation characteristics, 108 diets in 39 studies) and concentrate (amount of supplementation, 142 diets in 59 studies; concentration of crude protein, 215 diets in 82 studies; carbohydrate composition, 66 diets in 23 studies) factors on total diet digestibility. The diet digestibility of dairy cows was determined by total fecal collection or by using acid-insoluble ash as an internal marker. Diet organic matter digestibility (OMD) at a maintenance level of feeding (OMD_m) was estimated using sheep in vivo or corresponding in vitro digestibility values for the forage and reported ingredient and chemical composition values, with tabulated digestibility coefficients for the concentrate components of the diet. A mixed model regression analysis was used to detect the responses of different dietary factors on apparent total diet digestibility. Improved silage OMD_m resulting from earlier harvest was translated into improved production-level OMD in cows (OMD_p). The effects of silage fermentation characteristics on OMD_p were quantitatively small, although sometimes significant. Concentrate supple-

mentation improved total diet OMD_m, but this was not realized in lactating dairy cows because of linearly decreased neutral detergent fiber (NDF) digestibility as concentrate intake increased. Increasing the concentrate crude protein amount quadratically improved OMD_p in cows, with the response being mostly due to improved NDF digestibility. Replacement of starchy concentrates with fibrous by-products slightly decreased OMD_p but tended to improve NDF digestibility. The true digestibility of cell solubles (OM – NDF) estimated by the Lucas test both from all data and from the data subsets was not significantly different from 1.00, suggesting that responses in OMD_p of dairy cows are mediated through changes in the concentration and digestibility of NDF.

Key words: digestibility, diet composition, dairy cow, grass silage

INTRODUCTION

Diet digestibility is determined by intrinsic properties of the 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). The ultimate extent of fiber digestion cannot be reached in animals (Huhtanen et al., 2006), but as defined by Mertens (1993), a digestibility trial with sheep at a maintenance level of feeding gives the most consistent assessment of the intrinsic feed digestibility when eliminating animal influence (selection, intake level) and associative effects between dietary components. Consequently, in most current feed evaluation systems for dairy cattle, whole-diet digestibility is calculated from tabulated coefficients of individual dietary ingredients, which typically are determined with sheep fed at a maintenance level of feeding (INRA, 1989; Agricultural and Food Research Council, 1990; Yan et al., 2002). Alternatively, maintenance feed values can be derived from prediction equations by using chemical feed parameters or in vitro degradation data (Ministry of Agriculture, Fisheries and Food, 1975). When applied to mixed diets of dairy cows, this approach

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assumes that the digestion coefficients are equivalent between sheep and cattle and are additive, with no interactions between dietary components. However, research evidence indicates that these assumptions may not be valid, resulting in biased estimates of nutrient availability for dairy cows fed mixed diets at multiple levels of maintenance (Agricultural and Food Research Council, 1990; Colucci et al., 1990; Yan et al., 2002).

Many dietary factors and their interactions affect the ruminal fermentation, digestibility, and metabolism of the total diet in dairy cows. These include both forage (e.g., stage of maturity at harvest, fermentation characteristics) and concentrate [e.g., amount, concentrations of CP and fat, carbohydrate (**CHO**) composition] feeding factors. These associative effects between dietary components are usually negative and related to depressions in cell wall digestibility with increased concentrate feeding (Mould et al., 1983). The fundamental consequence of the negative associative effects is that digestibility of a feed mixture is less than that of the sum of the individual component feeds. There are also indications of positive associative effects, which typically result from supplementation of a nutrient that may restrict ruminal digestion and metabolism, for example, protein (Oldham, 1984). However, positive associative responses have not been quantified properly, and hence are not yet considered in any feed evaluation system. Quantitatively managing the associative effects on digestibility between dietary components would obviously be valuable both when improving the existing feed evaluation systems and in practical ration planning for dairy cows.

The objective of the present study was to estimate the effect of specific dietary factors (silage digestibility and fermentation quality, and amount and composition of concentrate supplementation) influencing diet digestibility in dairy cows by using data from production experiments in dairy cows. The meta-analysis presented herein focuses merely on grass silage-based diets, which are typically used in Northern Europe. The analysis uses specific data from dairy cow feeding trials in which confounding factors are minimized and only the feeding factors in question are varied. With this approach, we aimed to detect quantitatively the mechanisms of interaction of feed digestion in dairy cows. The digestibility values determined with sheep fed at a maintenance level of intake were used to describe the intrinsic digestibility (i.e., digestibility in vivo in optimal conditions; see Mertens, 1993) of the diet. In a companion paper (Huhtanen et al., 2009), these data are used to develop practical models predicting diet digestibility in cows fed mixed diets and digestibility discount factors for feeding-level effects.

MATERIALS AND METHODS

Data

Treatment mean data were collected from feeding trials with lactating dairy cows fed ad libitum either grass silage or legume (mainly red clover) or whole-crop silages (barley, wheat, or corn) partly or completely substituted for grass silage. The silages were supplemented with concentrate feeds differing both in amount and composition but offered at a flat rate within a single dietary comparison. 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, and NDF; fermentation acids; digestibility), and concentrate characterization (ingredients and concentrations of CP, fat, and NDF) were reported. Diet digestibility in cows was determined by the total fecal collection method ($n = 176$ diets) or by using acid-insoluble ash (**AIA**), determined as described by Van Keulen and Young (1977), as an internal marker ($n = 321$).

The total data included 497 diets in 92 studies (see the Appendix). In addition to the analysis of the whole data set, the data were divided into 5 subsets specifically to estimate the effects of silage digestibility (42 diets, 17 studies), silage fermentation characteristics (108 diets, 39 studies), amount of concentrate supplementation (142 diets, 59 studies), concentrate CP concentration (215 diets, 82 studies), and concentrate CHO composition (66 diets, 23 studies). Silage digestibility was influenced by the stage of maturity at harvest, silage fermentation quality by the type and amount of additive applications, concentrate CP amount by substituting protein supplements such as rapeseed (canola) feeds, soybean meal, or fishmeal for energy supplements (typically grain), and concentrate CHO composition by substituting fibrous by-products (e.g., sugar beet pulp and wheat middlings) for cereal grains.

Calculations

Diet OM digestibility (**OMD**) at a maintenance level of feeding (**OMD_m**) was determined using in vivo or in vitro digestibility for the forage component of the diet. In vivo digestibility was measured in sheep fed at a maintenance level of feeding using the total fecal collection method. In vitro digestibility was measured using rumen fluid (Tilley and Terry, 1963) or pepsin-cellulase incubations (Nousiainen et al., 2003). Silage digestibility estimated by the in vitro pepsin-cellulase method was corrected using forage type-specific equations (Huhtanen et al., 2006). Digestibility coefficients

were presented as grams per kilogram to produce regression coefficients with a convenient number of decimals. When silage indigestible NDF (**iNDF**) concentrations were not reported, the estimates were derived from regression equations based on Finnish data sets (Huhtanen et al., 2006). Digestibility (OMD_m) of the concentrate component of the diet was estimated from ingredient composition, analyzed chemical composition, and tabulated 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 values derived from unpublished MTT data sets. For the most common and quantitatively the most important concentrate ingredients, either one of these values was available. For some concentrate ingredients, the values were based on analyzed lignin concentration or lignin concentration published by NRC (2001) and in the Cornell Net Carbohydrate and Protein System feed libraries (Fox et al. 2003). The concentration of iNDF was estimated as 2.4 times the lignin concentration (Van Soest et al., 2005). Fecal metabolic OM was calculated as fecal OM – fecal NDF, and it was expressed as grams per kilogram of DMI. Fecal OM output was divided into 3 fractions: fecal iNDF, fecal potentially digestible NDF (**pdNDF**), and fecal neutral detergent solubles (**NDS**; calculated as $\text{OM} - \text{NDF}$). By definition, fecal iNDF per kilogram of DMI was assumed to be the same as dietary concentration. Fecal pdNDF (g/kg of DMI) was calculated as fecal NDF (g/kg of DMI) – iNDF (g/kg of DMI). The Lucas test (regression of the intake of the digestible dietary entity on the intake of the respective entity; see Van Soest, 1994) was performed for CP and NDS.

Statistical Analysis

Data were analyzed using the mixed model procedure of SAS (Littell et al., 1996). The model was $Y = B_0 + B_1X_{1ij} + b_0 + b_1X_{1ij} + B_2X_{2ij} + \dots + B_nX_{nij} + e_{ij}$, where $B_0, B_1X_{1ij}, B_2X_{2ij}, \dots, X_{nij}$ are the fixed effects and b_0, b_1 , and e_{ij} are the random experiment effects (intercept and slope), where $i = 1 \dots n$ studies and $j = 1 \dots n_i$ values. The subsets were analyzed separately to investigate the effects of single dietary factors on the digestibility of a dietary component when only this specific factor was variable in the experimental design (e.g., the amount of concentrate feeds, or the forage maturity at harvest).

To analyze interactions between the dietary factors on digestibility [such as OMD or NDF digestibility (**NDFD**)], digestibility estimated at a maintenance level of feeding and the ratio of pdNDF to NDF were used as random factors in the model. The ratio of pdNDF

to NDF was computed as $(\text{NDF} - \text{iNDF})/\text{NDF}$. The best-fit model was chosen based on the lowest residual mean square error (**RMSE**) and Akaike's information criterion (**AIC**). In the tables, RMSE values are adjusted for the random study effect. The rationale for and further details on using a mixed model analysis to integrate quantitative findings from multiple studies are described by St-Pierre (2001).

RESULTS

Data

The data displayed a large variation in intake, production, digestibility, and diet composition (Table 1). The proportion of concentrate was on average 0.41, ranging from 0 to 0.80. The mean NDFD was high, as is typical for Northern European diets based on grass silage. The standard deviation of NDFD was almost 2-fold that of OMD at a production level (**OMD_p**). Metabolic fecal output was on average 96 g/kg of DMI. When the random effect of trial was taken in account, the standard deviation was much smaller (6.9) than the overall standard deviation (14.6), which probably at least partly reflected differences in the analytical methods used to divide fecal OM between NDF and NDS.

Silage Maturity

As expected, higher silage digestibility associated with harvesting grass at an earlier stage of maturity affected the whole-diet OMD_p (Table 2). The effect was strongest on NDFD, but the pdNDF digestibility also increased with improved silage digestibility. Silage iNDF concentration predicted OMD_p in cows slightly better ($\text{RMSE} = 6.0$ g/kg; model not shown) than silage OMD_m . In the silage maturity data subset, the true digestibility of the NDS fraction estimated by the Lucas test was not significantly different from 1.00, whereas the respective value for true CP digestibility (0.876) was smaller ($P < 0.001$) than 1.00. Of the 3 fecal components, only iNDF decreased with increased silage OMD_m , whereas outputs of pdNDF and metabolic fecal OM (**MFOM**) per kilogram of DMI (mean 92.4 g/kg of DMI) were not significantly influenced.

Silage Fermentation Quality

Diet OMD_p decreased ($P < 0.05$) as the concentration of water-soluble carbohydrates (**WSC**) in silage increased (Table 3). The effect can mainly be attributed to reduced NDF and pdNDF digestibility. The effect of silage WSC concentration on diet digestibility was greater than that of total acids (**TA**), probably because

Table 1. Statistical description of the variables used in the data file for prediction of digestibility in dairy cows fed grass silage-based diets

Item	n	Mean	SD	Minimum	Maximum
Intake (kg of DM/d)					
Forage	497	10.9	1.93	4.6	17.4
Concentrate	497	7.7	2.47	0.0	18.4
Total	497	18.6	2.98	9.9	25.2
Production (kg/d)					
Milk	497	26.7	5.06	13.0	45.8
ECM	497	27.4	5.33	12.8	42.1
Digestibility (g/kg)					
OM	497	736	38.1	621	830
CP	471	691	41.1	542	782
NDF	388	627	71.8	408	830
pdNDF ¹	388	754	63.3	506	946
MFOM ² (g/kg of DMI)	388	96	14.6	61	147
Diet composition (g/kg of DM)					
CP	497	166	20.0	111	229
NFC	497	293	50.0	80	419
NDF	497	419	47.0	283	559
Indigestible NDF	497	68	22.4	19	148
Crude fat	497	44	10.1	22	112
Starch	497	141	56.3	0	292
Silage WSC ³	406	54	40.0	1	175
Silage TA ⁴	451	76	33.7	7	218
OMD _m ⁵ (g/kg)	497	774	31.5	658	846

¹pdNDF = potentially digestible NDF.²MFOM = metabolic fecal OM (calculated as OM – NDF).³WSC = water-soluble carbohydrates.⁴TA = total acids.⁵OMD_m = OM digestibility determined at a maintenance level of feeding in sheep or using a corresponding in vitro method.

VFA included in the TA had a negative effect on digestibility. The positive slope of lactic acid (0.08; model not shown) was numerically the same as the negative slope of WSC (–0.08). Although the differences were statistically significant, the magnitude of the responses was small. The effect of the extent silage fermentation on MFOM output (mean 96.6 g/kg of DMI; SE 1.67) was relatively small, although the slope of the Lucas equation for TA was significant.

Amount of Concentrate Supplementation

Increasing the amount of concentrate DMI (**CDMI**) had no effect on OMD_p of the total diet (Table 4). However, OMD decreased in cows when OMD_m was included as a second independent variable in the model. Consistent with this result, OMD_p in dairy cows increased by only 35% (i.e., the slope when using the random effect of trial in the model) of the expected

Table 2. Effects of silage OM digestibility determined at a maintenance level of feeding¹ on apparent diet digestibility, predicted true digestibility (slope of the Lucas test), and metabolic fecal OM (MFOM) production in lactating dairy cows

Response in dairy cows	n	Intercept	SE	Slope	SE	P-value	RMSE ²	AIC ³
Digestibility (g/kg)								
OM	44	372	42.4	0.515	0.050	<0.001	6.6	379.6
CP	36	461	45.8	0.308	0.053	<0.001	8.8	305.3
NDF	36	115	51.0	0.757	0.060	<0.001	8.2	342.4
pdNDF ⁴	36	634	45.3	0.202	0.057	0.002	12.3	350.7
Lucas test								
NDS ⁵	36	–82.8	29.0	0.973	0.056	<0.001	3.5	265.4
CP	36	–29.4	5.0	0.876	0.027	<0.001	0.7	161.3
MFOM (g/kg of DMI)	36	79.6	24.6	0.022	0.037	0.57	2.8	264.1

¹Determined with sheep at a maintenance level of feeding or with a corresponding in vitro method.²RMSE = residual mean square error.³AIC = Akaike's information criterion.⁴pdNDF = potentially digestible NDF (calculated as NDF – indigestible NDF).⁵NDS = neutral detergent solubles (calculated as OM – NDF).

Table 3. Effects of silage total acid (TA) and water-soluble carbohydrate (WSC) concentrations (g/kg of DM) on apparent diet digestibility and metabolic fecal OM (MFOM) output in lactating dairy cows

Response in dairy cows	n	X ₁	Intercept	SE	Slope ₁	SE	P-value	RMSE ¹	AIC ²
Digestibility									
OM	92	TA	726	5.0	0.03	0.044	0.53	5.9	772.9
OM	92	WSC	734	7.7	-0.08	0.033	0.02	7.3	770.5
CP	92	TA	681	8.1	0.14	0.045	0.004	8.5	825.8
CP	92	WSC	703	9.8	-0.15	0.038	<0.001	9.6	822.1
NDF	80	TA	617	11.3	0.18	0.060	0.005	12.3	764.2
NDF	80	WSC	646	12.6	-0.22	0.055	<0.001	12.8	762.6
pdNDF ³	80	TA	721	9.4	0.27	0.080	0.003	14.0	754.9
pdNDF	80	WSC	763	13.5	-0.32	0.079	0.001	12.8	747.0
MFOM ⁴ (g/kg of DMI)	80	TA	91	2.8	0.071	0.031	0.03	4.1	580.6
MFOM (g/kg of DMI)	80	WSC	99	4.1	-0.033	0.020	0.12	6.7	583.6

¹RMSE = residual mean square error.²AIC = Akaike's information criterion.³pdNDF = potentially digestible NDF (calculated as NDF - indigestible NDF).⁴MFOM = metabolic fecal OM (calculated as fecal OM - NDF).

improvements in diet digestibility as concentrate intake increased. Digestibility of both NDF and pdNDF decreased ($P < 0.001$) with increased concentrate supplementation. When the ratio of pdNDF to NDF was used with CDMI in the model to describe the quality of dietary fiber within the study, the slope of CDMI decreased from -10.3 to -7.9.

Increasing the amount of concentrate had no effect on CP digestibility, and the differences were related to changes in dietary CP concentration rather than to the amount of concentrate. Intake of digestible OM increased ($P < 0.001$) with concentrate supplementation, but the response tended to diminish at high levels of supplementation, as indicated by a negative quadratic term. The Lucas test showed that NDS were completely digestible when the amount of concentrates was increased. The output of MFOM averaged 91.4 (SE 1.42)

g/kg of DMI, and it was not influenced by the level of concentrate supplementation.

Protein Supplementation

Increasing dietary CP concentration by replacing energy supplements (generally cereal grains or fibrous by-products) with protein supplements was associated with improved diet digestibility (Table 5). The effect tended ($P = 0.06$) to be quadratic, with predicted maximum digestibility at a dietary CP concentration of 220 g/kg of DM. Although the effect was highly significant, quantitatively the improvements in diet OMD_p were rather small. Improvements in the OMD_p with increased protein supplementation were related to increased NDF and CP digestibility. True digestibility of the incremental CP estimated by the Lucas test was

Table 4. Effects of concentrate DMI (CDMI, kg/d) on apparent diet digestibility, true digestibility of neutral detergent solubles (NDS, g/kg), and metabolic fecal OM (MFOM) output (g/kg of DMI) in lactating dairy cows

Item	X ₁	X ₂	n	Intercept	SE	Slope ₁	SE	P-value	Slope ₂	SE	P-value	RMSE ¹	AIC ²
Digestibility (g/kg)													
OM	CDMI		142	721	4.9	-0.27	0.35	0.43				7.54	1,321.8
OM	CDMI	OMD _m ³	142	119	67.3	-3.2	0.60	<0.001	0.83	0.09	<0.001	5.58	1,208.4
CP	CDMI		125	700	6.6	-0.22	0.61	0.72				5.36	1,121.3
NDF	CDMI		110	717	10.2	-10.3	0.79	<0.001				11.3	1,091.8
NDF	CDMI	pdNDF ⁴ /NDF	110	-208	121.5	-7.9	0.63	<0.001	1.09	0.14	<0.0001	12.0	1,054.8
pdNDF	CDMI		110	842	10.2	-10.3	0.82	<0.001				13.6	1,099.8
Lucas test													
dNDS ⁵ (g/kg of DM)	NDS		110	-88.4	11.8	0.99	0.024	<0.001				5.12	865.1
MFOM ⁶ (g/kg of DMI)	CDMI		110	94.3	3.69	-0.33	0.32	0.30				4.80	855.2

¹RMSE = residual mean square error.²AIC = Akaike's information criterion.³OM digestibility determined at a maintenance level of feeding in sheep or with a corresponding in vitro method (g/kg).⁴pdNDF = potentially digestible NDF (calculated as NDF - indigestible NDF).⁵dNDS = digestible neutral detergent solubles.⁶MFOM = metabolic fecal OM (calculated as fecal OM - NDF).

Table 5. Effects of protein supplementation (energy supplement partly replaced with protein supplements; diet CP concentration as g/kg of DM) on apparent diet digestibility in lactating dairy cows

Item	X ₁	X ₂	n	Intercept	SE	Slope ₁	SE	P-value	Slope ₂	SE	P-value	RMSE ¹	AIC ²
Digestibility													
OM	CP		224	689	7.2	0.28	0.037	<0.001				4.0	1,817.6
OM	Ln CP		224	504	29.8	45.5	5.8	<0.001				4.0	1,805.4
OM	CP	CP × CP	224	638	27.0	0.93	0.330	0.01	−0.002	0.0010	0.06	3.9	1,826.5
CP	CP		220	457	13.6	1.47	0.076	<0.001				6.7	1,932.6
CP	Ln CP		220	−492	56.5	233.4	10.9	<0.001				6.4	1,898.8
CP	CP	CP × CP	220	169	48.2	5.09	0.589	<0.001	−0.011	0.002	<0.001	6.3	1,909.2
NDF	CP		182	508	14.3	0.77	0.077	<0.001				9.5	1,698.1
NDF	Ln CP		182	20.8	60.8	120.5	11.9	<0.001				9.5	1,687.6
pdNDF ³	CP		182	585	17.0	1.15	0.100	<0.001				12.5	1,757.7
pdNDF	Ln CP		182	−141	181	179	35.8	<0.001				8.7	1,810.7
Lucas test													
dCP ⁴	CP		220	−36.2	1.65	0.92	0.010	<0.001				1.0	1,090.0

¹RMSE = residual mean square error.²AIC = Akaike's information criterion.³pdNDF = potentially digestible NDF (calculated as NDF − indigestible NDF).⁴dCP = digestible CP.

0.92. The mean MFOM output was 95.4 (SE 0.91) g/kg of DMI, and it was not significantly influenced by dietary CP concentration.

Concentrate CHO Composition

Digestibility of OM decreased when starchy ingredients (generally cereal grains) were replaced with fibrous by-products (Table 6). Consequently, concentrate starch content was positively related to OMD_p. However, the goodness of fit was better on the basis of RMSE and Akaike's information criterion for concentrate NDF compared with starch concentration. Digestibility of CP decreased ($P = 0.001$), and that of NDF and pdNDF tended ($P = 0.06$) to increase with an increasing amount of concentrate NDF. Concentrate carbohydrate composition had no influence on MFOM output (mean 105.0 g/kg of DMI, SE 0.69), but fecal

output of both iNDF ($P = 0.03$) and pdNDF ($P < 0.01$) increased slightly as the amount of concentrate NDF increased. The coefficient of the Lucas equation was not significantly different from 1.00.

DISCUSSION

The total diet OMD_p in dairy cows was determined either by total fecal collection or by using AIA as an internal marker in the present data set. The relationship between OMD_p estimates based on total fecal collection or AIA in 5 studies including 21 diets was good, as indicated by a high R² value (0.82) despite a relatively narrow range of data. The mean OMD_p values estimated by total fecal collection and AIA were 736 versus 731 g/kg, with a mean squared prediction error of 16 g/kg. In 15 studies, the mean residual standard deviation of OMD_p was on average 13 g/kg, ranging from 5 to 20

Table 6. Effects of concentrate carbohydrate composition on apparent diet digestibility in lactating dairy cows

Response in dairy cows	X ₁	n	Intercept	SE	Slope ₁	SE	P-value	RMSE ¹	AIC ²
Digestibility (g/kg)									
OM	CNDF ³	66	769	11.2	−0.091	0.031	0.007	3.5	575.5
OM	CSta ⁴	66	729	11.1	0.053	0.019	0.011	7.6	594.3
CP	CNDF	66	711	11.6	−0.077	0.019	0.001	8.3	596.1
NDF	CNDF	38	604	29.0	0.178	0.084	0.055	8.4	381.3
pdNDF ⁵	CNDF	38	736	24.4	0.135	0.063	0.055	8.4	366.4
Lucas test									
Digestible NDS ⁶	NDS	38	−128	15.1	1.034	0.029	<0.001	5.3	291.7

¹RMSE = residual mean square error.²AIC = Akaike's information criterion.³CNDF = concentrate NDF concentration (g/kg of DM).⁴CSta = concentrate starch concentration (g/kg of DM).⁵pdNDF = potentially digestible NDF (calculated as NDF − indigestible NDF).⁶NDS = neutral detergent solubles (calculated as OM − NDF).

g/kg. The mean value is similar to that in sheep studies using total fecal collection (Huhtanen et al., 2006) and was below the 20 g/kg suggested by Van Soest (1994) as being the standard deviation of digestibility determination in carefully conducted experiments.

Digestibility of dairy cow diets is influenced by intrinsic digestion characteristics of the diet and extrinsic factors that influence the extent to which the intrinsic digestion potential is achieved. Digestibility of pdNDF in the present data set (0.754) was lower than that (0.85) in sheep fed grass silages alone at a maintenance level of feeding (Huhtanen et al., 2006). Metabolic fecal output in the sheep data (Huhtanen et al., 2006) was similar to the values in the present study (100 vs. 96 g/kg of DM). Because the digestibility of iNDF is zero by definition, most of the differences in diet digestibility between sheep fed at a maintenance level and dairy cows fed at a production level can be attributed to pdNDF digestibility, at least when the diets are based mainly on grass silage and small grain-based concentrates.

Effects of Silage Digestibility and Fermentation Quality

The response of silage digestibility on OMD_p in cows was 0.52 units per 1 unit in silage OMD_m as determined with sheep at a maintenance level of feed intake. Because the mean proportion of forage in the diet of lactating dairy cows was on average 0.60, the effect of silage digestibility on OMD_p of the mixed diet was comparable with that observed in sheep fed at maintenance. This is in agreement with the results of Gordon et al. (1995) and Rinne et al. (2002), who reported a close correspondence between silage OMD_m as determined with sheep fed at maintenance and whole-diet OMD_p in cattle fed mixed diets at multiple levels of maintenance. The improved apparent OMD_p in dairy cows can be attributed to the reduced NDF concentration of the diet and improved NDFD, whereas the MFOM output was not related to silage digestibility.

Improved total diet NDFD with improved silage digestibility is mainly derived from the reduced iNDF concentration. However, increased pdNDF digestibility with improved silage digestibility suggests a faster rate of pdNDF digestion in silages harvested at early rather than late stages of maturity. A faster digestion rate of NDF in early-harvested grasses has been demonstrated using in situ (Cone et al., 1999; Rinne et al., 2002), rumen evacuation (Bosch and Bruining, 1995; Rinne et al., 2002), and in vitro gas production techniques (Cone et al., 1999). A longer rumen residence time of early-harvested silages compared with late-harvested silages (Bosch and Bruining, 1995; Rinne et al., 2002) could also contribute to improved pdNDF digestibility of early-harvested grasses. Improved CP digestibility

with increased silage digestibility can be attributed to increased dietary CP concentration and a dilution of metabolic and endogenous fecal nitrogen. The true CP digestibility estimated by the Lucas test (0.88) was markedly lower than the corresponding value (0.97) estimated from our silage digestibility data from sheep (Huhtanen et al., 2006). This difference may be related to greater hindgut fermentation of early-harvested compared with late-harvested grass silages and an associated increase in metabolic fecal nitrogen.

The effects of the extent of silage fermentation on diet OMD_p were almost completely associated with the reduced NDFD of restrictively fermented silages because the changes in MFOM output were marginal (Table 3). The effect of silage fermentation on NDFD was significant, but quantitatively the responses were small, and significant effects have seldom been reported in single studies (Keady and Murphy, 1996; Heikkilä et al., 1998). Reduced NDFD can be attributed partly to an increased DMI of restrictively fermented silages (Huhtanen et al., 2007) and an associated increase in rumen passage rate, but it does not account for all the response. Indirect evidence from intraruminal infusion studies suggests that sugars depress NDFD (Rooke et al., 1987; Khalili and Huhtanen, 1991), whereas lactic acid had no effect on fiber digestion (Jaakkola and Huhtanen, 1992). The effects of silage fermentation on digestibility can partly counteract the effects of silage additives. Muck and Kung (1999) reported that inoculation improved the digestibility of silage DM in approximately one-third of the studies. Gordon (1989) reported a higher digestibility for inoculated silage in dairy cows compared with untreated and formic acid-treated silage, although no marked differences were observed in silage fermentation parameters.

The evidence from the present meta-analysis suggests that increased silage WSC concentration slightly depresses OMD because of reduced pdNDF digestion, but this evidently has only a minor contribution to the energy intake of dairy cows compared with the adverse effects of silage fermentation acids on silage DMI (Huhtanen et al., 2007). Different slopes for CP digestibility against TA and WSC concentrations could be related to rumen microbial protein synthesis. Fermentation of WCS in the silo to lactic acid and VFA has been associated with a reduced efficiency of microbial protein synthesis (Harrison et al. 2003), which will increase fecal CP excretion.

Effects of the Amount of Concentrate Supplementation

The lack of expected improvements in diet OMD_p with increased concentrate feeding in dairy cows clearly

indicates negative associative effects in digestion. Indeed, increased concentrate feeding depressed total diet OMD_p in dairy cows by 3.2 g/kg of DM when the higher intrinsic digestibility (OMD_m) was taken into account in the model. The present data agrees with the results of Tyrrell 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 digestibility of forages was lower than in our data set and the possibility for improved digestibility within increased concentrate feeding was greater. In agreement with this, Aston et al. (1994b) reported decreasing total diet OMD_p with high-quality grass silages in response to incremental concentrate supplementation, but with low-quality grass or lucerne silages, total diet OMD_p increased in a curvilinear manner. However, total diet NDFD was depressed with an increasing concentrate allowance irrespective of forage quality (Aston et al., 1994b).

The true digestibility of NDS was complete (0.99) and MFOM output (g/kg of DMI) was not influenced by increased concentrate feeding. Because dietary iNDF concentration decreased as CDMI increased, depression in pdNDF digestibility accounted for most of the negative associative effects on digestibility with increased concentrate feeding. Reduced NDFD resulted mainly from depression in pdNDF digestibility and partly from reduced potential NDF digestibility (0.0025 g/kg of CDMI); that is, a major part of the depression can be attributed to extrinsic factors.

Decreased pdNDF digestibility can result from reduced digestion rate, reduced ruminal residence time, or both. The evidence from in situ studies suggests that digestion rate of forage NDF is reduced with increased concentrate feeding. Lindberg (1981) studied the effects of oats fed proportionately at 0, 0.30, and 0.70 of diet DM on in situ digestion kinetics of forages. He found that an increased proportion of oats was associated with a decrease in both the rate and potential extent of NDF digestion. Similar effects were reported by Huhtanen and Jaakkola (1994), who incubated 6 grasses in the rumen of cattle fed grass silage or barn-dried hay with proportionally 0.25, 0.50, or 0.75 concentrates of diet DM.

The rumen evacuation-derived NDF digestion rate decreased with increased concentrate proportion in the diet (Huhtanen and Jaakkola, 1993; Stensig and Robinson, 1997). Reduced digestion rate was associated with a lower rumen pH and decreases in particle-associated enzyme activities. Stensig and Robinson (1997) supplemented an alfalfa or timothy silage diet with fiber-free concentrates at medium and high levels

(i.e., the depression in ruminal and total NDFD was entirely associated with forage fiber).

Both in situ and rumen evacuation studies suggest that reduced rumen pH, which inhibits cellulolytic microbes and depresses the rate of NDF digestion, is the main mechanism of observed negative associative effects of concentrate feeding on diet digestibility in dairy cows. Although the rumen retention time of forage particles seemed to decrease as CDMI increased (Colucci et al., 1990; Huhtanen and Jaakkola, 1993; Stensig and Robinson, 1997), increased rumen retention time was insufficient to compensate for the reduced digestion rate of pdNDF. Van Soest (1994) suggested that concentrate fiber contributes more to digestibility depression than forage fiber when concentrate is added to forage diets. This may be related to the faster passage rate of concentrate fiber typically present in small particles compared with forage fiber.

Effects of Protein Supplementation

The present meta-analysis demonstrated a positive effect of supplementary protein feeding on diet digestibility in dairy cows (Table 5). Quantitatively, the responses were not large; therefore, significant protein effects on digestibility have seldom been reported in single studies. The response was smaller (0.28 g/kg of OMD per 1 g/kg of DM increase in diet CP concentration) than the corresponding response of 0.70 in a meta-analysis reported by Oldham (1984). The smaller effect in the present analysis may be related to the use of rapeseed (canola) feeds, which contain more iNDF than soybean meal (Shingfield et al., 2003), as a protein supplement in a large number of studies. Oldham (1984) reported a slightly greater response to protein supplementation in DM digestibility in cows fed corn (*Zea mays*) silage compared with grass silage-based diets.

Several mechanisms may be associated with improved OMD_p in response to the replacement of energy supplements with protein supplements: overcoming a deficiency of RDP (1), a higher intrinsic rate and potential extent of fiber digestion of protein supplements (2), a buffering rumen pH and better rumen conditions for fiber digestion because of a reduced dietary starch concentration (3), and stimulation of cellulolytic bacteria by AA and peptides derived from supplementary protein (4).

The effect of protein supplementation on OMD was curvilinear, decreasing from 0.41 to 0.21 g/kg per 1 g/kg of DM when dietary CP concentration increased from 130 to 180 g/kg of DM, respectively. This implies that at least part of the response could be related to overcoming the limited supply of rumen-degradable nitrogen for rumen microbes. Soybean meal has some-

times increased diet digestibility more than isonitrogenous urea supplementation (Poos et al., 1979; Smith et al., 1980), suggesting that the form of nitrogen also may influence ruminal fiber digestion.

The concentration of iNDF increased ($P < 0.001$) with increased protein supplementation when the mixed model including the random study effect was used, precluding the notion that digestibility responses were attributed to improved intrinsic digestibility of the diet. Improved pdNDF digestibility can be related to increased digestion rate as dietary CP concentration is increased and, as a result, fecal output of pdNDF is decreased. The faster digestion rate could be attributed to reduced dietary starch concentration and associated improvements in rumen environment for fiber-degrading bacteria, differences in intrinsic NDF digestion rates between protein and energy supplements, and possible stimulatory effects of preformed AA and peptides on rumen cellulolytic bacteria.

Effects of Concentrate CHO Composition

Replacing starchy supplements with fibrous supplements has often improved NDFD in single studies (Mayne and Gordon, 1984; Huhtanen, 1988; Sutton et al., 1993). This effect has often been attributed to improved rumen cellulolytic activity associated with reduced starch content of the diet. A trend toward improved NDF and pdNDF digestibility with an elevated concentrate NDF concentration (Table 6) supports this hypothesis. However, in a ruminal *in situ* digestion study (Huhtanen, 1988), no difference in forage digestion kinetics was observed between barley and sugar beet pulp supplements.

Even though forage and total NDFD may be improved when starchy supplements are replaced with fibrous supplements, the overall effects on diet OMD_p are likely to be small or even negative, as indicated by the present meta-analysis (Table 6) and single studies (e.g., Aston et al., 1994a; Doherty and Mayne, 1996). At high levels of DMI, the depressions in NDFD of diets containing large proportions of fibrous concentrate ingredients can be a result of reduced concentrate fiber digestibility because of its fast passage rate. Woods et al. (1999) fed 6 different fibrous by-products to cattle at maintenance and at 2× maintenance, using high-concentrate diets (85% concentrate on DM basis). The average OMD_p decreased from 0.701 to 0.619 when the intake was doubled. The large depression in OMD_p is in contrast with the corresponding decreases of 0.030 and 0.022 in the studies of Huhtanen et al. (1995) with growing cattle and Volden (1999) with dairy cows, respectively. In these studies, the intakes of mixed diets

containing 40 or 60% of cereal grain-based concentrates were used.

Substituting cereal grains for fibrous by-products can produce positive associative effects in terms of improved forage digestibility and negative associative effects in terms of increased passage rate and decreased digestibility of concentrate fiber. Ipharraguerre and Clark (2003) suggested that positive associative effects with soyhulls are to be expected with diets with high concentrations of NFC and containing coarse forage, which alter the mat formation in the rumen and which in turn influence passage rate. Negative associative effects, on the other hand, are to be expected when soyhulls are used to replace forage in the diet.

CONCLUSIONS

The results of the present meta-analysis demonstrated that diet digestibility could be predicted accurately from values estimated with sheep fed at a maintenance level when different forage or concentrate factors of the diet were manipulated. Fecal output of metabolic matter was on average 96 g/kg of DMI and it was not influenced by any of the forage or concentrate feeding factors investigated. Consequently, most of the variation in diet digestibility in cows fed grass silage-based diets was related to dietary NDF concentration and NDFD. Improved forage intrinsic digestibility was almost totally realized in dairy cows fed at a production level of intake. Restricting silage fermentation decreased NDFD in cows, although the effect was quantitatively small. Increased concentrate feeding had significant negative associate effects on whole-diet digestibility mediated through decreased digestion of potentially digestible fiber. Increased concentrate protein feeding improved whole-diet digestibility in cows in a curvilinear manner because of positive effects on the ruminal environment for fiber digestion, but the magnitude of the effect was not very large.

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APPENDIX

List of Publications Describing the Experiments Used in the Data Analysis

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