Validation of an approach to predict total-tract fiber digestibility using a standardized in vitro technique for different diets fed to high-producing dairy cows

F. Lopes, K. Ruh, and D. K. Combs¹

Department of Dairy Science, University of Wisconsin, Madison 53706

ABSTRACT

The experimental objective was to validate an in vitro model to predict total-tract neutral detergent fiber (NDF) digestibility in dairy cattle. Twenty-one diets from 7 studies conducted at University of Wisconsin-Madison were analyzed for in vitro fiber digestibility. Forages varied among diets (corn, alfalfa, tall and meadow fescue, and wheat straw silages) and nutrient composition (ranges: NDF = 22.5 to 33.8%; crude protein = 15.8 to 18.9%; nonfiber carbohydrates = 38.0 to 51.0%). Total-tract NDF digestibility (TTNDFD) observed in in vivo trials was determined using different markers as described in the individual studies. The in vitro TTNDFD model predicted total-tract fiber digestibility from the proportion of total NDF potentially digestible (pdNDF), rate of pdNDF degradation, and rate of passage of pdNDF. The model predicted TTNDFD similar to in vivo measurements. The relationship between TTNDFD measured in vivo and TTNDFD predicted by the in vitro assay was significant ($R^2 = 0.68$). The relationship between in vitro 30-h NDF digestibility values and in vivo total-tract NDF digestibility values was not significant, whereas in vitro 48-h NDF digestibility values were correlated ($R^2 = 0.30$) with in vivo TTNDFD measurements. Indigestible NDF (iNDF) showed a negative relationship ($R^2 = 0.40$) with TTNDFD in vivo. Each 1-percentage-unit increase of iNDF resulted in a decrease of 0.96 percentage units of total-tract NDF digestibility; however, iNDF by itself was not a good predictor of TTNDFD because of the difference among the means. This study showed that an in vitro TTNDFD model that uses iNDF, pdNDF, and rates of pdNDF digestion and passage can predict ($R^2 = 0.68$) total-tract NDF digestibility. Most importantly, we demonstrated the ability to predict total-tract fiber digestibility from a model based on in vitro NDF degradation, which could

improve our ability to optimize forage utilization and milk production.

Key words: neutral detergent fiber (NDF), in vitro, total-tract NDF digestibility

INTRODUCTION

In vitro and in situ methods are widely used as an alternative to in vivo methods for estimating and comparing rumen fiber digestion. Oba and Allen (1999) reviewed several feeding studies with dairy cattle and concluded that a 1-percentage-unit change in in vitro or in situ NDF digestibility (NDFD) was correlated with a 0.17-kg increase in voluntary DMI and 0.25-kg increase in 4% FCM yield. Change in in situ or in vitro fiber digestibility within a study was correlated with intake and milk production, but no significant correlation was found between the absolute measures of fiber digestion and intake or milk yield across studies.

Most in vitro fiber digestion assays are based on the Tilley and Terry (1963) 2-stage method. Tilley and Terry (1963) reported that in vitro DM digestibility was correlated to in vivo DM digestibility in sheep fed at maintenance intake. In this procedure, samples are incubated anaerobically in rumen fluid for 48 h; then, the residue is incubated in a pepsin-HCl solution for 48 h. Van Soest et al. (1991) modified the Tilley and Terry method to measure the potential extent of NDF digestion. Presently, neither in vitro technique has been validated at ad libitum intake in dairy cattle. As a result, differences in in vitro NDFD measures between forages have little inference to in vivo measures of fiber digestion (Goesser, 2008). Allen (2011) suggests that fiber digestion rates determined from in vitro methods (traditional in vitro method; Van Soest et al., 1991) results in over-estimating in vivo fiber digestibility and that in vitro measures are useful only to compare relative differences of fiber digestibility among forages.

The objective of the present study was to validate an in vitro model used to predict total-tract NDF digestibility compared with in vivo measurements of total-tract NDF digestibility from 7 trials conducted at the University of Wisconsin-Madison.

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¹Corresponding author: dkcombs@wisc.edu

MATERIALS AND METHODS

All procedures involving animals were approved by the University of Wisconsin College of Agriculture and Life Science animal care and use committee.

Twenty-one diets from 7 studies conducted from 2009 to 2013 by researchers from the Dairy Science department at University of Wisconsin–Madison were used to validate an approach to predict total-tract NDF digestibility (**TTNDFD**; Combs, 2013) using a laboratory in vitro technique. Diets varied in ingredients and nutrient composition, as presented in Table 1. Forage type varied across studies and included brown midrib (**BMR**) corn silage, conventional corn silage, alfalfa silage, and grass silage.

The 21 TMR samples were analyzed for NDF, indigestible NDF (**iNDF**; according to Krizsan and Huhtanen, 2013, with modifications), and in vitro NDF digestibility (**ivNDFD**). Other nutrients reported (Table 1) were analyzed according to each individual study publication. The TMR samples were dried in a forced-air oven at 60°C for 48 h and ground using a Wiley mill (Arthur H. Thomas, Philadelphia, PA) with a 1-mm screen before nutrient analysis. Neutral detergent fiber was determined using a modified Goering and Van Soest (1970) technique. Approximately 0.5 g of sample was weighed into a 125-mL Erlenmeyer flask, and 50 mL of neutral detergent solution and 0.5 g of sodium sulfite were added. Samples were heated to 100°C for 5 min before 2 mL of amylase solution was added. The sample and solution were then boiled for an additional 55 min. After 1 h, flasks were filled half full with hot water, 2 mL of amylase solution was added, and the flask was swirled while the sides were scraped with a rubber scraper to prevent particles from clinging to the flask. The solution was emptied, filtered through a glass microfiber filter (3.1-μm pore size, Ahlstrom Corp. 141; Ahlstrom, Helsinki, Finland), and rinsed twice more with boiling water. The sample remaining on the filter paper was then rinsed twice with 30 mL of acetone, allowing for a 2-min soak with each rinse. Samples with glass filter paper were placed in porcelain crucibles, dried at 100°C for 24 h, and weighed. Then, NDF (% of DM) was calculated using the following equation:

$$\text{NDF (\% of DM)} = (\text{dry NDF residue weight}) / (\text{initial dry sample weight}).$$

Indigestible NDF used in the model to predict **TTNDFD** was determined by rumen fluid in vitro incubation for 240 h without rumen re-inoculation. The in vitro method procedure used to prepare samples and rumen fluid

was as described by Goeser and Combs (2009), and after a 240-h incubation, the NDF concentration was determined on the residue as described above. Then, **iNDF** (% of total NDF) was calculated as follows:

$$\text{iNDF (\% of DM)} = (\text{dry iNDF 240-h residue weight}) / (\text{initial dry sample weight}),$$

$$\text{iNDF (\% of NDF)} = (\text{iNDF, \% of DM}) / (\text{NDF, \% of DM}).$$

The in vitro **TTNDFD** model is based on the concept that fiber digestion is a 2-step process, beginning in the rumen followed by fiber digestion in the hindgut. Ruminant digestion of NDF is often described as time-dependent and is a competitive process affected by the amount and rate of degradation (**kd**) of potentially digestible NDF (**pdNDF**), similar to the cellulose digestion model described by Waldo et al. (1972), and rate of passage of **pdNDF** (**kp**). The model inputs include fraction of a single pool of **pdNDF** digested in a first-order fractional rate (**kd**) and **iNDF**. Pool of **pdNDF** was calculated from the difference of total NDF and **iNDF** pool (**pdNDF** = NDF – **iNDF**). The **pdNDF kd** was calculated from NDF residue measurements taken at 24, 30, and 48 h of in vitro incubation in rumen fluid (Goeser and Combs, 2009) using a first-order kinetics model with an indigestible fraction, as described by Mertens (1993), which assumes that the indigestible residue does not disappear and that **pdNDF** residue disappears at a rate proportional to its mass at any time. The **pdNDF kp** was predicted from a regression model (Krizsan et al., 2010) for **iNDF**, which was adjusted to account for the selective retention of **pdNDF** (Lund et al., 2007) determined using the flux/compartiment pool method described by Ellis et al. (1994). The predicted fiber digestibility in the **TTNDFD** model was indexed to a 630-kg dairy cow consuming 23.4 kg/d DM of a diet containing 30% NDF. This index sets the **kp** of **pdNDF** at 2.67%/h.

To calculate total-tract NDF digestibility from concentrations of nutrients in the TMR and feces, external or intrinsic marker techniques were used in each in vivo study. Analysis of NDF, rare-earth markers, lignin, and indigestible NDF were conducted as described in the individual papers (Table 1).

Analysis of variance was used to measure the difference between **TTNDFD** in vivo, **TTNDFD** in vitro, 30-h **ivNDFD**, 48-h **ivNDFD**, and **iNDF** using the paired *t*-test with Proc Means of SAS (SAS Institute, 2009). Regressions to determine linear relationships between **TTNDFD** in vitro, **TTNDFD** in vivo, **iNDF**,

Table 1. Summary of trial diets used to predict total-tract NDF digestibility in lactating dairy cows using the total-tract NDF digestibility (TTNDFD) model

Study	Diet ¹ (TMR)	DMI, kg/d	% of DM				NDFD, ³ % of NDF				% of total NDF	
			CP	Starch	NFC	NDF	iNDF, ² % of NDF	24 h	30 h	48 h	TTNDFD in vitro ⁴	TTNDFD in vivo ⁵
Arndt et al., 2009	High forage + corn grain	25.5	16.9	25.6	41.9	29.6	29.3	32.3	40.3	46.0	37.7	36.6
Ferraretto et al., 2014	Low forage + corn grain	27.0	16.7	34.1	49.0	23.0	33.5	23.3	30.5	43.1	33.5	32.5
	LFY	26.1	16.8	22.1	40.7	30.1	29.6	37.9	44.1	54.1	39.8	39.7
	BMR	27.8	17.3	22.8	40.8	29.4	28.6	44.0	50.9	51.6	39.6	40.4
Lopes et al., 2013a	BMR-restricted intake	22.2	16.6	20.8	45.7	26.9	25.9	15.5	26.4	55.0	52.8	49.4
	BMR-ad libitum intake	25.4	15.9	21.9	45.9	28.3	24.1	15.5	17.6	48.4	47.6	50.1
	CS-restricted intake	26.9	15.8	22.2	47.3	26.1	31.5	17.6	30.5	47.0	43.2	48.3
Lopes et al., 2013b	CS-ad libitum intake	25.5	16.6	21.2	45.5	27.5	31.1	17.0	30.5	46.5	42.7	50.2
	100CS:0ALF	25.0	15.9	25.1	51.0	24.9	31.1	20.8	34.4	45.7	38.0	38.3
	67CS:33ALF	25.1	16.3	23.3	48.9	25.5	31.6	22.0	40.8	52.4	41.0	40.9
Fredin et al., 2013b	33CS:67ALF	24.1	17.2	19.5	47.9	24.6	31.8	25.0	40.6	51.4	41.0	39.4
	100ALF:0CS	21.9	18.6	17.9	43.7	25.5	32.3	30.6	45.9	57.8	45.0	43.8
	High corn grain	25.2	18.4	28.5	40.3	29.9	24.1	43.8	45.6	51.9	43.8	49.0
Fredin et al., 2013a	High corn silage	24.1	18.4	23.8	40.3	29.9	24.3	41.5	39.8	47.8	40.2	49.0
	Low corn silage	25.4	18.9	22.0	38.0	32.1	17.1	52.4	58.2	61.1	51.7	55.6
	Normal starch/fine ground	23.4	17.1	26.5	45.7	26.5	30.1	14.1	26.7	42.8	40.5	41.0
Verbeten et al., 2011	Reduced starch/coarse ground	23.1	17.3	18.2	37.9	33.8	24.1	16.2	41.6	55.7	51.3	51.1
	Corn silage + alfalfa	25.1	17.1	—	48.6	22.5	28.1	30.0	40.0	47.0	36.8	26.3
	Meadow fescue silage	24.8	17.7	—	43.6	25.4	22.1	44.3	49.9	57.3	39.1	43.0
Mean	Tall fescue silage	23.3	17.0	—	43.7	25.9	26.0	38.0	41.9	51.7	38.1	43.2
	Wheat straw	23.5	18.0	—	45.0	25.4	39.2	22.7	21.6	31.5	33.8	32.4
		24.8	17.2	23.6	44.4	27.3	28.4	28.8	38.0	49.8	41.8	42.9
SD		1.50	0.87	3.72	3.66	2.84	4.68	11.51	9.90	6.33	5.28	7.14

¹LFY = leafy corn; BMR = brown midrib; CS = corn silage; ALF = alfalfa.
²iNDF = indigestible NDF measured from 240 h in vitro rumen fluid incubation.
³NDFD = NDF digestibility measured using an in vitro method (Goesser, 2008).
⁴Total-tract NDF digestibility predicted from the in vitro TTNDFD model (Combs, 2013).
⁵Total-tract NDF digestibility as measured in each study using indigestible markers and fecal spot sampling.

Table 2. Differences between observed and predicted total-tract NDF digestibility using different parameters

Item	Difference	SD ¹	P-value
TTNDFD in vivo – TTNDFD in vitro ²	1.09	4.21	0.24
TTNDFD in vivo – 30-h NDFD ³	4.87	11.6	0.07
TTNDFD in vivo – 48-h NDFD ³	–6.93	6.60	<0.01
TTNDFD in vivo – iNDF ⁴	14.5	11.0	<0.01

¹SD = standard deviation of the means.

²TTNDFD = predicted total-tract NDF digestibility using TTNDFD test.

³In vitro incubation for 30 and 48 h to measure NDF digestibility (NDFD).

⁴iNDF = indigestible NDF measured from 240-h in vitro rumen fluid incubation.

30-h ivNDFD, and 48-h ivNDFD were performed using Proc Reg of SAS (SAS Institute, 2009). Statistical significance and trends were declared at $P \leq 0.05$ and $P > 0.05$ to $P < 0.10$, respectively.

RESULTS AND DISCUSSION

Mean diet DMI, nutrient concentrations, and TTNDFD are reported in Table 1. Dry matter intake ranged from approximately 22.0 to 28.0 kg/d, consistent with intake for high-producing cows. The nutrient composition ranged from 15.8 to 18.9% (SD ± 0.87) CP, 17.9 to 34.1% (SD ± 3.72) starch, 37.9 to 51.0% (± 3.66) NFC, 22.5 to 33.8% (± 2.84) NDF, and 17.1 to 39.2% (± 4.68) iNDF (Table 1). The NDF and iNDF concentrations suggested a broad range in fiber composition in diets that were used to test the TTNDFD model.

The total-tract NDF digestibilities predicted from an in vitro assay and measured from each in vivo study for each diet are presented in Table 1. The mean values for in vitro and in vivo TTNDFD were 41.8 and 42.9% of total NDF, respectively. Total-tract NDF digestibility values predicted from the in vitro technique were similar to values observed in vivo; only one diet (corn silage + alfalfa; Verbeten et al., 2011) was poorly predicted by the TTNDFD model. Verbeten et al. (2011) noted that this diet was low in NDF and high in NFC compared with the other diets tested and observed a reduction in TTNDFD for this HOT diet. The TTNDFD observed in vivo was 26.3% compared with 33.5% TTNDFD for in vitro prediction; we believe that the higher TTNDFD in vitro may be related to the greater buffering capacity of the in vitro system. Among the parameters used to predict total-tract NDF, the TTNDFD model was the only parameter for which the difference of the means was similar ($P = 0.24$) to total-tract NDF digestibility observed in vivo (Table 2). The compilation of the TTNDFD data ($n = 21$) resulted in the following equation: TTNDFD in vivo (%) = $15.6 (\pm 4.19) + 0.60 (\pm 0.09) \times \text{TTNDFD in vitro} (\%)$; $R^2 = 0.68$, root mean square error = 4.27%, $P < 0.01$ (Figure 1). The results from this regression and the difference of the means

showed that the in vitro TTNDFD model predicted total-tract NDF digestibility observed in in vivo studies using indigestible markers and spot fecal sampling to estimate fiber digestibility.

When in vitro 30-h NDF digestibility values were compared with in vivo total-tract NDF digestibility data, the relationship was not significant ($P = 0.54$; Figure 2). This lack of relationship between in vitro 30-h NDF digestibility and in vivo total-tract NDF digestibility data (Figure 2) suggests that the use of a single-timepoint incubation to predict NDF digestibility is not adequate. The differences between TTNDFD in vivo and 30-h ivNDFD means are presented in Table 2. The 30-h NDFD incubation tended ($P = 0.07$) to similar means to TTNDFD in vivo, although it had higher standard deviation and was almost 5 percentage units different, on average, from the total-tract NDF digestibility observed in vivo (Table 2). A better relationship was observed for 48-h ivNDFD compared with in vitro 30-h ivNDFD to predict total-tract NDF digestibility. The in vitro 48-h NDF digestibility coefficients related to the in vivo measurements ($R^2 = 0.30$, $P = 0.01$; Figure 3). However, when the differences of means were compared between TTNDFD in vivo and 48-h ivNDFD (Table 2), the NDF digestibility measured after 48-h in vitro incubation could not be used to predict total-tract digestibility.

Although the digestibility of NDF measured by a single timepoint (30 or 48 h) in vitro incubation is often reported in the literature and by commercial laboratories, the values for 30-h ivNDFD are usually higher than the values observed in in vivo studies measuring total-tract NDF digestibility (Goesser, 2008). The problem with single-timepoint in vitro NDFD assays is that the value is not a direct measure of pdNDF or rate of fiber digestion. A single-timepoint in vitro NDFD assay simply indicates how much residual fiber remains after a specific period of exposure to rumen fluid. The residue measured includes the indigestible fiber fraction plus the residual potentially digestible fiber remaining. None of the in vitro single incubation timepoints accurately reflect how different forages would be utilized

Figure 1. Relationship between total-tract NDF digestibility (TTNDFD) measured in vivo and total-tract NDF digestibility predicted in vitro using the TTNDFD model. The regression equation to describe the relationship was $\text{TTNDFD in vivo (\%)} = -3.62 (\pm 7.45) + 1.11 (\pm 0.17) \times \text{TTNDFD in vitro (\%)}$ with $R^2 = 0.68$, root mean square error = 4.27, P -value < 0.01; $n = 21$ diets.

by the animal because none of the time points chosen in the laboratory assay reflect the mean retention time of the forages in vivo (Combs, 2013). In this trial, the compilation of iNDF data from 21 diets showed a moderate relationship ($R^2 = 0.40$; $P < 0.01$; Figure

4) between total-tract NDF digestibility measured in vivo and indigestible NDF measured by in vitro 240-h incubation. Figure 4 shows that for each 1-percentage-unit increase of iNDF, there was a decrease of 0.96 percentage units of total-tract NDF digestibility. This

Figure 2. Relationship between total-tract NDF digestibility (TTNDFD) measured in vivo and NDF digestibility measured using the in vitro standardized (Goessens and Combs, 2009) method for 30-h incubation. The regression equation to describe the relationship was $\text{TTNDFD in vivo (\%)} = 39.0 (\pm 6.43) + 0.10 (\pm 0.16) \times 30\text{-h in vitro NDF digestibility (\%)}$ with $R^2 = 0.02$, root mean square error = 7.43, P -value = 0.54; $n = 21$ diets.

Figure 3. Relationship between total-tract NDF digestibility (TTNDFD) measured in vivo and NDF digestibility measured using the in vitro standardized (Goeser and Combs, 2009) method for 48-h incubation. The regression equation to describe the relationship was $\text{TTNDFD in vivo (\%)} = 12.03 (\pm 10.87) + 0.61 (\pm 0.21) \times 48\text{-h in vitro NDF digestibility (\%)}$ with $R^2 = 0.30$, root mean square error = 6.28, P -value = 0.01; $n = 21$ diets.

relationship confirms the importance of an accurate measurement of iNDF in determining forage quality and developing mechanistic models to predict NDF

digestibility (Krizsan and Huhtanen, 2013). However, iNDF by itself should not be used as the only parameter to predict total-tract NDF digestibility, because

Figure 4. Relationship between total-tract NDF digestibility (TTNDFD) measured in vivo and indigestible NDF measured in vitro 240-h incubation. The regression equation to describe the relationship was $\text{TTNDFD in vivo (\%)} = 70.19 (\pm 7.80) - 0.96 (\pm 0.27) \times \text{indigestible NDF (\%)}$ with $R^2 = 0.40$, root mean square error = 5.81, P -value = 0.002; $n = 21$ diets.

the difference of means between iNDF and TTNDfD in vivo differed significantly ($P < 0.01$; Table 2).

The values of iNDF and kd of pdNDF per se do not accurately reflect the digestibility of NDF or the digestible energy value of the forage at ad libitum intake. The most useful value is one that uses digestion kinetic parameters to calculate a digestibility coefficient for NDF. Huhtanen et al. (2008) noted that a good model to predict NDF digestibility should consider the following: separation of NDF into iNDF and pdNDF; use of digesta passage models, including selective retention of feed particles in the rumen; and a model that accurately and precisely predicts kd by an in vitro system. All of these parameters are used to predict total-tract NDF digestibility using the TTNDfD model developed by University of Wisconsin–Madison researchers.

CONCLUSIONS

The in vitro test of diets from 7 different studies demonstrated that the TTNDfD model can provide important insights into fiber utilization by dairy cattle. Being able to predict total-tract fiber digestibility from a model based on in vitro NDF degradation and incorporating this information into rations could improve our ability to optimize forage utilization and milk production.

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REFERENCES

- Allen, M. S. 2011. Mind over models. Pages 29–44 in Proc. 2011 Tri-State Dairy Nutrition Conf., Fort Wayne, IN. Accessed Sep. 1, 2011. <http://tristatedairy.osu.edu/proceedings.html>.
- Arndt, C., L. E. Armentano, and M. B. Hall. 2009. Corn bran vs. corn grain at two levels of forage: Intake and production responses by lactating dairy cows. *J. Dairy Sci.* 92(E-Suppl. 1):94. (Abstr.)
- Combs, D. K. 2013. TTNDfD: A new approach to evaluate forages. Pages 113–125 in Proc. 2013 Cornell Nutrition Conf. Dept. Anim. Sci., Cornell Univ., Ithaca, NY.
- Ellis, W. C., J. H. Matis, T. M. Hill, and M. R. Murphy. 1994. Methodology for estimating digestion and passage kinetics of forages. Pages 682–756 in Forage Quality, Evaluation and Utilization. G. C. Fahey Jr., M. Collins, D. R. Mertens, and L. E. Moser, ed. Am. Soc. Agron., Crop Sci. Soc. Am., Soil Sci. Soc. Am., Madison, WI.
- Ferraretto, L. F., A. C. Fonseca, C. J. Sniffen, A. Formigoni, and R. D. Shaver. 2014. Effect of corn silage hybrids differing in starch and NDF digestibility on lactation performance and total-tract nutrient digestibility by dairy cows. *J. Dairy Sci.* 97(E-Suppl. 1):151. (Abstr.)
- Fredin, S. M., S. J. Bertics, and R. D. Shaver. 2013a. Effects of dietary starch content and corn particle size on intake, digestion and milk production by dairy cows. *J. Dairy Sci.* 96(E-Suppl. 1):149. (Abstr.)
- Fredin, S. M., L. F. Ferraretto, M. S. Akins, and R. D. Shaver. 2013b. Effects of dietary starch content and NDF source on intake and milk production by dairy cows. *J. Dairy Sci.* 96(E-Suppl. 1):34. (Abstr.)
- Goering, H. K., and P. J. Van Soest. 1970. Forage Fiber Analyses (Apparatus, Reagents, Procedures, and Some Applications). Agric. Handbook No. 379. Agricultural Research Service-USDA, Washington, DC.
- Goeser, J. P. 2008. Improvement of rumen in vitro NDF digestibility techniques and data analysis. PhD Thesis. University of Wisconsin, Madison.
- Goeser, J. P., and D. K. Combs. 2009. An alternative method to assess 24-h ruminal in vitro neutral detergent fiber digestibility. *J. Dairy Sci.* 92:3833–3841.
- Huhtanen, P., A. Seppälä, M. Ots, S. Ahvenjärvi, and M. Rinne. 2008. In vitro gas production profiles to estimate extent and effective first-order rate of neutral detergent fibre digestion in rumen. *J. Anim. Sci.* 86:651–659.
- Krizsan, S. J., S. Ahvenjärvi, and P. Huhtanen. 2010. A meta-analysis of passage rate estimated by rumen evacuation with cattle and evaluation of passage rate prediction models. *J. Dairy Sci.* 93:5890–5901.
- Krizsan, S. J., and P. Huhtanen. 2013. Effect of diet composition and incubation time on feed indigestible neutral detergent fiber concentration in dairy cows. *J. Dairy Sci.* 96:1715–1726.
- Lopes, F., D. E. Cook, R. W. Bender, and D. K. Combs. 2013a. Effect of source of corn silage and level of dry matter intake on rumen digestion kinetics and total-tract digestibility in dairy cows. *J. Dairy Sci.* 96(E-Suppl. 1):523. (Abstr.)
- Lopes, F., D. E. Cook, and R. W. Bender. 2013b. Effect of changing ratios of alfalfa and corn silage on rumen digestion kinetics and total-tract digestibility in dairy cows. *J. Dairy Sci.* 96(E-Suppl. 1):16. (Abstr.)
- Lund, P., M. R. Weisbjerg, and T. Hvelplund. 2007. Digestible NDF is selectively retained in the rumen of dairy cows compared to indigestible NDF. *Anim. Feed Sci. Technol.* 134:1–17.
- Mertens, D. R. 1993. Kinetics of cell wall digestion and passage in ruminants. Pages 535–570 in Forage Cell Wall Structure and Digestibility. H. G. Jung, D. R. Buxton, R. D. Hatfield, and J. Ralph, ed. American Society of Agronomy, Madison, WI.
- Oba, M., and M. S. Allen. 1999. Evaluation of importance of digestibility of neutral detergent fiber from forage: effects on dry matter intake and milk yield of dairy cows. *J. Dairy Sci.* 82:589–596.
- SAS Institute. 2009. SAS/STAT 9.3 User's Guide. Version 9.3 ed. SAS Institute Inc., Cary, NC.
- Tilley, J. M. A., and R. A. Terry. 1963. A two-stage technique for the in vitro digestion of forage crops. *J. Br. Grassl. Soc.* 18:104–111.
- Van Soest, P. J., J. B. Robertson, and B. A. Lewis. 1991. Methods of dietary fiber, neutral detergent fiber and non-polysaccharides in relation to animal nutrition. *J. Dairy Sci.* 74:3583–3597.
- Verbeten, W. D., D. K. Combs, and D. J. Undersander. 2011. Partially replacing alfalfa and corn silage with fescue silages maintained fat corrected milk production. *J. Dairy Sci.* 94(E-Suppl. 1):556. (Abstr.)
- Waldo, D. R., L. W. Smith, and E. L. Cox. 1972. Model of cellulose disappearance from the rumen. *J. Dairy Sci.* 55:125–129.