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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. Combs[1]
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 (Goeser, 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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[1]Corresponding 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. Ruminal 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/compartment 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,