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Modification of a rumen fluid priming technique for measuring in vitro neutral detergent fiber digestibility[1]

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ABSTRACT

Recently, we developed an alternate method to measure in vitro neutral detergent fiber (NDF) digestibility (ivNDFD) based on a primed rumen fluid inoculum. Pretreating rumen fluid inoculum with cellulose and holding the inoculum until it generated 0.3 mL of gas/mL of rumen fluid before inoculating forage samples improved ivNDFD assay repeatability but depressed ivNDFD means. Our objective in this study was to determine if pretreating rumen fluid with a mixture of carbohydrates and urea would affect the ivNDFD mean and variance. We also used the modified procedure as a reference assay to calibrate near-infrared reflectance spectroscopy (NIRS) to predict 24-, 30-, and 48-h ivNDFD. Two experiments were completed. In experiment A, 3 ivNDFD assays modified from the method of Goering and Van Soest were evaluated over 24, 28, 48, 54, and 72 h by using dried, ground alfalfa (1 mm) or wheat straw (0.5 g) sealed in Ankom F57 forage fiber bags. Bags were placed individually in 125-mL Erlenmeyer flasks and incubated with Goering and Van Soest media and 10 mL of rumen fluid. Rumen fluid was collected before feeding from 2 cannulated cows fed a high-forage diet and was prepared in 1 of 3 ways: 1) pooled rumen fluid was strained and used immediately to inoculate flasks (modified Goering and Van Soest method); 2) strained, pooled fluid was combined with buffer, reducing solution, and 1.25 mg of primer/mL of rumen fluid and allowed to produce 0.12 mL of gas/mL of rumen fluid before sample inoculation [Combs-Goeser (CG) method]; or 3) the CG method was used without the primer mixture (unprimed method). The assay was repeated 5 times, with 5 time points (24, 28, 48, 54, and 72 h) and 2 subsamples per time point for each method. Neutral detergent fiber was analyzed using an Ankom^{200} forage fiber analyzer and ivNDFD was determined as follows: ivNDFD (% of NDF) = 100 × $[(NDF_{0h} - NDF_{residue})/(NDF_{0h})]$. Results were analyzed using a mixed model procedure, and data were blocked by method to obtain repetition sums of squares, which were compared by an *F*-test to assess interassay error. Repetition sums of squares were reduced with the CG method compared with the Goering and Van Soest method (19 vs. 228), and mean ivNDFD estimates were similar at 28, 48, and 54 h. In experiment B, 24-, 30-, and 48-h ivNDFD data for 54 feeds were determined in triplicate using the CG method, and corresponding samples were then scanned with an NIRS instrument. Calibrations were computed using partial least squares regression techniques. The NIRS calibration equation R^2 values were 0.93, 0.93, and 0.89 for 24-, 30-, and 48-h ivNDFD. Results suggest that the modified ivNDFD method using rumen fluid primed with a mixture of carbohydrate and urea (CG method) reduced interassay error.

Key words: neutral detergent fiber digestibility, in vitro, forage

INTRODUCTION

To be certified by the National Forage Testing Association (NFTA, 2008), commercial forage testing laboratories must meet standards for inter- and intraassay errors for DM, CP, and NDF assays. However, no such standard exists for in vitro NDF digestibility (**ivNDFD**; NFTA, 2008). Fiber digestibility is an important indicator of forage quality. Forage fiber digestibility can range from approximately 20 to nearly 80% of the total fiber (Oba and Allen, 1999) and can account for as much as 75% of the digestible energy of forage DM (NRC, 2001). Methods for measuring fiber digestibility are not standardized, and tend to be imprecise relative to assays for DM, CP, and NDF (Mentink et al., 2006). Research and commercial forage testing laboratories most commonly use in vitro procedures that are modifications of the procedure of Goering and Van Soest (1970). Estimates of NDF digestibility vary from laboratory to laboratory because of differences in the laboratory assay, which makes it difficult to compare results between laboratories or to incorporate fiber digestibility into ration-balancing software. In addition, in vitro estimates of fiber digestibility tend to vary from

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run to run within a laboratory because of the variable activity of rumen fluid. Others have recognized significant interassay error with in vitro techniques (Schofield and Pell, 1995; Hall et al., 1998; Rymer et al., 2005).

Goeser and Combs (2009) recently developed an alternative technique to measure in vitro NDF digestibility. Interassay variation was significantly reduced when ground cellulose was added to rumen fluid inoculum and the mixture was allowed to reach a standard gas pressure before sample inoculation. The priming technique resulted in reduced interassay error, or improved precision, relative to a modified Goering and Van Soest (1970) ivNDFD assay. However, estimates of 24-h ivNDFD were lower for the priming technique than estimates based on the modified Goering and Van Soest assay.

In commercial laboratories, most forage analyses are done by near-infrared reflectance spectroscopy (**NIRS**). The precision of NIRS calibration equations depends on the precision of the analytical technique used to calibrate the NIRS instrument (Shenk and Westerhaus, 1994) and previous attempts to calibrate NIRS to ivNDFD for diverse feeds have been unsuccessful because of imprecision in the laboratory technique (Andres et al., 2005; Mentink et al., 2006). An ivNDFD analytical assay with lower interassay error may allow for NIRS calibrations with improved calibration statistics.

The purpose of this study was not to determine which method may be more accurate, but to compare the precision of the methods. We compared precision by measuring intra- and interassay error for the 3 ivNDFD methods. The modified priming technique was then used to calibrate NIRS by using 24-, 30-, and 48-h ivNDFD data for validation of the modified priming technique precision.

MATERIALS AND METHODS

Two experiments were completed. Experiment A compared 3 in vitro NDF digestion techniques. Experiment B evaluated NIRS calibration statistics when 24-, 30-, and 48-h ivNDFD were predicted by using the modified priming technique as a reference procedure.

Experiment A—Comparing ivNDFD Estimates, and Intra- and Interassay Precision of 3 ivNDFD Methods

Each of the 3 ivNDFD techniques evaluated in this experiment used rumen fluid inoculum collected and pooled from 2 ruminally cannulated, lactating dairy cows. The first method evaluated was a modified Goering and Van Soest (1970) ivNDFD technique (**GV**) described by Goeser and Combs (2009). The second method was a modification of the priming technique described by Goeser and Combs (2009; **CG**), and the third method used unprimed inoculum that had been held until it reached the same gas pressure as in the CG method (**UN**).

Two forages, alfalfa silage and wheat straw, were analyzed with each of the 3 ivNDFD methods. Both forages were analyzed by Dairyland Laboratories Inc. (Arcadia, WI) by AOAC (2006) methods for DM (method 930.15), CP (method 954.01), and ash (method 942.15). The methods described by Goering and Van Soest (1970) were used to sequentially determine ADF and sulfuric acid-lignin. Both forages were dried at 60°C for 48 h in a forced-air oven and ground to pass a 1-mm Wiley mill screen (Arthur H. Thomas, Philadelphia, PA) before submission for chemical analysis or for in vitro fiber digestibility. The in vitro NDF digestion assays were conducted as follows.

Approximately 0.5 g of dried, ground forage sample was weighed into tared, labeled filter bags with a mean pore size of 35 μm (F57, Ankom Technology, Macedon, NY). The CG and UN samples were prepared and digested in a manner similar to the rumen fluid priming method, and the GV samples were prepared and digested in a manner similar to the GV technique described by Goeser and Combs (2009). The forage fiber bags remained sealed for the entire procedure for each of the 3 methods. Five repetitions were completed and each repetition included zero hour, 5 digestion time points (24, 28, 48, 54, and 72 h), and blank samples analyzed in triplicate for each of the 3 techniques described.

Although the forage samples were prepared in a similar manner, the CG and UN methods used different rumen fluid inoculum preparation procedures based on the methods of Goeser and Combs (2009).

Rumen Fluid Collection and GV Flask Inoculation. The Research Animal and Resource Center of the College of Agriculture and Life Sciences, University of Wisconsin-Madison, approved the animal experimental protocol. At approximately 0630 h on the day of inoculation, all flasks were subjected to continuous CO_2 flow, and 2 mL of reducing solution was added to each flask designated to the GV. At 0645 h, approximately 1 L of rumen fluid was collected from each of 2 cannulated, lactating cows into prewarmed, glass-lined Thermoses. The donor cows were fed a 97% forage and 3% concentrate diet ad libitum, once daily at 0700 h. The rumen fluid inoculum was strained through 4 layers of cheesecloth while under CO_2 flow, and fluid from each cow was pooled in a 2,000-mL Erlenmeyer flask. Approximately 800 mL of strained, pooled rumen fluid was used to inoculate the GV flasks immediately, with 10 mL of rumen fluid inoculum per flask. The period from rumen fluid collection to GV flask inoculation was approximately 15 min.