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Low-infrastructure filter bag technique for neutral detergent fiber analysis of forages

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ABSTRACT

We tested a water bath filter bag technique (WB) for analysis of neutral detergent fiber (NDF) versus a standard filter bag technique (ANKOM). The principal difference between WB and ANKOM was absence of a pressurized chamber for WB. The NDF method we used (aNDF) did not include sodium sulfite. One-hundred and ninety-six diverse forage and silage samples were gathered from Vietnam and New York State including: 40 C_3 grasses, 122 C_4 grasses, 21 legumes and 13 silages. Samples were completely randomized for parallel processing in ANKOM and WB. Water bath aNDF levels were strongly correlated with ANKOM ($r^2 = 0.995$) with an overall mean difference of 6.93 g/kg dry matter, and can be described by the equation: ANKOM aNDF (g/kg DM) = 0.9963 × Water Bath aNDF − 4.536. Intercept and slope were not different from zero ($P = 0.1953$) and one ($P = 0.4828$), respectively. Unique intercepts and slopes by sample classification (C_3 grasses, C_4 grasses, legumes, silages) were significant in a multivariate model, but may not be necessary based on the strong overall relationship between ANKOM and WB aNDF. Furthermore, duplicate repeatability for ANKOM and WB was not different. The water bath method is viable for NDF analysis of diverse forage and silage samples, and could provide a low-infrastructure efficient alternative for low-budget laboratories.

1. Introduction

Neutral detergent fiber (NDF) has been adopted worldwide by laboratories, universities, agricultural extensionists and nutritionists as a key indicator of forage nutritive value for use in ration balancing and to support ruminant nutrition research and practitioner decisions. Benefits of NDF over crude fiber (*e.g.*, association with dry matter intake, rumination, fill, passage, and feed intake) are well known (Van Soest, 1994; Mertens, 2003). However, NDF adoption has lagged in many developing countries, particularly in tropical regions where crude fiber is especially poor for prediction of nutritive value. Resistance to NDF adoption has been attributed to the low cost of the crude fiber technique and a large body of proximate analysis-centric ruminant nutrition data in these regions that would become obsolete upon adoption of the detergent system due to poor association between NDF and crude fiber (Van Soest, 1994). Another potential reason is the lack of an accurate, efficient NDF technique with minimal required investment in infrastructure and expertise.

Abbreviations: NDF, neutral detergent fiber; aNDF, neutral detergent fiber with α-amylase inclusive of residual ash; WB, water bath filter bag technique; DM, dry matter; NDS, neutral detergent solution; BBC, overall blank bag correction factor; OD, outer diameter.

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Table 1
Forage and silage samples included in the study.

Species	Origin	*n*
C_3 grasses		**40**
Orchardgrass (*Dactylis glomerata* L.)	New York State	11
Quackgrass (*Elytrigia repens* (L.) Desv. Ex Nevski)	New York State	3
Tall Fescue (*Festuca arundinacea* Schreb.)	New York State	3
Rice straw (*Oryza sativa* L.)	Vietnam	3
Reed Canarygrass (*Phalaris arundinacea* L.)	New York State	11
Timothy (*Phleum pratense* L.)	New York State	9
C_4 grasses		**122**
Big Bluestem (*Andropogon gerardii* Vitman)	New York State	6
Brachiaria Cv. Mulato II (*Brachiaria ruziziensis* x *B. decumbens* x *B. brizantha*)	Vietnam	75
Guinea Grass (*Panicum maximum* Jacq. (TD 58))	Vietnam	3
Switchgrass (*Panicum virgatum* L.)	New York State	26
Paspalum atratum Swallen	Vietnam	3
Elephant Grass (*Pennisetum purpureum* Schumach.)	Vietnam	5
Maize (*Zea mays* L.)	New York State	4
Legumes		**21**
Birdsfoot Trefoil (*Lotus corniculatus* L.)	New York State	3
Alfalfa (*Medicago sativa* L.)	New York State	14
Stylosanthes guianensis (Aubl.) CIAT 184	Vietnam	1
Red Clover (*Trifolium pratense* L.)	New York State	3
Silages		**13**
Alfalfa silage (*Medicago sativa* L.)	New York State	3
Maize silage (*Zea mays* L.)	New York State	10
Whole Set		**196**

Numerous modifications of the original NDF technique (Van Soest and Wine, 1967) have been suggested (Mascarenhas Ferreira et al., 1983; Van Soest et al., 1991; Giger-Reverdin, 1995; Mertens, 2002). An important modification that enabled rapid processing while reducing technician error was a high throughput NDF technique in filter bags (Komarek, 1994). A newer version of Komarek's original approach consists of a pressurized chamber and more automated processing. Several studies have provided evidence that filter bag techniques correlate well with conventional NDF analysis, often with lower variation, and can be implemented as an acceptable alternative (Komarek, 1993; Vogel et al., 1999; Fay et al., 2005; Ferreira and Mertens, 2007). However, the initial investment in infrastructure can be cost prohibitive, especially for laboratories in developing countries and other low budget operations. Pereira et al. (2009) evaluated an alternative filter bag NDF technique in a shaker water bath. Significant differences were not detected between their method and ANKOM for a limited NDF range of ryegrass, rye, and oats samples.

Development of the technique proposed in this paper was motivated by an observed need in developing countries for an efficient, low-budget NDF analysis alternative using materials that are often readily available in laboratories. Consequently, the primary objectives of this study were: (1) to develop a low-infrastructure filter bag NDF technique, and, (2) to test the technique versus a standard filter bag NDF technique (ANKOM) using a diverse set of temperate and tropical forages.

2. Methods

Forage samples ($n = 196$) were collected in New York State and Vietnam between 2010 and 2012 (Table 1), dried to stable weight at 60 °C in a forced air oven, and ground in a Wiley Mill (Arthur H. Thomas Co., Philadelphia, PA) to pass a 1-mm screen. Samples (0.25 g) were weighed to nearest 0.0001 g, transferred into filter bags (ANKOM F57, 25 μm porosity), and analyzed separately for aNDF concentration (without sodium sulfite) in duplicate using a standard technique (ANKOM Technology, 2011) and the proposed water bath technique (WB). Sodium sulfite was not added, because samples consisted of forages and silages that did not contain high protein material, material of animal origin or heat damaged samples, and to eliminate the possibility of sodium sulfite attacking and solubilizing lignin (Van Soest et al., 1991; Hintz et al., 1996). Results were not ash corrected, but were blank bag corrected using the overall blank bag correction factor for each technique. The blank bag correction factor for individual blank bags was calculated as the post-extraction 105 °C dry weight for each bag divided by its pre-extraction weight. The overall blank bag correction factor (BBC) was the mean of individual blank bag correction factors for each technique. Dry matter (DM) was determined for 1 g subsamples dried overnight in a forced air oven at 105 °C, the temperature recommended in National Forage Testing Association Method 2.2.2.5 (Undersander et al., 1993). aNDF concentration was calculated as: g aNDF/kg DM = $((R - BW \times BBC)/(SW \times SDM)) \times 1000$, where R = post-extraction 105 °C dry weight of bag and residue, BW = pre-extraction blank bag weight, BBC = blank bag correction factor for ANKOM or WB, SW = pre-extraction sample weight, and SDM = sample 105 °C DM. A standard *Dactylis glomerata* L. sample was also included in each batch for both techniques to assess variability among batches and techniques for the same sample. Sample processing order was completely randomized. ANKOM and WB batches (48 filter bags or 22 duplicate samples + 2 blank bags + 2 *D. glomerata* L. standards) were completed on the same days using the same neutral detergent solution (NDS) batch. ANKOM guidelines (ANKOM Technology, 2011) for NDS preparation, equivalent to the procedure described by

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