

## Technical Note: Comparison of three analytical procedures to estimate the acid detergent fiber concentration in feeds of widespread use in Argentina

J. L. Danelón<sup>1</sup>, M. S. Guaita<sup>2</sup>, P. Fay<sup>1</sup>, S. Chifflet<sup>3</sup>, M. Wawrzekiewicz, and H. M. Fernández

Departamento de Producción Animal, Facultad de Agronomía,  
Universidad de Buenos Aires. Buenos Aires, Argentina.  
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**Abstract:** The objective of this study was to compare the values of acid detergent fiber (ADF) content in feeds widely used in Argentina obtained using three different analytical procedures. Two of these involved use of the ANKOM® filter bag technique: direct treatment of the feed samples with acid detergent solution (D-ADF) in one case or sequential treatments with neutral detergent followed by acid detergent solution (S-ADF) in the other. The third procedure was the traditional Van Soest using filtering glass crucibles (VS-ADF). Materials analyzed, chosen because of their extensive use for cattle feeding in Argentina, were: soybean meal, whole corn grain, whole sunflower seed, fresh forages of tall wheatgrass (*Thinopyrum ponticum*), alfalfa (*Medicago sativa*), guinea grass (*Panicum maximum*), barley grass (*Hordeum vulgare*), weeping lovegrass (*Eragrostis curvula*) and Italian ryegrass (*Lolium multiflorum*), and silages of corn and mixed legume-grass forages. Four runs were performed for each procedure and feed. Statistical comparisons showed that S-ADF results differed ( $P < 0.001$ ) from those of both VS-ADF and D-ADF. High linear relationships ( $R^2 > 0.99$ ) were found between both VS-ADF and D-ADF values and those of S-ADF. However, between-procedure differences found for some of the feeds analyzed indicate that the methodology used for ADF determination should be clearly identified when reporting results.

**Key words:** ANKOM technology, FDA determination, Filter bag technique, Traditional procedure

## Nota Técnica: Comparación de tres procedimientos para estimar la concentración de fibra en detergente ácido en alimentos de uso masivo en Argentina

**Resumen:** El objetivo de este trabajo fue comparar la concentración de fibra insoluble en detergente ácido (FDA) en alimentos muy utilizados en Argentina, obtenida mediante tres procedimientos analíticos diferentes. Los procedimientos comparados fueron dos involucrando la técnica de las bolsas filtrantes ANKOM®: consistentes en un caso en el tratamiento directo de las muestras con solución detergente ácido (D-ADF) y otro el tratamiento secuencial primero con detergente neutro seguido de detergente ácido todo sobre la misma muestra (S-ADF). El tercer procedimiento fue la técnica tradicional de Van Soest utilizando crisoles de capa filtrante (VS-FDA). Los materiales analizados, elegidos debido a su gran difusión para la alimentación del ganado, fueron: harina de soja, granos de maíz y semillas de girasol, pasto fresco de agropiro alargado (*Thinopyrum ponticum*), alfalfa (*Medicago sativa*), pasto guinea (*Panicum maximum*), cebada (*Hordeum vulgare*), pasto llorón (*Eragrostis curvula*) y raigrás anual (*Lolium multiflorum*), y ensilajes de maíz y de pasturas perennes coasociadas. Por cada procedimiento y alimento se practicaron cuatro corridas analíticas. Los análisis estadísticos mostraron que los resultados de S-ADF difirieron ( $P < 0.001$ ) tanto de VS-FDA como de D-FDA. Se encontraron relaciones lineales estrechas ( $R^2 > 0.99$ ) entre VS-ADF y D-ADF con S-ADF. Sin embargo, las diferencias encontradas entre los procedimientos analíticos para algunos de los alimentos indican que la

<sup>1</sup>Autor para la correspondencia, email: [jdanelon@agro.uba.ar](mailto:jdanelon@agro.uba.ar)

<sup>2</sup>Departamento de Producción Animal INTA, Estación Experimental Regional Agropecuaria, Balcarce

<sup>3</sup>Facultad de Ciencias Agrícolas, Universidad Católica Argentina, Buenos Aires

metodología utilizada para evaluar la concentración de ADF deberá ser identificada con claridad al reportar resultados.

**Palabras clave:** Determinación de FDA, Técnica tradicional, Tecnología ANKOM, Técnica de las bolsas filtrantes

## Introduction

Determination of the acid detergent fiber (ADF) content in feeds can be done by different analytical procedures. One of these is the traditional procedure developed by Goering and Van Soest (1970) (VS-ADF). In 1993 ANKOM® Technology (Macedon, NY, USA) introduced a semi-automatic instrument that uses filter bags of a standardized pore size (FBT), which has gained worldwide acceptance. In the FBT methodology, the feed samples are placed inside polyester filter bags and treated therein with an acid detergent (cetyl trimethyl ammonium bromide) solution. The residue of the treated sample is considered the ADF. This method is referred to here as direct treatment (D-ADF). The VS-ADF and the D-ADF procedures have been compared in several studies and similar results have been obtained by either procedure. In recent years, determining ADF after

first recovering the NDF residue in a sequential analysis in a single filter bag (S-ADF) has been described by Grabber and Mertens (2006). This method has been used to determine the ADF content of different forages. In Argentina, many laboratories have adopted the S-ADF procedure since it reduces the cost and the labor involved in the analyses, when compared with the D-ADF technique, if both NDF and FDA contents are to be determined on the same sample(s). However, there is no information available on the results rendered by each procedure when the same Argentinean feeds are evaluated. The goal of this work was to compare the results obtained by means of the S-ADF vs those obtained by the D-ADF and the VS-ADF, when these procedures are used to analyze the ADF concentrations in feeds widely used to feed ruminants in Argentina.

## Materials and Methods

### Analytical procedure

The experiment took place in the Animal Nutrition Laboratory at Balcarce Agricultural Research Station of the National Institute of Agricultural Technology (INTA). To obtain the ADF concentrations of the feeds, three different procedures were used: a) The technique (VS-ADF) developed by Goering and Van Soest (1970), in which the residues obtained after treating the feeds samples with acid detergent are recovered by means of glass filtering crucibles; b) A direct method (D-ADF) that uses polyester filter bags to both retain the feed samples while they are being treated with the acid detergent, and to retain the residues resulting from this treatment; c) A sequential procedure (S-ADF) in which the feed sample is placed in a polyester filter bag, and remains therein while it is consecutively treated with neutral (to obtain the NDF residue) and then acid (to obtain the ADF residue) detergents (Marichal *et al.*, 2006). Thus, both the NDF and the ADF values of the feed are obtained using the same single filter bag.

### Feeds

The feeds analyzed are among those widely used to feed ruminants in Argentina: soybean meal,

corn grain, sunflower seed, fresh forages of tall wheatgrass (*Thinopyrum ponticum*), alfalfa (*Medicago sativa*), weeping lovegrass (*Eragrostis curvula*) and Italian ryegrass (*Lolium multiflorum*), and silages of corn and mixed legume-grass pastures. Further, two standard feeds obtained in the USA were analyzed: guinea (*Panicum maximum*) and barley grasses (*Hordeum vulgare*). Samples were dried at 60°C for 48 h and ground to 1 mm in a Wiley mill. The ground samples were dried at 105 °C to constant weight and 0.5 g were placed in ANKOM polyester filter bags which were sealed by heat. Treatment with the detergents was done in an ANKOM<sup>220</sup> fiber analyzer (ANKOM Technology, Macedon, New York). Values of ADF obtained were expressed as percentages of the dry matter.

### Statistical Analysis

ADF values for each technique and feed were obtained from four duplicate sets. Comparisons of S-ADF values against VS-ADF and D-ADF values was by t-test of paired means ( $\alpha = 0.01$ ) considering values for each feed as a pair. In addition, regression analyses of S-ADF vs VS-ADF values and of S-ADF vs D-ADF values were carried out.

### Results and Discussion

The linear relationships between S-ADF and VS-ADF values, and between S-ADF and D-ADF values, were highly significant:

$$\text{VS-ADF} = -1.095 + 1.184 * \text{S-ADF}; R^2 = 0.995; n = 11$$

$$\text{D-ADF} = -1.659 + 1.200 * \text{S-ADF}; R^2 = 0.997; n = 11$$

The global comparison of S-ADF values against VS-ADF and D-ADF values revealed differences among them. Means obtained were 24.58, 28.01 and 27.83%, respectively. Comparisons for each feed separately are presented in Table 1. Differences ( $\alpha = 0.01$ ) between values of ADF obtained by the S-ADF method and by the other two methods were detected for most of the feeds, with the exception of alfalfa, soybean meal, and corn grain. The differences between S-ADF and VS-ADF in ryegrass values, and between S-ADF and D-ADF in grassland silage values, were significant at  $P < 0.05$ .

For most of the feeds, D-ADF values were higher than S-ADF values, the exception being those of corn grain. This difference can be attributed to the removal by the neutral detergent

of feed components (e.g. tannins, pectic substances) that are not removed by the acid detergent (Cassida *et al.*, 2007). The discrepancies between procedures should be taken into account when the ADF residues will be subsequently used for other analyses such as acid detergent insoluble nitrogen (Fay *et al.*, 2005) or lignin (Grabber and Mertens, 2006).

With respect to the variability between duplicate samples and among different runs the S-ADF method showed greater variability than the VS-ADF and D-ADF methods, although the variability was rather small in all three methods. Average variances for S-ADF, VS-ADF, and D-ADF between duplicate samples were 0.453, 0.172, and 0.267 and those among runs were 1.023, 0.649, and 0.374, respectively.

Due to the differences found in FDA values according to the analytical procedure used a precise citation or a description of the technique used in each case is very important when reporting results.

Table 1. Means and standard deviations of acid detergent fiber (ADF) concentration in common Argentine feeds as determined by three different analytical procedures

Feeds	VS-ADF <sup>a</sup>		D-ADF <sup>b</sup>		S-ADF <sup>c</sup>		p-Values	
	Mean	SD	Mean	SD	Mean	SD	VS-ADF vs. S-ADF	D-ADF vs. S-ADF
	(%)							
Barley grass	32.60	0.87	33.81	0.54	29.57	0.90	0.00	0.00
Alfalfa	19.16	0.23	18.93	0.37	18.22	0.96	0.11	0.22
Ryegrass	24.96	0.64	25.61	0.34	22.59	1.50	0.03	0.01
Tall wheatgrass	38.10	1.56	38.09	0.49	33.26	0.92	0.00	0.00
Corn silage	29.57	0.89	28.49	0.61	24.59	1.08	0.00	0.001
Grass silage	26.65	0.78	26.67	1.00	24.27	1.07	0.01	0.02
Weeping lovegrass	44.38	0.49	43.59	0.73	37.44	0.76	0.00	0.00
Soybean meal	8.34	0.78	7.62	0.17	7.56	0.91	0.24	0.90
Corn grain	3.33	0.71	2.80	0.47	3.11	0.91	0.72	0.56
Sunflower seed	35.66	0.69	34.63	1.03	30.89	1.04	0.00	0.00
Guinea grass	45.41	0.52	45.89	0.33	38.88	0.89	0.00	0.00

<sup>a</sup>Traditional Van Soest's method (Goering and Van Soest, 1970)

<sup>b</sup>Filter bag method (ANKOM Technology)

<sup>c</sup>Sequential method (Marichal, Carriquiry, and Trujillo, 2006)

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