Reprod. Nutr. Dev. 43 (2003) 431–436

DOI: 10.1051/rnd:2003035

Short communication

FibreBags vs. FibreCaps for acid and neutral detergent fibre analysis

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(Received 16 December 2002; accepted 8 August 2003)

Abstract — A new procedure for determining acid detergent fibre and neutral detergent fibre (ADF and NDF) was developed to reduce the need for filtration and to allow for batch processing of forage samples. The FibreBag system is an economically necessary evolution of the earlier FibreCap system. The purpose of this enquiry was to determine if the FibreBag is a suitable replacement for the FibreCap. The FibreBag method produced very similar results to the FibreCap system of analysis and ranked the various forage samples ion the same order. All the results suggest that the FibreBag is suitably similar in performance to the FibreCap system.

NDF, neutral detergent fibre / ADF, acid detergent fibre / sainfoin, *Onobrychis viciifolia* / birdsfoot trefoil, *Lotus corniculatus*

1. INTRODUCTION

The evolution of detergent fibre methods has produced several amendments that have been applied to improve the methods [1]; however, they are still very time consuming and require careful individual handling, which limits the efficiency of the procedure [2]. Work by Komarek et al. [2, 3] developed a method of fibre analysis that allows for multiple forage samples to be analysed at the same time by sealing the samples into individual "filter bags", and then "batch processing" them, using the ANKOM filter bag system. These bags also allow for more rapid filtration after refluxing and therefore make work easier for the analyst [2].

Based on this concept of batch processing, the FibreCap system of forage analysis was developed [4]. It is a further refinement of the Van Soest method of fibre analysis, allowing a lab technician to analyse more samples in a shorter period of time utilising standard laboratory equipment and reducing experimental error resulting from the older method [4, 5]. In several studies Kitcherside et al. [4] found that FibreCaps were a very good substitute to more laborious methods. They were also able to obtain lower residual variation with the FibreCap method.

The FibreCap system is much faster, and therefore cheaper than traditional methods of fibre analysis, with a technician being able to process six times as many samples in

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an hour than with conventional methods, and 3.75 times more samples than with the ANKOM system [4]. There is also a large reduction in solvent needed for this improved method. However, the costs per sample can still be further reduced. So as a result, Gerhardt UK Ltd (Brackley, Northants) developed the FibreBag.

The FibreBag is an open topped mesh bag that uses much less plastic in its construction than the closed top FibreCap. This means that they are both cheaper to produce, and more cost effective for the manufacturer to ship over large distances to different forage testing laboratories. Since they use the same laboratory equipment as the FibreCap they also have the same benefits of the number of samples per hour relative to the older methods.

This study was initiated to compare the effectiveness of FibreBags against that of FibreCaps to determine if they are a suitable replacement for the FibreCaps in forage testing laboratories. Four different temperate forage legumes were used for this evaluation: birdsfoot trefoil (*Lotus cornicultus* L.), sainfoin (*Onobrychis viciifolia* Scop.), red clover (*Trifolium pratense* L.), and lucerne (*Medicago sativa* L.). The lucerne and red clover was produced from three harvests from demonstration areas on the campus of the Royal Agricultural College. The sainfoin and birdsfoot trefoil came from a single harvest of two replicates of a variety trial that included 7 birdsfoot trefoil varieties and 7 sainfoin varieties. These samples were harvested in the autumn of the establishment year. All samples were grown on plots at the Royal Agricultural College, Cirencester, UK (lat. 52° 42' 30" N, long. 01° 59' 40" W).

2. MATERIALS AND METHODS

The samples were first oven dried at 80 °C, ground using a Glen Creston mill with a 0.75 mm screen, and stored in resealable plastic bags. The use of a higher drying temperature for the forages, 80 °C, as opposed to 60 °C, the normal drying temperature for samples used for forage quality analysis [1] can be justified because this study was a relative comparison of two methods of analysis and not an absolute comparison of the quality of forage samples, where the higher drying temperatures would have created distortions in the forage quality. However, because this is only a relative comparison of two methods, the author argues that as long as the two methods use the same population of samples the effect of the higher temperature is negated. Before the fibre analysis, the FibreBags (FB) and FibreCaps (FC) were numbered, dried at 100 °C for 1 h, desiccated for 5 min, and weighed. An excess of each of the forage materials, approximately seven grams, was re-dried at 100 °C for 24 h and then the individual samples were weighed before analysis. The samples were re-dried because in the case of lucerne and red clover it was possible that several months may have passed between the harvest date and the analysis. Sample dry weights for the ADF analysis were from 0.65 to 0.75 g (weighed to 0.1 mg), for the NDF analysis the sample size was from 0.45 to 0.55 g (weighed to 0.1 mg). Immediately after weighing, the samples were placed into the correct FibreBag or FibreCap.

The FibreBags require a plastic insert to keep the bag open to allow the reflux action to work properly. The FibreBags and FibreCaps were then placed in a carousel with six per carousel. These carousels were in turn placed in a 1000 mL beaker, with 360 mL of acid detergent solution or neutral detergent solution. The carousels were agitated in the solution to thoroughly wet the forage samples and remove excess air from the FibreCaps. The beakers were placed onto a heating element under a reflux condenser, and brought to a boil within 5 to 10 min. The heat was then reduced to maintain a gentle boil and boiled for 60 min. After 30 min of boiling, the sides of the FibreBags were washed down using a syringe [4].

Table I. Mean squares of acid detergent fibre (ADF) and neutral detergent fibre (NDF) concentration comparing FibreBags (FB) versus FibreCaps (FC) of the samples grouped as all the samples and the four forage legume species.

	Mean squares for traits								
	All samples			Lucerne			Birdsfoot trefoil		
Source	df	ADF†	NDF	df	ADF	NDF	df	ADF	NDF
		g·kg^{-1}			g·kg^{-1}			g·kg^{-1}	
Method	1	2 691***	41 646***	1	179*	1 691*	1	665*	19 227***
Entry	33	19 433***	10 055***	2	23 876***	5 755***	13	18 381***	14 032***
Method × Entry	33	186	512	2	102	42	13	253	568
Residual	134	141	505	10	32	226	54	160	469
	Red Clover			Sainfoin					
	df	ADF	NDF	df	ADF	NDF			
		g·kg^{-1}			g·kg^{-1}				
Method	1	244	1 045**	1	1 733**	21 132***			
Entry	2	36 286***	3 194***	13	10 672***	6 919***			
Method × Entry	2	10	174	13	193	588			
Residual	10	57	112	54	151	705			

*, **, *** Significant at 0.05, 0.01 and 0.001 levels of probability, respectively.
† ADF = acid detergent fibre, NDF = neutral detergent fibre, FB = FibreBag, FC = FibreCap.

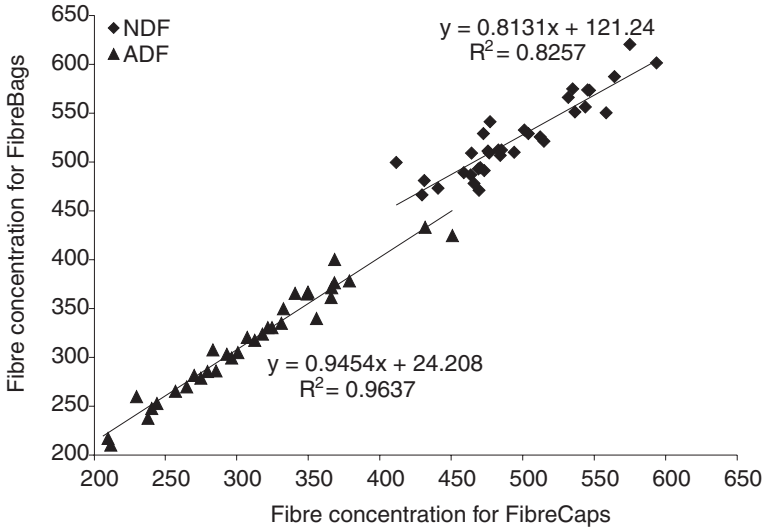

Figure 1. Comparing FibreBags and FibreCaps mean forage acid detergent fibre (ADF) and neutral detergent fibre (NDF) concentration across four forage legume species ($g \cdot kg^{-1}$).

Table II. Pearson product moment and Spearman rank correlation coefficients (*r*) of FibreBag and FibreCap analysis of acid detergent fibre (ADF) and neutral detergent fibre (NDF) for the forage samples.

Method	*n*	Pearson	Spearman
		r	
All samples			
ADFa	34	0.982***	0.986***
NDF	34	0.909***	0.916***
Lucerne			
ADF	3	0.997*	1.000***
NDF	3	0.987	1.000***
Birdsfoot trefoil			
ADF	14	0.975***	0.998***
NDF	14	0.929***	0.995***
Red clover			
ADF	3	0.999**	1.000***
NDF	3	0.900	1.000***
Sainfoin			
ADF	14	0.985***	0.998***
NDF	14	0.855***	0.993***

*, **, *** Significant at 0.05, 0.01 and 0.001 levels of probability.

The FibreBags and FibreCaps were rinsed in hot water (95 to 100 ºC) 4 times to remove all of the detergent solution. The FibreCaps were removed from the carousels, and oven dried at 100 ºC for 24 h and re-weighed. The FibreBags require an extra step of removing the plastic insert; this was done by gently rinsing the insert with water as it was removed from the FibreBag, to prevent loss of the sample material. The re-dried samples were weighed; the ADF and NDF concentrations were calculated in the manner detailed in Vogel et al. [6].

A randomised complete block design was used in this study with three replicates. Similar to the Vogel et al. [6] design, methods and entries were fixed effects. Analysis of variance was applied to all samples as well as to each of the perennial forage legumes separately, lucerne, birdsfoot trefoil, red clover, and sainfoin by using GenStat [7] (Tab. I). Linear regression between two sample containers was performed with a separate lines for both ADF and NDF using GenStat 5 release 4.2 [7] (Fig. 1). A randomised complete block design was used in this study with three replicates. The Pearson product and Spearman rank correlation were also performed on the data (Tab. II).

3. RESULTS AND DISCUSSION

The analysis of variance (Tab. I) comparing all ADF samples, lucerne samples and birdsfoot trefoil samples and the sainfoin samples shows that there was at least a ($P < 0.05$) difference for the method and entry as sources of variation. Only the ADF analysis of the red clover samples had no difference between the two methods. NDF had ($P < 0.05$) differences for the method and entry across all of the samples and the species specific groupings of samples (Tab. I). There were ($P < 0.001$) differences between the FibreBag and FibreCap methods for all samples for both ADF and NDF. There were no significant differences in the mean squares of the interaction between method and entry.

The ADF analysis provided a difference in means between the FibreBags and the FibreCaps of only 8 $g \cdot kg^{-1}$, and the NDF analysis was 28 $g \cdot kg^{-1}$. These small differences were comparable to the results obtained with the ANKOM system [1–3]. For both ADF and NDF, the mean concentrations of fibre of the FibreBag were higher than the FibreCaps. In this analysis the FibreBags were made with a 7 micron smaller mesh than the FibreCaps, possibly suggesting that some of the smaller material would be able to escape from the FibreCaps that was not able to escape from the FibreBags. This coupled with the fine grinding standard 0.75 mm explains why the FibreBags had higher mean concentrations of ADF and NDF. An increase in the grind size from 0.75 mm to 1 mm might improve the tightness of fit of the results. Both ADF and NDF had a ($P < 0.001$) correlation coefficient between FibreBag and FibreCap results (Fig. 1). The slope of the equation for the mean results of the ADF analysis, 0.9454x, illustrates that there was a strong ($P < 0.001$) relationship between the two different techniques. In the case of the NDF analysis, the correlation is not as strong, 0.8257x, but is still highly significant ($P < 0.001$). The mean standard deviations for NDF and ADF were similar, both for the FibreBags and FibreCaps, suggesting that a consistent result is possible when using the FibreBag for fibre analysis.

The Spearman rank correlation was used to see if the FibreBags and FibreCaps ranked the samples in a similar fashion. In all samples and the legume subsets there is a ($P > 0.001$) correlation between FibreBags and FibreCaps for both ADF and NDF (Tab. II). The results indicate that both procedures of fibre analysis ranked samples in the same relative order for all of the legume subsets.

The Pearson product moment correlation was also performed on the samples and yielded similar results to the Spearman rank correlation with two exceptions. The NDF

analysis for both lucerne and red clover did not appear to be significant, even with large correlation coefficients (Tab. II). This is a result of the small sample size (n = 3) and may have been different with a larger number of samples.

The slightly higher mean yields for ADF and NDF in the FibreBags (Fig. 1) could be the result of flow constrictions produced by the inserts in the FibreBags and would be solved by the modification of the insert to improve solution-solid contact. There was, however, enough of a similarity in the results of this investigation to suggest that forage analysis with FibreBags (NDF and ADF) after some more modifications and refinements will be a suitable replacement for the FibreCaps.

When the small standard deviation and the high correlation coefficients are considered along with the price advantage of FibreBags over FibreCaps and the increases in efficiency that they offer a technician, the FibreBag appears a far superior alternative to the FibreCaps. Like the ANKOM filter bags, the FibreBags require little lab space and the bags are disposable, so there is little maintenance required. For those who already use the FibreCap system of analysis there are no major changes in laboratory equipment required, and therefore no large capital outlays required. For others, most of the equipment required is in most forage laboratories, i.e. heating elements, cold water condensers, and 1000 mL beakers with handles. The only additional equipment required is the carousels and FibreBag inserts. In short, this manipulation of the established method of forage fibre analysis (Van Soest fibre analysis) will enable a larger number of samples to be processed in a short period of time by fewer technicians with lower capital and consumable costs.

ACKNOWLEDGEMENTS

The author would like to thank C. Gerhardt UK Ltd, for providing materials and funding for this project. We would also like to thank S. Duncan for her help and support with the laboratory portion of this project. Finally, thanks to Dr G. Smith for his advice and help with the statistical analysis of this study.

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