In Vitro and Chemical Determination of Fibre and Digestibility of common European Feedstuffs Using the ANKOM Fibre Analyzer and the ANKOM Daisy *In Vitro* Incubator

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SUMMARY: ANKOM Fibre analyzers and In vitro incubators have been shown as a reliable and accurate method for performing Acid Detergent Fibre (ADF), Neutral Detergent Fibre (NDF), Crude Fibre (CF) and Acid Detergent Lignin (ADL) determinations. Batch processing of samples greatly increases laboratory efficiency and reduces technician-to-technician variability. The result of this work indicates that ANKOM systems are a rigorous and precise alternative to performing the labor intensive conventional methods for like analysis on feedstuffs common in the European market. NDF studies produced a median standard deviation (SD) of .0484, while CF and ADF median SD's are reported at 0.212 and 0.187 respectively. ADL determinations produced a median SD value of 0.120. Additionally, numerous studies of NCGD determinations were made to examine ANKOM systems ability to produce equally precise results using M.A.F.F. approved procedures and enzymes. Neutral Cellulase Gamanase Digestibility (NCGD) studies indicated that the ANKOM systems produce high precision even with modifications to both (time and enzyme ratio) factors. Standard deviations as low as 0.001 and bias' well within M.A.F.F. guidelines indicate great promise. The ability to increase laboratory efficiency without a loss of precision and the potential to reduce the cost of consumables indicates that the ANKOM systems are a valuable asset to be considered.

Introduction

The information in this paper was prepared to document the results of numerous studies designed to examine the effectiveness of the ANKOM Filter Bag Technique (FBT) in determining NDF, ADF, CF, ADL and NCGD on samples obtained from Europe.

Due to time constraints, work demands on laboratories contacted for comparative studies and reports of inter-lab bias, we were unable to obtain comparative data from a European laboratory source. As a result, this paper is a release of an internal ANKOM Technology research report to record preliminary findings evaluating system precision and rigorousness.

The FBT utilizes a specially manufactured filter bag that retains particles $\geq$25 microns in size. Each filter bag allows for the encapsulation of a quantitative aliquot for solublization and/or extraction.

The 3-D filtration matrix produced by the filter bag creates filtration efficiency equal to or greater than that produced by the standard gooch crucible.

Comparative studies have demonstrated that results obtained via the FBT are consistent with those achieved through conventional means as defined by the AOAC and/or USDA publications.

The ANKOM Fibre Analyzer was first introduced to the marketplace in 1993 for the analysis of NDF and ADF. Initially researchers from colleges and universities recognized the potential of the instrument to increase their analytical capacity and efficiency. Use of the FBT grew as the method proved itself in these research laboratories.

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Introduction (con't)

The accuracy of the FBT was verified through numerous studies conducted with Dr. P.J. Van Soest (the originator of fibre analysis) at Cornell University. The comparison of the standard Van Soest methods and the FBT using the same solutions indicated that the two methods were equivalent for a wide variety of feeds (Komarek, Robertson and Van Soest, 1993 and Komarek, et. al. 1994).

As data accumulated on the effectiveness of the FBT, commercial laboratories evaluated the method and adopted it as their standard method recognizing the efficiencies and cost effectiveness provided.

It was obvious that the efficiencies of batch processing of filter encapsulated samples could be applied to other analyses such as CF and *In Vitro* procedures. Several users adapted AOAC method 962.09 for the analysis of CF to the ANKOM system and validated it in their laboratories. ANKOM initiated a series of studies to expand the database and refine CF using the FBT (Komarek et. al. 1996). Today laboratories in over fifty countries utilize ANKOM systems to make precise and accurate determinations of fibre.

The development of the ANKOM Daisy^{II} Incubator was the result of some cooperative work with several ANKOM users that had expressed a need for a more efficient method for determining *In Vitro* True Digestibility (IVTD) or *In Vitro* Apparent Digestibility. J. M. A. Tilley and R. A. Terry (1963) developed the most common International reference method for *in vitro* digestibility so research was focused in this area.

Comparative studies for IVTD were performed at Cornell University. In these studies eight forage samples (including a haylage) were analyzed for IVTD. The 48-hour method followed by a NDF procedure demonstrated that there was no statistically significant difference (Table 1/Graph 1) between methods.

Since it's introduction in late 1994, users have further validated its use in additional studies. The majority of known research on the Daisy Incubator revolves around 48 hour IVTD and rate studies using rumen innoculum (Traxler, 1995) (Cohen, 1998).

Materials and Methods

Twelve - fifteen samples covering a wide range of fibre, fat/oil and starch compositions were provided by Central Laboratories of Banbury UK for analysis. The objective was to evaluate the broadest range of European sample types. Problematic samples [high (18%) oil and (60%) starch] were included to evaluate the limits of the technique. Although the objective was to evaluate ANKOM systems for determining NDF and NCGD additional time was spent to further validate CF, ADF and ADL.

A complete description of the methods used is included in Appendix A - E. These methods describe the complete process utilized by the FBT. To evaluate the impact of the acetone pre-extraction, one analytical run was performed without the pre-extraction step. To evaluate the impact of the sodium sulfite addition, one analytical run was performed without sodium sulfite. In all other analyses all samples were subjected to a pre-extraction with acetone.

A series of NCGD studies were accomplished to evaluate the precision and modifications to time and enzyme to sample ratios. In one study four runs in duplicate were completed with three runs following the 40 hour digestion time defined in the M.A.F.F procedure and one run reducing the digestion time to 36 hours.

Materials and Methods (con't)

A second NCGD study was performed with two runs following the prescribed enzyme to sample ratio defined by M.A.F.F. and two runs reducing the enzyme ratio to 28ml per 0.5g sample and 26ml per 0.5g sample respectively. These studies also began to examine the minimum number of samples that can be analyzed per the systems digestion vessels. In total, over 200 NCGD assays were completed.

Results and Discussion

CF determinations (Table 2/Graph2) using the FBT produced precise results in evaluating fifteen sample types. Standard deviations (SD) ranged from a high of 0.892 to a low of 0.021. The median SD was 0.212. The CF content ranged from a low (Wheat 72Kg/Hl) of 3.36 to a high (Nutritionally Improved Straw) of 38.98

NDF determinations (Table 3) were equally precise. With seven sample types analyzed in six analytical runs SD values ranged from a low of 0.229 for a wheat sample to a high of 0.722 for a Nutritionally Improved Straw. The median was 0.484. Over 100 assays were performed with no re-runs required.

It has been determined that pre-extraction with acetone is necessary for soy bean and high fat samples (>5%). Pre-extraction with acetone had no adverse effect on low fat or non-soy bean samples.

Study 7 (Table 3) provides results of samples analyzed without the addition of sodium sulfite during extraction. The lack of sodium sulfite appears to effect only two samples, Beet MLSD pulp and Croda Rape Ext. As reported by Hintz et al. (1996) sodium sulfite is needed to "reduce nitrogenous contamination in fibre analysis." Sodium sulfite has the greatest impact when analyzing processed samples or samples subjected to heat levels above 60° C.

Table 4 presents the ADF results of ten sample types run in duplicate. The agreement between duplicates was excellent. ADF results are used in some energy models and are is precursor step to ADL determination. Likewise, the ADF result can be used to further isolate the hemicellulose component of the plant cell wall. The ADL (72% H_2SO_4 method) results (Table 5) demonstrates excellent agreement between duplicates.

NCGD Results

As can be seen in Table 6, there was no difference between the values determined at thirty-six and forty hours of digestion when using the ANKOM FBT. SD values ranged from a low of 0.001 to a high of 0.011 with a median of 0.004. The NCGD values determined ranged from a low of 39.2% to a high of 93.7%.

Table 7 depicts the results of 128 assays that were performed to determine if the ratio of enzyme to sample could be reduced without negatively impacting the expected results. The ratio of enzyme to sample for digestion jars 1 and 2 being consistent with the 30ml per sample requirements listed in the M.A.F.F. procedure. The results of digestion jars 3 and 4 depict the results when the enzyme ratio is decreased to 28ml and 26ml per sample respectively. The reduction in the ratios of enzyme to sample did not reduce the digestibility values.

With the reduction of enzymes, the tests also confirmed that each digestion jar is capable of as little as 720ml of buffer/enzyme solution to 24 samples (30ml/0.5g) or as many as 39 samples to 1000ml of solution. This further suggests that the system may be able to handle as many a 78 samples per digestion jar (308 samples per run), as each jar is capable of 2000ml of a solution.

Conclusions

The FBT offers a precise and rigorous method for the efficient determination of NDF, CF, ADF and ADL. For NCGD studies the FBT:

- Produced excellent precision.
- Permitted the reduction of incubation times.
- Supported the reduction of enzyme to sample ratios.
- Reduced the technician time requirements.
- Greatly increased capacity
- Reduced laboratory space requirements

It is the conclusion of this report that the FBT presents a viable alternative to the conventional means used in the United Kingdom for determining NDF, CF, ADF, ADL and NCGD.

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