Effect of Method Variation on the Determination of aNDF Using the ANKOM Filter Bag System

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Introduction

The ANKOM system is a semiautomatic method for measuring fiber that allows 24 samples to be analyzed simultaneously. Samples are sealed in filter bags that are extracted in a pressurized chamber with vertical agitation. Pressure is achieved by heating reagent solutions in the extraction chamber after it is sealed. This pressure prevents the filter bags from ballooning which helps to ensure that reagent solutions pass through the filter bags during extraction. The ANKOM system has the advantage that filtering difficulties associated with the use of crucibles are eliminated, the number of samples that can be analyzed daily is increased, and imprecision due to variation among technicians in analytical technique may be reduced because the system is semiautomatic. However, the effects of variation in the procedure used with the ANKOM system have not been evaluated or compared with the traditional crucible reflux method. The objective of this research was to determine which steps in the ANKOM procedure have a significant impact on the determination of amylase-treated neutral detergent fiber (aNDF).

Materials and Methods

Three experiments were conducted to identify critical steps and evaluate the effects of small differences in the recommended procedure (ruggedness) on the analysis of aNDF using the ANKOM filter bag system. Each experiment was an incomplete factorial design. In experiment 1, alfalfa silage, corn silage, red clover hay, barley hay, corn stover, alfalfa pellets, citrus pulp, wheat midds, corn grain, roasted soybeans, and expeller soybean meal were analyzed in duplicate within batches. Duplicate samples were separated to be on either the top and bottom trays in each batch and a specific set of seven differences in method were evaluated in one of 8 batches:

- (**A**) mixing sodium sulfite and amylase in neutral detergent (ND) solution before adding it to the extraction chamber vs. (**a**) adding ND solution, sulfite and amylase to the chamber sequentially without mixing,
- (**B**) using 1800 ml of ND vs. (**b**) using 2100 ml of ND,
- (**C**) start with extraction chamber at room temperature or cooled with cold tap water vs. (**c**) start with extractor warm after a previous extraction,
- (**D**) mixing amylase with hot wash water before adding to the chamber vs. (**d**) adding hot water and amylase sequentially,
- (**E**) using boiling water and heating chamber with lid sealed vs. (**e**) using hot water (80-90 ºC) with no heating,
- (**F**) soaking with water for 5 min each time (first 2 with amylase followed by 2 without) vs. (**f**) soaking for 3 min (first 2 with amylase followed by 1 without), and
- (**G**) after ND extraction, soaking with 240 mls of acetone for 5 min with swirling at 0, 2, and 4 min. vs. (**g**) soak in minimum amount of acetone (about 200 mls) for 3 min. without swirling.

In experiment 2, the effects of mixing sodium sulfite and amylase (treatments A vs. a and D vs. d) were investigated using a washing method that maximized pressure in the chamber during the water soakings:

- (**H**) using 2100 mls of hot water (80-90 ºC) and heating the chamber with the lid sealed vs. (**h**) using boiling water with no heat.

In this experiment, 2000 mls of ND was used and other factors were held constant using treatments C, F, and G. In addition to the materials used in experiment 1 (except citrus pulp, roasted soybeans, and expeller soybean meal), single samples of brewer's grains, grass silage, hominy feed, distiller's grains, wheat straw, meat meal, oat grain, SoyPlus, bermudagrass hay, birdsfoot trefoil hay, corn gluten feed, sunflower meal, and high moisture ear corn were evaluated in each batch and the tray location of samples within the extractor was varied among batches. Materials were selected to have a greater proportion of feeds that were heated or contained starch which should be most sensitive to sulfite and amylase mixing.

In experiment 3, factors A, C, D, F, and G were held constant and deviations in soaking (treatments H vs. h) were evaluated with variations in pre-extraction methods:

- (**I**) pre-extract all samples twice in 240 mls of acetone that was shaken 10 times then soaked for 10 min vs. (**i**) pre-extract all samples once in 240 mls of acetone for 10 min without shaking or swirling, and
- (**J**) increase last soaking of treatments I and i to 6 hr vs. (**j**) no 6 hr soaking of treatments I and i.

Materials were the same as in experiment 2 except that alfalfa pellets, wheat midds, hominy feed, meat meal, and high moisture corn were replaced with raw soybeans, a feed mixture containing fat, roasted soybeans, Puma cottonseed, and rice mill feed (containing fat) to increase the number of materials that contained fats.

In all three experiments, statistical analysis was done on results expressed as deviations from the traditional crucible reflux method for each material. Least square means for each sample-treatment combination were tested with all other treatments adjusted to their mean response.

Results and Discussion

In experiment 1, the effect of post-extraction with acetone (G vs. g) was highly significant overall primarily due to its effect on roasted soybean material (G = +2.10 vs. g = +13.29). The high deviation and large variation in aNDF caused by this treatment on roasted soybeans that were not pre-extracted, tended to mask all other treatments; therefore, the roasted soybean material was removed from the data set for the remaining statistical analyses. Deviations in aNDF from the crucible method was different for soaking time (F = +0.58 vs. f = +0.85) and location in the extraction chamber (bottom half = +0.62 vs. top half = +0.82). The ANKOM system contains 8 vertical trays that each hold 3 bags. In experiment 1, there was a linear effect due to tray with a change in aNDF of -0.063 per tray from top to bottom. The effect of soaking time (F vs. f) was due primarily to significant differences for alfalfa pellets, alfalfa silage, and red clover hay (average F = +0.25 vs. f = +0.98 for these legume materials). Although mixing amylase and sulfite with ND (A vs. a) was not significant over all materials, this treatment was significant for two starch containing feeds, corn silage and wheat midds (average A = -0.01 vs. a = +0. 70). When both crucible and ANKOM aNDF were adjusted for blanks, mixing amylase in the soaking water before adding it to the chamber (D vs. d) was significant (D = +0.08 vs. d = +0.32).

In experiment 2, the overall deviations in aNDF were different when water was heated during the soaking procedure (H = +0.64 vs. h = +0.98) and there was a significant linear effect due to tray location from top to bottom (-.090/tray). Heating affected deviations in aNDF for corn stover and sunflower meal (average H = -.06 vs. h = +1.45). Although aNDF measured by the ANKOM method had large deviations from the crucible method for distiller's grains and meat meal, heating the water during soaking reduced the magnitude of the discrepancy (average H = +3.48 vs. h = +4.48). The lack of an overall effect of mixing sulfite and amylase with ND may have been related to an unexplained significant inverse effect for distiller's grain, meat meal, and sunflower meal (average A = +3.57 vs. a = +2.27) compared to all other materials. Mixing of amylase with soaking water was not significant overall or for any single material.

In experiment 3, shaking of materials in acetone during pre-extraction significantly lowered the deviations from aNDF measured by the crucible reflux method (I = -0.41 vs. i = +0.27) apparently due to the loss of particles from many materials, especially those that were ground finely. Of the materials containing fat, shaking improved the measurement of aNDF only for Puma cottonseed (I = +1.32 vs. i = +2.86). Soaking materials in acetone for 6 hr did not improve the measurement of aNDF in Puma cottonseed (J = +2.78 vs. j = +1.27), but it lowered the deviation in aNDF from the crucible method for brewer's grains, one of the roasted soybeans and wheat straw (J = -0.64 vs. j = +0.76). Heating the water during soaking significantly lowered the deviations in aNDF when corrected for blanks (H = -0.46 vs. h = -.14) for all samples, and also for one of the roasted soybeans and sunflower meal (average H = -1.23 vs. h = +.06). The negative overall average deviation for treatment H was due to losses of fiber on many samples during pre-extraction. There was linear effect due to tray location (-.085/tray) from top to bottom.

Summary and Conclusion

It appears that small differences in volume of ND (B vs. b) or starting temperature of the extraction chamber (C vs. c) had little effect on aNDF analyses using the ANKOM system. Treatment E may have been ineffective because using boiling water and heating it in a sealed chamber did not create enough pressure to collapse the filter bags and aid the washing of fiber residues. The lack of significant difference for most treatments and the small magnitude of the difference (about 0.3%-unit of aNDF) when they were statistically significant indicates that the ANKOM system is relatively rugged for the average material. To be valid, however, an analytical method must obtain accurate results for each type or sample of material. Averaging results across all samples can mask the need for a particular procedural treatment for a specific sample or type of material. Significant differences for procedural treatments for individual samples or materials were often greater than 0.7%-units. The procedural treatments (and the material affected by it) that are recommended for the ANKOM system include: A (starchy materials), D (all materials), F (all materials, especially legumes), G (fatty materials that are not pre-extracted), and H (all materials, especially corn stover, sunflower meal, roasted soybeans, distiller's grains, and meat meal). Treatment I improved aNDF analysis for only one of the fat-containing materials, but resulted in a significant loss of fiber when used for all materials and cannot be recommended. Treatment J did not improve aNDF analysis for fatty materials and caused several feeds to have deviations from the crucible reflux method that went from significantly positive to significantly negative and cannot be recommended.