# Crude Fiber Determinations Using the ANKOM System

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## INTRODUCTION

Crude fiber (CF), as defined by Thaer (1) and Henneberg (2), was originally developed to fractionate feeds between nutritionally available and unavailable components. The Conventional Technique (CT) for determining CF involves successive refluxing in 0.255N $H_2SO_4$ and 0.313N NaOH. It quantitatively recovers cellulose while hydrolyzing the majority of non-structural carbohydrates along with most of the hemicellulose and lignin (3). Though the CF fraction is neither chemically nor nutritionally uniform it is important as a relative value and is a regulatory requirement on feed labels. The present CT is laborious because it requires samples to be individually processed. The filtration steps and the associated sample handling are time consuming and are main factors contributing to loss of precision.

This paper describes the Filter Bag Technique (FBT) that was originally developed for determining acid and neutral detergent fiber (4,5). The FBT involves enclosing a discrete sample in a filter bag and processing the sample throughout the entire reflux and rinsing steps. The use of filter bags permitted batch processing of samples and reduced sample handling. A comparison between the FBT and the CT for determining CF was conducted on a wide range of forage and feed samples. Samples were chosen to represent a broad section of feeds typically analyzed for CF.

## MATERIALS AND METHODS

Twenty-two feed samples were analyzed for CF using the CT and the FBT. Seven AAFCO check samples were evaluated along with pelleted alfalfa and mixed feeds. Samples were oven dried and ground through a 1mm screen. The CF determinations for both techniques were performed at South Dakota State University. The sample size and reagents were the same for both techniques. The ratio of acid and base solution to sample size for the CT and FBT were 200 ml/g and 90 ml/g respectively. All samples were extracted with ether prior to refluxing and presented on a ash-free basis.

### Conventional Technique

The conventional CF method was performed according to AOAC 978.10 protocol (6). Most of the CT results were run in duplicate.

### Filter Bag Technique

The FBT was performed using the ANKOM$^{200}$ Fiber Analyzer and #F57 filter bags. The FBT used to determine CF was as follows:

A. Prepare Filter Bags/Samples
   1) Weigh filter bag (ANKOM Tech. Corp.- #F57) record tare and zero balance. The bags have negligible moisture content and do not need to be pre-dried.
   2) Weigh 1.0 g (±0.05g) of sample directly into filter bag.
   3) Seal the bag closed within 1cm from the open edge using a heat sealer (ANKOM Tech. Corp. #1915).
   4) Spread the sample uniformly inside the filter bag. This should be done by shaking the bag to eliminate clumping.

B. Extract fat from samples by placing 24 bag/samples with samples into a 3L beaker and add 250 ml of petroleum ether. Place 2L beaker inside 3L beaker to keep bags submerged. Initially agitate bags by pushing 2L beaker up and down. Let samples sit for 5 minutes and then repeat step with new ether and air dry.

C. Place 24 bags in the Bag Suspender (ANKOM Tech. Corp. #F11). Place three bags per basket; 24 bags total. Stack baskets on center post with each basket rotated 120 degrees. The 9th basket remains empty and acts as a top for the 8th basket. The spring is placed on top of the 9th basket to keep the Bag Suspender submerged.

D. Add 2000 ml of ambient temperature acid (.255 N $H_2SO_4$) solution into ANKOM$^{200}$ digestion vessel. Turn Agitation and Heat ON (CLOSE, BUT DO NOT TIGHTEN LID). Heat acid solution to 95°C. WARNING: Do not allow solution to rise above 95°C; boiling solution will cause overflow on instrument. Submerge Bag Suspender and start 30 minute timer. Seal lid and allow temperature to rise to 100°C. NOTE: Less bags and solution can be used per study but a minimum of 1600 ml of solution is required in the digestion vessel.

E. After 30 minutes turn **Heat** and **Agitation** OFF. Open the exhaust valve and release hot solution before opening lid. WARNING: The solution in the vessel is under pressure. The exhaust valve needs to be opened to release the pressure prior to opening the lid.

F. After the solution has been exhausted close valve and open the lid. Add approximately 2L of hot (90°-100°C) rinse water and turn Agitator ON and leave the Heat OFF. Close the lid but do not tighten. Agitate the bags in rinse water for 3-5 minutes. Repeat hot water rinse twice.

G. Remove Bag Suspender and add 2000 ml of ambient temperature Base (0.313 N NaOH) solution into ANKOM$^{200}$ digestion vessel. Turn **Agitation** and **Heat** ON. Heat base solution to 95°C and then submerge Bag Suspender and set/start timer for 30 minutes. Maintain Heat and Agitation at 100°C until timer is complete..

H. After 30 minutes turn Heat and Agitation OFF. Open the exhaust valve and release hot solution before opening lid. Add approximately 2L of hot (90°-100°C) rinse water and turn Agitator ON and leave the Heat OFF. Close the lid but do not tighten. Agitate the bags in rinse water for 3-5 minutes. Repeat hot water rinse three times.

I. Remove filter bags from bag suspender and gently press out excess water. Place bags in a 250 ml beaker and add methanol to cover bags. Allow bags to soak 2-3 minutes then remove and lightly press out excess alcohol.

J. Spread bags out and let air dry. Complete drying in oven at 125°C for 2 hours. Remove from oven and place in a Desiccant Pouch (large zip-lock bag with small desiccant pacts inside) until cooled to ambient temperature and weigh. Ash entire bag/sample in pre-weighed crucible for 2 hours at 550°C, cool in desiccator and weigh.

$$\% \text{ Crude fiber} = \frac{( C - ( A \times 0.992 ) ) \times 100}{B}$$

A = Bag weight
B = Sample weight
C = Loss in weight on ignition of bag/sample
0.992 = Blank bag ash correction

## RESULTS AND DISCUSSION

The FBT and the CT correlated well and showed no significant difference between techniques ($P<0.10$). The Filter Bag Technique was performed in triplicate while the CT values are single or double data points. The mean values of all the samples for the CT=16.41% and FBT=16.47% (Table #1). The filter bags retain a small amount of inorganic material during refluxing. Therefore, a bag ash correction factor of 0.992 was used to account for the foreign inorganic material.

Table 1. Crude fiber comparison between the FBT and the CT for twenty-two feed samples.

| | Technique | |
|---|---|---|
| | Conventional[1] | Filter Bag[2] |
| Gilt Finisher | 2.17 | 1.94 |
| Poultry Food | 3.67 | 3.56 |
| Chick Feed | 3.87 | 3.67 |
| Extruded Supplement | 5.15 | 5.06 |
| Baby Pig Starter | 6.60 | 6.50 |
| Nutrena Herd Feed | 7.86 | 8.04 |
| Beef Cattle Feed | 8.23 | 10.15 |
| Beef Feed | 9.81 | 10.53 |
| Guinea Pig Pellets | 11.04 | 10.75 |
| Cottonseed Cake, 30% | 12.58 | 12.76 |
| Range-N-Gro 601 | 13.52 | 13.45 |
| Dakota Pride Cake | 14.99 | 15.06 |
| Calf Starter Feed | 16.14 | 15.75 |
| Rabbit Ration | 17.50 | 17.26 |
| Molasses & Roughage | 19.42 | 18.33 |
| Zip Cattle Cocci-Ban | 22.52 | 21.99 |
| Med-Flex BAN | 26.41 | 29.52 |
| Alfalfa Suncured Pellets | 29.58 | 29.07 |
| Alfalfa Suncured Pellets | 29.87 | 29.69 |
| Alfalfa Bulk Pellets | 29.27 | 28.96 |
| Alfalfa Dehydrated Pellets | 30.56 | 31.29 |
| Mixed Hay | 40.17 | 38.92 |
| Mean Crude Fiber | 16.41 | 16.47 |

[1]Value is from single data point.
[2]Value is average of three data points.

The FBT demonstrated good precision; the average CV was only 2.64% (Table 2). By removing the manual filtration required by the CT and eliminating most of the sample handling and transfer steps the FBT demonstrated high reliability. There were no outliers in the sixty-six CF determinations performed using the FBT. Contrary to the CT, the FBT allows soluble components to be removed through the filter bag during refluxing. This is beneficial in that these soluble components, if precipitated, are outside of the filter bag and cannot influence the fiber value. Precipitation of soluble components during filtration in the CT can cause clogging and result in erroneously high values.

Table 2. Filter Bag Technique replication results.

| | Replication | | | | | |
|---|---|---|---|---|---|---|
| Sample Type | #1 | #2 | #3 | Average | sd | CV |
| Gilt Finisher | 1.78 | 1.96 | 2.07 | 1.94 | 0.15 | 7.56 |
| Poultry Food | 3.53 | 3.54 | 3.62 | 3.56 | 0.05 | 1.38 |
| Chick Feed | 3.50 | 3.75 | 3.77 | 3.67 | 0.15 | 4.10 |
| Extruded Supplement | 5.00 | 5.13 | 5.04 | 5.06 | 0.07 | 1.32 |
| Baby Pig Starter | 6.38 | 6.54 | 6.58 | 6.50 | 0.11 | 1.63 |
| Nutrena Herd Feed | 8.60 | 7.77 | 7.76 | 8.04 | 0.48 | 5.99 |
| Beef Cattle Feed | 9.97 | 10.41 | 10.06 | 10.15 | 0.23 | 2.29 |
| Beef Feed | 10.36 | 10.40 | 10.82 | 10.53 | 0.25 | 2.42 |
| Guinea Pig Pellets | 11.13 | 10.43 | 10.70 | 10.75 | 0.35 | 3.28 |
| Cottonseed Cake, 30% | 12.58 | 12.62 | 13.08 | 12.76 | 0.28 | 2.18 |
| Range-N-Gro 601 | 13.27 | 13.23 | 13.84 | 13.45 | 0.34 | 2.54 |
| Dakota Pride Cake | 15.16 | 14.89 | 15.14 | 15.06 | 0.15 | 1.00 |
| Calf Starter Feed | 15.81 | 15.48 | 15.97 | 15.75 | 0.25 | 1.59 |
| Rabbit Ration | 17.77 | 16.96 | 17.04 | 17.26 | 0.45 | 2.59 |
| Molasses & Roughage | 18.31 | 18.09 | 18.59 | 18.33 | 0.25 | 1.37 |
| Zip Cattle Cocci-Ban | 22.70 | 21.34 | 21.92 | 21.99 | 0.68 | 3.10 |
| Med-Flex BAN | 29.69 | 29.12 | 29.75 | 29.52 | 0.35 | 1.18 |
| Alfalfa Suncured Pellets | 28.23 | 28.87 | 30.10 | 29.07 | 0.95 | 3.27 |
| Alfalfa Suncured Pellets | 29.72 | 29.02 | 30.32 | 29.69 | 0.65 | 2.19 |
| Alfalfa Bulk Pellets | 28.69 | 28.50 | 29.68 | 28.96 | 0.63 | 2.19 |
| Alfalfa Dehydrated Pellets | 31.47 | 30.13 | 32.28 | 31.29 | 1.09 | 3.47 |
| Mixed Hay | 38.36 | 38.89 | 39.5 | 38.92 | 0.57 | 1.47 |
| Mean | 16.46 | 16.23 | 16.71 | 16.47 | 0.39 | 2.64 |

The FBT required less reagents than the CT (90 ml versus 200 ml). The ability to analyze twenty-four samples at one time increased capacity over the CT threefold. By sealing the sample inside the filter bag the FBT eliminates handling and transfer errors. The CT required individual handling of samples and a separate rinsing step. In the FBT the filtration step was eliminated and samples were digested and rinsed in a batch process.

## CONCLUSION

The FBT accurately and precisely determines CF in a broad range of sample types. Great efficiency gains were realized when using the FBT versus the CT. Because the ANKOM$^{200}$ instrument automatically controls the temperature, agitation, and filtration the time constraints on the technician are greatly reduced. Using the ANKOM$^{200}$ instrument to perform the FBT will potentially improve the precision and accuracy of CF determinations for many laboratories.

## REFERENCES


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