



LECO FP 2000 Protein analyzer analyses nitrogen by the Dumas combustion method. The sample is combusted at 600 C and all gases except nitrogen is removed by a series of scrubbers. The nitrogen is then equilibrated with Helium at NTP and measured as percent nitrogen. Automation enables doing 40 samples at the rate of 5 - 6 minutes/sample.

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Errors can occur in the analysis of aqua feeds and ingredients due to various reasons. A brief description of possible reasons for errors and possible remedial measures are suggested where applicable. Confusion can occur when one compares the array of standard and official methods published by various government and private agencies through out the world. A typical example as in Table 1 lists three American and one European official method for analysis of moisture that also can include volatile matter other than water. This table only goes to show the complexities in a simple procedure such as determining the moisture content in a sample. When approved standard methods are followed they are accepted without question by the industry and peer reviewed publications and may be valid for legal settlements. However under a research laboratory condition one has the latitude to select the most applicable method and even make logical modifications. No attempt is made here to compare and contrast standard or official methods with methods used outside of these regulated methods. The article presents a logical approach what is expected of each of the analysis, likely problems and allows the reader to think about what is best.

Table 1: Methods for analysis of moisture

Name of standard	Number of methods	Suggested air oven methods
AOAC	More than 20	More than 5
AOCS	6	30 min. at 130°C±1
AACC	10	103-104°C for 3 hours
IUPAC	1	3 hours at 130°C±2

AOAC: Official methods of analysis of the American Official Analytical Chemists.

AOCS: Official methods and recommended practices of the American Oil Chemists' Society

AACC: Approved methods of the American Association of Cereal Chemists

IUPAC: Standard methods for the analysis of oils, fats and derivatives, International Union of Pure and Applied Chemistry

Errors in the Analysis of Aqua Feeds and Feed Ingredients

Accuracy and precision are both possible for single component samples such as pure proteins, fats, carbohydrates etc. and for samples with a known assortment of many components. Aquaculture feeds and feed ingredients contain an array of undefined components from which one finds out the composition of the desired analytes. The analysis required for making a feed from ingredients or for determining the characteristics of ingredients to make a feed are listed under the term "Proximate analysis". Analysis for dry matter (DM), crude protein (CP), crude fat (EE), ash and crude fiber (CF) are usually grouped under the term proximate analysis. Proximate analysis data are important as they fulfill the criteria for the labeling of nutritional indicators as specified by the Association of American Feed Control Officials Inc. Techniques used for proximate analysis vary from one standard method to another and such variations are invariably due to the differences in the characteristics of the materials analyzed. Whichever method is adapted it is important to write down strict protocol to follow a single method when comparing samples, or when analyzing a large number of samples as in a feed mill receiving various ingredients and manufacturing feeds. It will benefit the customer if a short statement of the method used is provided along with the results as outlined in the example below.

- Moisture was determined by heating the ground sample to 103°C for 16 hours and results are expressed on a dry matter basis.
- Crude protein was determined by Kjeldahl and % nitrogen multiplied by the factor 6.25.
- Crude fat was determined by Soxhlet extraction with petroleum ether for 6 hours.
- Ash was determined by ashing at 600°C for 6 hours.
- Crude fiber was determined by the AOAC method.

Sample homogeneity

Before analyzing a sample it is important that the sample matrix is homogenous. Coarse samples must be ground, sieved and reground to obtain a sample representative of the entire content of the sample. Ingredients such as meat and bone meal, feeds containing meat and bone meal, shrimp meal, poultry feather meal, etc. tend to separate into heavier and lighter layers when ground due to different bulk densities. Such gravity separation increases during transport. Sampling errors can occur if the entire sample is not mixed and a homogenous sample obtained for analysis. One way to minimize errors is to use larger samples when ever possible.

Dry matter

The purpose for determining DM (see also Table 1) is to eliminate the variations in moisture content that can vary the results of other proximate analyses. For example if the CP content of fishmeal with 8% moisture is 36.8%; the CP content on a dry matter basis will be 40%. If the moisture content of the fishmeal changes to 10% the CP content will reduce to 36%. Expressing results of analysis on a DM basis provides a definite number that can be used by the consumer to calculate the CP content at any other level of moisture in the sample. One should also remember that when a sample is heated to remove moisture other volatile materials that will also be lost depending on their volatility. Such volatile mater includes free fatty acids, some amines, alcohols and esters that give characteristic odor to ingredients such as fish meal. We can already infer that moisture free or DM basis could be in error when the volatile matter content is high.

Crude protein

Feeds and feed ingredients contain a variety of non-protein nitrogenous substances and their proteins are generally insoluble in water or mild alkali and may be highly pigmented. Therefore colorimetric methods used by biologists such as Lowry are not useful. Since the protein content is calculated based on the estimation of total nitrogen (N) content by the Kjeldahl acid hydrolysis method or the Dumas combustion

Table 2: Array of solvents used for fat extraction and characteristics of the extracts

Solvent	Extract characteristics	Remarks
Petroleum ether	True lipids	Extraction may incomplete
Diethyl ether	True and oxidized lipids.	Same as for Pet. ether. Solvent is explosive and unsafe to use.
Chloroform: Methanol (2:1)	Fats, phospholipids, sterols, some amino acids and	Induce chemical changes to the extract. Toxic hazard.
Acetone	Non-polar lipids and pigments	Works well if combined with acid hydrolysis. Will extract several non-fat solvent soluble
n Butanol (water saturated)	Ideal for cereals such as wheat and corn flour, bran, gluten.	Strong undesirable odor. High boiling point
Liquid carbon dioxide	Clean and selective extraction possible	Considered lab. environment friendly. Needs Supercritical extraction equipment.

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Dionex Accelerated Solvent Extractor is used for determination of crude fat. Petroleum ether (10 mL is used as a solvent and the extraction takes place in a hermetically sealed metal container at 105 C at 140 psi. One sample is completed in less than 10 minutes and this model does 21 samples automatically.

method it is clear that the result is a combination of protein and non-protein nitrogen and hence the term CP. Errors in CP analysis can occur due to sample inhomogeneity discussed earlier and using the same sample used for DM. Kjeldahl method for CP that depends on measurement of distilled

ammonia can give higher readings even when traces of ammonia contaminate the room such as from a sewer or even cigarette smoke. Multiplying % nitrogen by 6.25 is based on the assumption that all proteins contain 16% nitrogen (100/16 = 6.25). This is not true. Total N content of ingredients can vary anywhere from 15 to 18%, for example: casein has 15.7% N, wheat protein 17.54 % N and gelatin 18 % N etc. Feeds contain several plant and animal protein ingredients in varying

proportions each of which can vary in their % protein nitrogen content. To the dismay of a nutritionist it is now obvious that no single multiple factor can be used to provide an accurate estimate of the protein content of the feed thus the term crude protein seems most appropriate when the factor 6.25 is used.

Crude fat

Crude fat is usually abbreviated as EE (Ether Extract) so as not to confuse with CF used as an abbreviation for Crude Fiber. Methods for the determination of EE has gone through a revolution in the last two decades due to concerns on toxicity of solvents used for extraction and use of less material and solvent have become important. Solvent extraction should yield % EE useful for understanding the real fat content and be able to assign it nutritional value. Certain ingredients such as fishmeal require a combination of acid hydrolysis (4 N HCl for 4 hours) and solvent extraction for the determination of % EE. Room or below temperature methods using a combination of chloroform, methanol and water are commonly used for biological materials and claimed to provide extracts ideally

suiting for fatty acid analysis. More recently Accelerated Solvent Extraction is gaining popularity since extraction can be automated, uses less solvent and yields fairly clean samples suitable for nutritional analysis although its value again depends on the choice of solvent. Table 2 shows an array of solvents used for fat extraction and some characteristics of the extracts.

Ash

Ash is typically the inorganic residue from incineration of all the organic matter in the feed or feed ingredient. Ashing normally done in a muffle furnace between 550 and 600°C for six hours to obtain ash content of the sample seems simple and obvious. Ashing process converts all the remaining organic materials into their oxides. This means the relative weight of various elements that were originally in chemical forms such as chlorides would have been converted to their oxides, thus ash does not truly represent the true weight of all inorganic materials in the sample since their molecular weights have changed. Ashing at 600°C may not be enough for certain samples such as casein that may still have residual carbon. Elements such as arsenic, mercury, and boron as such; cadmium, iron and zinc as chloride; and copper, nickel, and

vanadium as porphyrins are lost during ashing at 600°C. Wet ashing using a mixture of perchloric and nitric are used if true mineral composition of the inorganic residue is required and complex equipment designs are marketed to accomplish safe and efficient wet ashing.

Crude fiber

Crude fiber (CF) is used as an important index of nutritional value of plant based ingredients, the higher the CF content the lower the nutritional value. Originally CF was used to measure non-digestible cellulose in non-ruminant feeds. The methods for CF determination are greatly affected by minor variations in the procedure followed and especially when analyzing high protein feeds. More recently Total Dietary Fiber (TDF) measurement is rapidly replacing CF at least in the food industry. The methods developed for TDF are far more accurate and provide more useful information on the nutritional value than CF, however, it should be noted that TDF in human diets is considered beneficial. The TDF measures inorganic constituents, lignin, cellulose, hemicelluloses, pectins, gums, mucilages, algal polysaccharides and modified cellulose. ■



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