CHAPTER 6

Evaluation of Feedstuffs and Aquafeeds

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Introduction

The use of quality feeds is important in the success of any aquaculture venture. Feed quality is highly dependent on the quality of raw material and the processing technique. A formulated feed which makes use of low quality raw materials will not give the fish farmer any significant benefit. Feedstuffs and finished feeds should, therefore, undergo the process of evaluation and quality control in order to produce high quality feed. Systematic evaluation of feedstuffs and feed using physical, chemical, microbiological, and biological methods is necessary to assure their effectiveness when fed to fish. This procedure starts from procurement of feedstuffs and continues to feed processing until manufacture and storage of the finished product. The finished feed must contain all the nutrients required by the fish in adequate amounts and proper proportions.

Different methods are used to evaluate feedstuffs and feed quality. In carrying out all these methods, a standard sampling procedure is necessary to obtain a representative sample. A spear probe is inserted diagonally and horizontally from one corner of the feed bag to the other. Samples are taken from all bags in case of smaller lots while only 10% are considered in bigger lots. There are four methods of feedstuff and feed evaluation: physical, chemical, microbiological, and biological. Physical method involves senses of smell, taste or sight to detect the presence of adulterants in feedstuffs and feeds. Chemical method quantifies the amount of given compound present in the feed. Microbiological method involves the use of microorganisms in the evaluation of nutrients. Biological method involves actual feeding experiment. This method is more tedious and expensive than the first three methods but gives a more accurate estimate of feed utilization.

This chapter discusses how to evaluate feedstuffs and feeds. The results of feed evaluation will be used to ensure the production of high quality feeds for fish, crustaceans, and shellfish.

Physical Evaluation

A. Use of the Senses

Rancidity and off-odors in the feed can be detected by sense of smell. Off-flavors of the main ingredients contained in the ration can be detected by sense of taste. The presence of extraneous materials like small stones, scrap metal, dirt, pieces of wood, and seeds that are added to increase the weight of the product, as well as presence of insects and molds can be detected by sight. Finally, the wetness, dryness or hardness of a feed or feed ingredient can be detected by touch. Feedstuffs and feeds of acceptable quality must be dry, free flowing, and uniform in appearance.

B. Feed Microscopy

The microscope identifies the physical composition of a feedstuff or feed ingredient that either confirms or denies the presence of unwanted materials. A high-powered compound microscope can detect even the finest ground adulterants in a sample. This method is more accurate than the use of the senses in checking adulteration in feeds and feed ingredients.

There are two types of microscope used in feed microscopy: the compound microscope, to identify the internal structure of feed components; and the stereomicroscope, to identify the external structure.

Techniques in sample preparation for feed microscopy

1. Screening. Feedstuffs or mixed feed of different particle sizes are separated by hand screen using sieves of no. 10, 20, and 30 meshes. Sieving separates fine starchy dust from the larger particles in the feed for better identification.
2. Flotation. Feedstuff or feed sample is soaked in a solvent (either carbon tetrachloride or chloroform), stirred, and allowed to settle to separate the organic portion (top fraction) from the inorganic portion (lower fraction). Each fraction is removed and placed in a petri-dish and allowed to dry at room temperature.

C. Measurement of Feedstuffs Bulk Density

The bulk density of the sample is compared with that of a pure feedstuff. If contaminants or adulterants are present, the bulk density will either be higher or lower than the values of the pure feedstuffs. Bulky feeds are less efficient in producing fish flesh. The use of bulky feed ingredients in a feed mixture instead of heavier ones lowers the total digestible nitrogen of the mixture. Bulky feed yields low biologically available energy. The bulk density is computed as weight of samples in gram per liter after the sample has been placed and poured off in a 1 l cylinder.

D. Attractability

Attractants are important components in any feed formulation since they determine how fast and how much of a feed will be taken in by the aquatic animal. A well formulated feed will be useless if the animal does not accept it. Attractability tests are carried out with a single animal and effectiveness is measured usually with a stopwatch based on how fast the animal is attracted to the pellet.

E. Water Stability

Feeds should be stable in water for a certain period to increase their availability to aquatic animals. The feed has to maintain its integrity in the water so that the entire feed is consumed. This is especially true if the species to be fed are slow feeders such as crustaceans. If feeds are not water stable, they disintegrate rapidly losing much of the nutrient content and resulting in feed wastage and degradation of water quality. The use of efficient binders and processing techniques, such as steaming and extrusion, produces a more stable pellet. A simple test to determine whether a feed is water stable or not is by crumbling the pellet by hand or checking for rough edges. Another method for testing water stability is by determining weight loss of the pellet after it is placed in water for a specific period. Correct interpretation of the results is necessary in testing other binders to improve water stability. Proper processing condition for the formulated diet and the binders used are equally important in achieving a water stable pellet. The higher the percentage of pellet disintegration at a given time, the higher the weight loss, and the lower the pellet stability.

A simple method of determining water stability is as follows:

1. Wire baskets are totally oven-dried at 100°C (1-3 h), cooled in a dessicator, and weighed to constant weight.
2. A certain amount of feed (about 5 g) with known moisture content is then placed in the wire basket.
3. The wire baskets with feed are then allowed to stay in the water under conditions similar to those of the experimental tanks at designated times (2, 4, 6, and 8 h).
4. The wire baskets are then oven-dried, cooled in a dessicator, and weighed to constant weight.
5. Percent dry weight loss is calculated after subtracting the basket weight.
6. Percent water stability is then computed as:

$$\% \text{ Water Stability} = \frac{Fo}{Io} \times 100$$

where: Io = Initial dry weight of feed
Fo = final dry weight of feed

Example: Compute the water stability of a feed

$$\begin{aligned} \text{wt feed (as is)} &= 5.26 \text{ g} \\ \text{dry matter (DM)} &= 95\% \\ \text{wt feed (dry basis)} &= \text{wt feed (as is)} \times \frac{\%DM}{100} \end{aligned}$$

$$\text{Io, initial dry wt of feed} = 5.26 \times \frac{95}{100}$$

$$\text{Io} = 5.0 \text{ g}$$

After the indicated immersion time:

$$\begin{aligned} \text{wt feed + basket} &= 12.5 \text{ g} \\ \text{wt empty basket} &= 8.0 \text{ g} \end{aligned}$$

$$\text{Fo, final dry wt of feed} = \text{wt basket + feed} - \text{wt basket}$$

$$\begin{aligned} \text{Fo} &= 12.5 - 8.0 \\ &= 4.5 \text{ g} \end{aligned}$$

$$\%\text{ water stability} = \frac{\text{Fo}}{\text{Io}} \times 100 = \frac{4.5}{5.0} \times 100 = 90\%$$

Chemical evaluation

A. Proximate analysis

The proximate composition is an index to the nutritive value of feeds and feedstuffs. This analytical technique is designed to differentiate between nutritive and non-nutritive components and analyze moisture/dry matter content, crude protein, crude fat or ether-extract, crude fiber, and ash. Nitrogen-free extract (NFE) is obtained by subtracting the sum of these constituents from 100. Although proximate analysis gives a general indication of the feed value, it does not deal with specific nutrients. This method establishes the category in which a feedstuff belongs and is a useful descriptive device in establishing the characteristics of feeds (Figure 6.1).

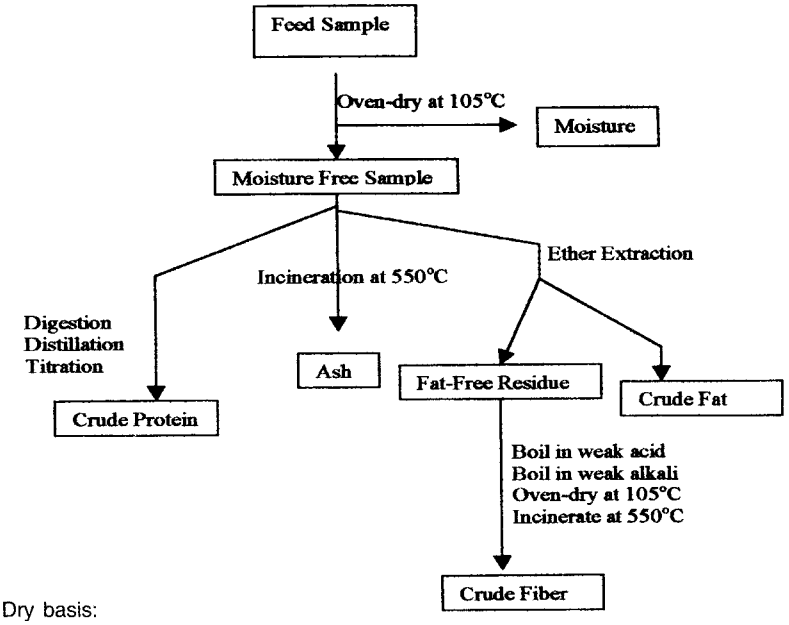

Figure 6.1
Flow diagram for the proximate analysis of feedstuffs and feeds.

1. **Moisture/dry matter content.** It is important to determine how much of the feed sample weight is actually made up of dry matter especially in formulating rations using ingredients with high moisture content. Moisture is a diluent of the nutrient in feedstuffs and feed and has an effect on their stability and shelf life. Feedstuffs and feeds should be dried to contain less than 10% moisture prior to storage especially in tropical countries. High moisture content can lead to growth of molds during storage of feedstuffs and feeds.

   Moisture content determination involves drying the feed sample to constant weight using a drying oven or a moisture balance (Figure 6.2). It is important to interconvert feed analysis data from dry-matter basis to as-received basis for a more accurate feeding management using the following formulae:

Figure 6.2
A moisture balance used to determine moisture content of feedstuffs and aquafeeds.

$$\% \text{ nutrient (as received)} = \% \text{ nutrient (dry basis)} \times (\% \text{ dry matter})/100$$

$$\% \text{ nutrient (dry basis)} = \% \frac{\text{nutrient (as received)}}{\% \text{ dry matter}} \times 100$$

2. **Crude Protein**. Crude protein determination is done using the Kjeldahl method. The sample is digested in concentrated sulfuric acid, resulting in the complete oxidation of all organic materials and the nitrogen is converted to ammonium sulfate. Excess sodium hydroxide is then added to liberate ammonia, which is absorbed in boric acid and titrated with standard hydrochloric acid. The procedure for protein analysis using Kjeldahl method is in Appendix B1. A picture of the Kjeldahl distillation-titration (Kjeltec™) instrument used to analyze the crude protein content of feedstuffs and feeds is shown in Figure 6.3. The term crude protein means that other nitrogenous materials, which are not true proteins, such as urea, amides. nucleic acids, and amino sugars may be present. Since fish has very limited ability to utilize non-protein nitrogen (NPN), the measurement of the true amino acid protein should be done on feedstuffs.

Figure 6.3
The Kjeldahl distillation-titration (Kjeltec™) apparatus used to analyze crude protein.

3. **Crude fat or ether extract.** The crude fat content of feedstuffs and feeds is determined by extraction of ground samples with ether. Ether-soluble materials include a variety of organic compounds but only a few have nutritional significance such as the true fats, fatty acid esters, compound lipids, and fat soluble vitamins or pro-vitamins such as the carotenoids. This method does not remove all lipids, especially the phospholipids and other fats bound to proteins.

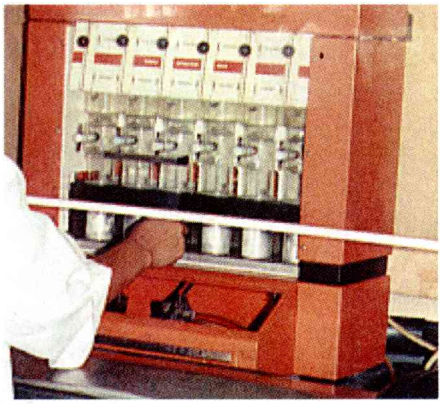

Figure 6.4
The Soxtec™ apparatus used for crude fat analysis.

Figure 6.5
A muffle furnace used to analyze the ash content of feedstuffs and aquafeeds.

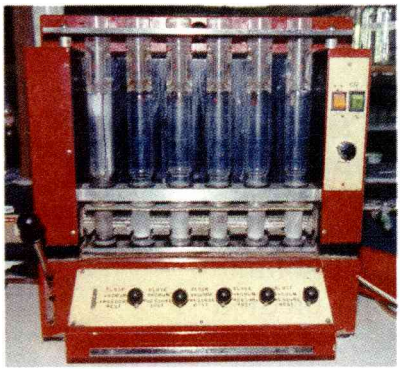

Figure 6.6
The Fibertec™ used for crude fiber determination.

If the ether extract contains a large percentage of fats and fatty acid esters, this method of evaluation is valid because it aims to isolate a fraction of feedstuff which has a high caloric value. However, if the extract contains a large percentage of plant waxes, essential oils, resins or similar compounds, this analysis has little meaning, as these compounds have little value to fish. A procedure for fat analysis is shown in Appendix B2. Soxtec™ apparatus used in crude fat analysis of feedstuffs and feed is shown in Figure 6.4.

4. **Ash or mineral matter.** Ash or the total mineral content is measured by burning the feed sample in a muffle furnace at 550-600°C. This burns all organic matter, leaving a residue of ash or inorganic mineral salts. Excessively high ash values may indicate contamination, or dilution of feedstuffs with such substances as limestone and salt. The content of calcium, phosphorus, magnesium, and other minerals in feeds and feedstuffs are determined from the ashed sample. Figure 6.5 shows a picture of a muffle furnace used for ash determination.

5. **Crude fiber.** In crude fiber determination the ether-extracted sample is boiled in dilute acid, then in dilute base, dried, and burned in a furnace. The difference in weight before and after burning is the crude fiber fraction. This method simulates digestion occurring in the gastric stomach and in the small intestines of the fish. Crude fiber is made up primarily of plant structural carbohydrates, such as cellulose and hemicellulose, and it also contains some lignin, a highly indigestible material associated with the fibrous portion of plant tissues. Figure 6.6 shows a picture of the Fibertec™, an instrument used for crude fiber determination.

6. **Nitrogen-free extract (NFE).** NFE is derived by subtracting the sum of the other proximate components, crude protein, crude fat, ash, crude fiber on a dry weight basis from 100. It represents mainly starch, sugars, and other readily soluble carbohydrates. It may also include hemicelluloses and some of the more soluble lignin.

B. Methods of Protein Evaluation

1. **True protein value determination** (Spectrophotometric methods).
   a. Biuret method. This method is applicable to extracted liquid fish protein aliquots, with a protein concentration of between 0.1 to 0.5 mg N/ml. The method is based on the reaction of Cu^{+2} with peptides in alkaline solution to yield a purple complex that has a peak absorption at 545 nm.
   b. Lowry method. This method estimates total protein of feedstuffs and feeds. It uses Folin-Ciocalteu reagent and has a blue end color with extinction at 660 nm. The principle behind this method is the reduction of the phosphomolybdic acid-phosphotungstic acid (Folin-Ciocalteu) reagent by tyrosine and tryptophan residues in the protein.

2. **Measurement of protein quality.** The usefulness of feeds as sources of protein depends primarily on the total concentration of protein and the composition of amino acids making up the protein. Imbalance among amino acids in a formulated feed results in inadequate protein nutrition. The imbalance decreases growth and feed efficiency or may result in fish mortality. The relative usefulness of the protein of a particular feed in meeting the needs of the fish is known as its quality.

   a. **Amino acid composition.** Amino acid analysis is done using either an amino acid analyzer (AAA) or high performance liquid chromatograph (HPLC) with fluorescence detector. Figure 6.7 shows a picture of HPLC, an instrument used to analyze amino acid composition of a protein. HPLC utilizes o-phthalaldehyde/N-acetylcystine (OPA/AcCys) and Na hypochlorite reagent. Amino acids react with OPA/AcCys to form a fluorescent substance with an excitation wavelength of 350 nm and fluoresces at 450 nm. Protein samples are hydrolyzed and injected into a column with cation-exchange resin. Buffers of different pH and ionic strengths are pumped through the column to bring about the separation of various amino acids. The amount of fluorescent compound is directly proportional to the amino acid concentration in the eluate. The amino acid is identified in the resulting chromatogram by the retention time of the peak and quantified by the area under such peak. In the amino acid analysis by AAA, ninhydrin solution is used for color development instead of OPA/AcCys.

Figure 6.7
The high performance liquid chromatograph (HPLC) used for analysis of amino acid composition of a protein.

   b. **Chemical scores for protein.** This method considers the relative amounts of amino acids present in the protein as determined by chemical analysis. The quality of protein is affected by a relative deficiency of one or more essential amino acids. Whole egg protein is considered as standard or ideal in terms of amino acid composition and is commonly used as a "reference protein". The

amino acid usually in greatest deficit in a protein is called the "limiting" amino acid. The percentage of amino acid of greatest deficit is subtracted from 100 to give the chemical score for specific protein source. For example: A protein with lysine content of 40% of that in egg protein will have a chemical score of 60, provided that this is the amino acid in greatest deficit.

Table 6.1
Essential amino acid indices (EAAI) of some common feedstuffs for shrimp

Feedstuffs	EAAI values
White fish meal	0.96
Peruvian fish meal	0.92
Slipmouth fish meal	0.94
Tuna fish meal	0.92
Herring fish meal	0.95
Shrimp meal	0.98
Squid meal	0.96
Soybean meal	0.87
Ipil-ipil leaf meal	0.54
Sweet potato meal	0.53

Source: Penaflorida 1989

c. Essential amino acid index (EAAI). The EAAI method of chemically evaluating a protein considers all essential amino acids rather than the most limiting amino acid with respect to some standard. EAAI is the geometric mean of the amino acid found by comparing the content of the ten essential amino acids in a feed protein with that found in fish tissue protein. The closer the EAAI value to 1.0 the better is the protein quality. This equation is expressed algebraically as:

$$EAAI = 10\sqrt{\frac{100a}{a_e} \times \frac{100b}{b_e} \cdots \frac{100j.}{j_e}}$$

in which a, b, ... j are the percent of essential amino acids in the food protein and a_e, b_e,.... j_e are the percent of the respective amino acids in whole egg protein. For computation, it is convenient to express the equation in logarithmic form as:

$$\log EAAI = \frac{1}{10} \log\left(\frac{100a}{a_e} + \log \frac{100b}{b_e} + \cdots \log \frac{100j.}{j_e}\right)$$

Examples of essential amino acid indices of feedstuffs are in Table 6.1. Thus protein from ipil-ipil leaf meal and sweet potato meal are of poor quality compared to fish meals, shrimp meal, squid meal, and soybean meal.

Figure 6.8
The gas chromatograph (GC), an instrument used for analysis of fatty acid composition.

C. Methods of Lipid Evaluation

1. Measurement of lipid quality. The quality of lipid contained in feedstuffs and feeds is determined by its fatty acid composition which is obtained thru gas chromatographic (GC) analysis (Figure 6.8). It is a technique of separating sample into its constituents and then measuring or identifying components in same way. The separation technique involves two phases, the stationary and mobile.

The sample is carried by the mobile phase, usually an inert gas, through the stationary phase (column), where

separation takes place. Since the technique requires that samples should be volatile, lipids are converted into fatty acid methyl esters (FAME) before injection.

The measurement of fatty acids uses detectors and the most popular is the Flame Ionization Detector. Sample components eluting from the column in the gas stream are ionized creating a current that is measured by an electrometer. The peaks indicate a measure of the fatty acid concentration in the sample. The peak retentions times are used to identify the component fatty acids based on a standard fatty acid mixture.

Sample preparation involves lipid extraction from the feeds or feedstuffs (Appendix B3) followed by saponification and esterification processes, as described in Appendix B4. The output from this GC is represented by a chromatogram shown in Figure 6.9.

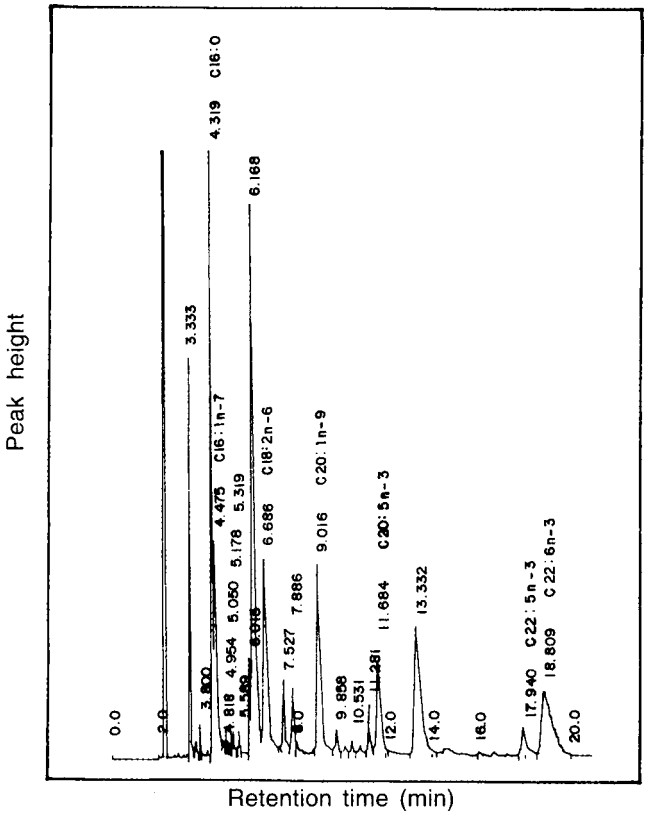

Figure 6.9
A sample gas chromatographic analysis showing retention times of various fatty acids in a feed sample.

2. **Tests for lipid rancidity.** Oxidative deterioration of lipids has been shown to cause rancid flavor in stored feedstuffs and feeds. Lipid sources such as fish and vegetable oils which are commonly used in the preparation of feeds are rich in polyunsaturated fatty acids and are susceptible to oxidation. The degree of oxidation in feedstuffs and feeds can be determined through the following methods:

   a. **Peroxide value (PV).** Peroxide value is defined as the reactive oxygen content expressed in terms of milliequivalents (meq) of free iodine per kg of fat. Peroxides are precursors of breakdown products that cause rancid flavors in fat. The concentration of peroxides indicates oxidation during early stages of lipid deterioration. This index becomes less reliable during the later stage of deterioration, because peroxide degradation increases. PV is determined by titrating the iodine liberated from potassium iodide with sodium thiosulfate solution. Details of the method of peroxide value determination is shown in Appendix B5.

   b. **Free fatty acid value (FFA).** Free fatty acid values may be determined using the titrimetric method. The free fatty acid value is usually calculated as oleic acid by dividing the acid value by 2. The presence of these free fatty acids can speed up oxidative deterioration. Oils with high content of free fatty acids will develop undesirable color and flavor. For details of the procedures, see Appendix B6.

c. **Thiobarbituric acid number (TBA).** This method involves heating the oil with thiobarbituric acid in the presence of a strong acid. A red coloration is produced as a result of a reaction between the thiobarbituric acid and malonaldehyde. The latter may be present in the oil or it may be produced from an oxidation product during the course of the reaction. The intensity of the red color is proportional to the concentration of malonaldehyde in the mixture and can be measured in a spectrophotometer. Results may be expressed as mg of malonaldehyde per kg of oil. It is necessary to carry out the reaction in the absence of oxygen in order to obtain reliable results with marine oils. This is a sensitive test and can be correlated with the development of off-odors and flavors. It is especially suited for the detection of oxidative rancidity in lipids which are unsaturated and contain 3 or more double bonds. For details of the procedure, see Appendix B7.

The absorbance of a 1 g sample in 100 ml reagent multiplied by the factor 46 is the TBA number, or the mg of malonaldehyde per 1000 g of sample. As the amount of reagent used is only 20 ml, the result must be multiplied by 0.2 to give the absorbance of the sample in 100 ml reagent as specified by the definition.

D. Method of Vitamin Evaluation

High Performance Liquid Chromatography (HPLC). The HPLC is a powerful analytical method or tool with a wide range of applications. It provides high resolution, sensitivity, and automatic operation, and is efficient in determining the amount of vitamins contained in feedstuffs and feeds (Figure 6.10).

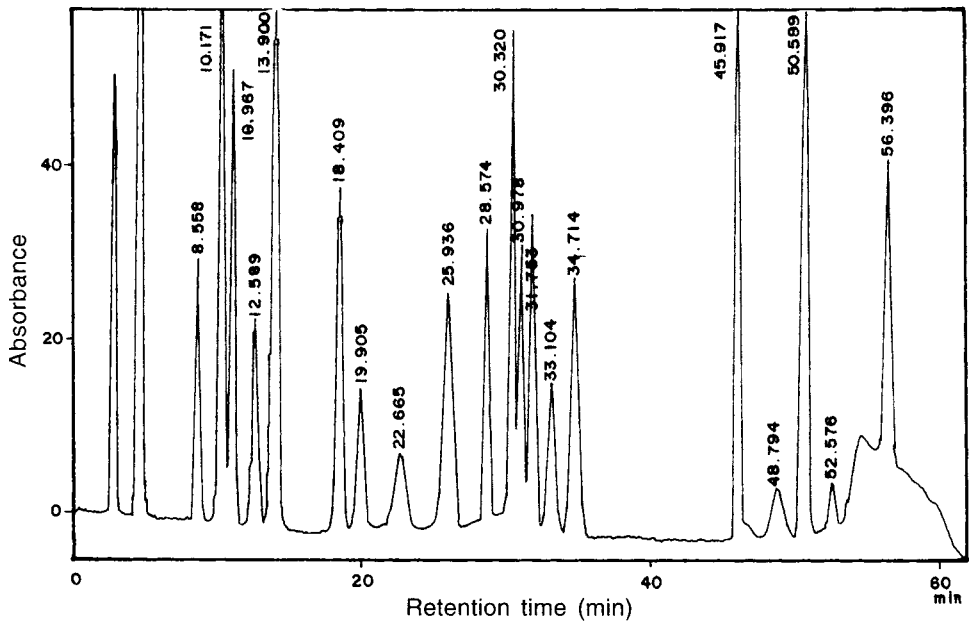

Figure 6.10
A sample HPLC chromatogram of a vitamin mixture.

E. Methods of Mineral Evaluation

There are qualitative and quantitative methods for determining minerals in feedstuffs. Some of the qualitative methods are:

1. **Spot tests for presence of some minerals.** The minerals or inorganic compounds used in animal feed either occur in nature or are chemically compounded. To test for the presence of minerals qualitatively, a spot test is usually applied. The minerals in mixed feed are powdered and sieved. The fine portion is placed in chloroform in a beaker. The floating material is poured off and the residue is sprinkled in the filter paper with a small spatula for spot test of the following minerals.

   a. **Cobalt, Copper, and Iron**. To test for the presence of these minerals, a filter paper is moistened with solution A (100 g sodium potassium tartrate dissolved in 500 ml distilled water). The sample is sprinkled in the filter paper and 2-3 drops of solution B (1 g of 1-nitroso-2-hydroxynaphthalene-3,6-disulfonic acid) are added. The filter paper is then dried. Cobalt gives a pink color; copper gives a light brown colored ring; and iron gives a deep green color.

   b. **Manganese.** To test for the presence of manganese oxide, sulfate, and carbonate, a filter paper is moistened with solution A (2 N NaOH). The test sample is sprinkled in the paper and 2-3 drops of solution B (0.07 g benzidine dihydrochloride in 10 ml of glacial acetic acid is diluted with 100 ml distilled water). Manganese oxide gives a dark blue color with black center and manganese sulfate gives a larger spot of lighter blue which appears quickly.

   c. **Iodine, Magnesium, and Zinc**. To test for the presence of iodine, magnesium, and zinc, the same procedure as above is followed, except that starch paper moistened with bromine solution (1 ml of saturated bromine water made up to 20 ml with distilled water) is the one sprinkled with sample for the iodine test. Iodine then gives a blue-purple color.

      In the case of magnesium, solution A (1 N KOH) and B (12.7 g iodine and 40 g KI dissolved in 25 ml distilled water and then diluted to 100 ml) are mixed to give a very dark brown color. A small part of this is taken and again 2-3 drops of solution A is added until it turns pale yellow and this is sprinkled in the filter paper together with the sample. Magnesium gives yellow brown spots.

      For zinc, the filter paper is moistened with solution A (2 N NaOH). The sample is sprinkled in the filter paper and 2-3 drops of solution B (0.1 g dithizone in 100 ml of carbon tetrachloride) is added. Zinc gives a raspberry red color.

2. **Mineral composition.** The resulting ash from the crude ash analysis is used for mineral analysis of the sample. The quantitative methods for mineral evaluation are:

   a. **Titrimetric method.** In the titrimetric method, permanganate titration is done. In calcium analysis, calcium is precipitated as oxalate at pH 2.5-3, dissolved in sulfuric acid, and the liberated oxalic acid is titrated with standard potassium permanganate solution. The level of phosphorus in the feed is determined based on Misson's reaction.

   b. **Spectrophotometric method**. The phosphorus present as orthophosphate reacts with vanadate-molybdate reagent to produce a yellow-orange complex which is measured spectrophotometrically at 400 nm. Calcium and phosphorus determinations are conducted on feeds such as fish meals, bone meal, calcium phosphate, and calcium carbonate sources.

   c. **Atomic emission spectroscopy.** This method utilizes high-temperature atomization sources to determine the concentration of about 70 elements in feedstuffs. This operates on the principle that when a substance is excited by a plasma or electric discharges, elements present emit light at wavelengths that are specific for each element. The light emitted is dispersed by a prism monochromator. The spectral lines produced are recorded on a photographic plate that is linked directly to a computer-driven data processing system. The samples are pre-concentrated and most elements can be determined at the low parts per billion level.

   d. **Flame spectrometric methods.** The qualitative and quantitative means of these methods can be applied to plant materials, plant nutrients, soils, and other biological fluids. The specific frequency of radiation, emitted or absorbed, identifies the element. The intensity of emitted or absorbed radiation at the specific frequency is proportional to the amount of the element present.

   e. **Atomic absorption spectrometry (AAS).** This method uses combustion and is used to observe the atomic vapor that is produced when a sample solution is nebulized and passed into the flame in an atomic absorption spectrometer. This instrument consists of a centralized hooded area in which a large flame, often 6 in wide by 6 in high, is located. This can be used to identify mineral elements present in feeds and feed ingredients.

F. Methods of Energy Determination

The caloric content of the feed sample is determined through the use of a bomb calorimeter. The principle involved in bomb calorimetry is that the heat of combustion is measured, when the heat exchange process takes place in the water contained inside the calorimeter jacket. Bomb

calorimetry generally measures BTU's, calories, or joules in solid or liquid fuels and combustible samples as well. It is a system that determines directly the caloric value or gross energy of the sample.

To determine the fraction of the gross energy that the fish can actually utilize, a metabolism trial is conducted to determine, digestible, metabolizable, and net energy values. The use of a bomb calorimeter is necessary to avoid difficulties and inaccuracies inherent in determining the NFE. Subsequently, similar determinations on the fecal residue from such diets make it possible to correct the gross caloric value for the apparent loss in digestion and obtain values for digestible energy. This is a better measure than gross energy for expressing the useful energy of feedstuffs.

1. **Digestible energy (DE).** An inert material, chromium oxide, is added to the food at a level of 0.5-1%. The fish are fed the diet for several days for fecal collection, and the chromium oxide levels of both the feed and feces are determined. The digestible energy (DE) is defined as follows:

$$\text{DE (kcal/100 g diet)} = \text{energy of diet} - \left(\text{energy of feces} \times \frac{\text{mg } Cr_2O_3\text{/g dry diet}}{\text{mg } Cr_2O_3\text{/g dry feces}}\right)$$

2. **Metabolizable energy (ME).** The ME is the portion of the gross energy consumed by the fish for growth, fattening, or heat production. It does not appear in the feces or in the urine of fish. The digestible energy minus the energy in the urine and feces is the ME, which provides a better measure of the energy value of the feed than the digestible energy. The large differences in the efficiency of utilization of ME are due to wide variations in heat losses.

3. **Net energy (NE).** This represents the most precise measure of the energy needs of fish and the capacity of different feeds to meet these needs. Not only do NE values allow for the energy lost in the urine and feces, but they also take into consideration the energy lost as heat during nutrient utilization. However, actual NE values have been determined for only a limited number of feeds. Thus, most available NE values are only estimates.

G. Analysis of Toxins in Feeds

Some feed ingredients contain natural toxins that are growth inhibitory at high levels and may be deleterious to fish. Methods of determining these toxins are:

1. **Urease activity**. The enzyme urease usually found in raw soybeans produces toxicity through the hydrolysis of urea to ammonia. Heat treatment of soybean meal at 120°C for 20 min is adequate to remove this enzyme. Quantitative analysis to determine urease activity level in feedstuffs is patterned after Chow (1980).

2. **Gossypol.** This is an endogenous toxin present in the gland of cottonseed which persists during meal production unless removed by a special process, or, unless the cottonseed is a glandless variety. The gossypol level in a feed ingredient is determined spectrophotometrically.

3. **Aflatoxin**. This is a class of potent toxins produced by the mold *Aspergillus flavus* and is usually present in feed materials such as groundnut cake, copra, peanuts, corn, rice, and legumes produced and stored under hot and humid conditions. The chromatographic methods most widely and routinely used for aflatoxin analysis are one- and two-dimensional thin layer chromatography (TLC) and high performance thin layer chromatography (HPTLC). In view of the potential deleterious effects of these antinutritional factors on the growth of fish, analysis should be done on feedstuffs known or suspected to contain these materials.

Microbiological Evaluation

Amino acid composition. Another method of determining amino acid composition is through the microbiological method. This method is valuable in analyzing mixtures of amino acids because of the speed and reproducibility of results obtained. A nutrient medium which contains all of the essential compounds needed for the growth of a particular microorganism except the amino acid to be assayed is prepared. Addition of this amino acid results in growth of the microorganism in proportion to the amount of amino acid added. Culture tubes are set up and graded amounts of the unknown are added to a series of tubes. Standards are set up at the same time with graded amounts of the pure amino acid. The unknown can be compared with standards by measuring the rate of growth of the microorganism. With organisms which form acid such as *Lactobacilli*, titration of the acid formed can be used as a measure of the number of cells present. Pure cultures need to be used in this kind of evaluation.

Biological evaluation

A feeding experiment is conducted to test the efficacy of formulated feeds. It is usually done in tanks, ponds, or cages. In a laboratory experiment, environmental conditions are easily kept constant. In carrying out a feeding experiment, the following factors have to be considered:

a. The objective of the study has to be clearly defined.

b. Experimental treatments and statistical design appropriate to the objective of the experiment have to be carefully selected. A completely randomized design (CRD) or randomized complete block design (RCBD) is usually applied in most feeding experiments. However, when there are more variables, a factorial design is used. Prior to a feeding experiment the number of replications per

treatment and the number of fish per replicate should be chosen such that a meaningful statistical analysis of data can be accomplished at a set level of significance. It is best that the data to be gathered, sampling frequency, number of samples per replicate, and statistical methods to be used in the data analysis are known beforehand. A statistician should be consulted for the design of the experiment.

c. The experimental fish species have to be identified with its scientific and common names, together with its strain, source, size, age, and previous nutritional history. Acclimation of the test animal is usually done for at least a week to enable the fish to adjust to its new environment. Initial body weight and length have to be measured during stocking. It is best to use an equal number of fish of approximately the same size and age per replicate. In some studies, body composition (initial and final) has to be known. This is usually determined in three groups of fish in three replicates. Fish are usually weighed, sacrificed, chopped, dried in a freeze drier, and oven-dried a 60°C to constant weight. Results of proximate composition is then related to the initial weights of the fish to obtain absolute weights of the nutrient components. The same procedure is followed to determine the final body composition of the fish. The data is used to determine nutrient retention.

d. The experimental tanks should be large enough to allow substantial fish growth (Figure 6.11). Experimental tanks, dimensions, water source, volume, and depth should be clearly defined. Water may be static but replaced regularly, recirculating, or flow-through depending on the available resources. Filtration system should be adequate and efficient in removing particulate matter and metabolites that may influence the response of fish to treatments. Water temperature is usually ambient and sometimes controlled. Water quality (temperature, dissolved oxygen, pH, ammonia, etc.) should be monitored daily and should be favorable for maximum growth.

e. The duration of a feeding experiment should be long enough, at least 8 weeks, to allow fish to manifest definite growth trends and significant differences in response parameters as affected by the dietary treatments.

Figure 6.11
A laboratory set-up for a feeding experiment using 250 l tanks.

f. Growth, the most widely accepted response parameter for evaluating treatments in feeding, is a sensitive and practical indicator of the adequacy of essential nutrients in the diets. Other parameters such as survival, feed efficiency, etc. should also be taken into account.

Parameters to be monitored in a feeding experiment:

1. Growth

a) Absolute growth

wt gain = wf – wi,
where: wf = final wt; wi = initial wt

Absolute growth rate

$$\text{wt gain/day} = \frac{\text{wf} - \text{wi}}{\text{days of culture}}$$

b) Relative growth

$$\% \text{ wt gain} = \frac{\text{wf} - \text{wi}}{\text{wi}} \times 100$$

Relative growth rate

$$\% \text{ wt gain/day} = \frac{\text{wf} - \text{wi}}{(\text{wi})\ (\text{days of culture})} \times 100$$

Specific Growth Rate (SGR)

$$\text{SGR} = \frac{\ln w2 - \ln w1}{t_2 - t_1}$$

where: w1 and w2 are weights at periods 1 (t_1) and 2 (t_2), respectively.

2. Efficiency of feed utilization

$$\text{Feed conversion ratio (FCR)} = \frac{\text{dry feed consumed}}{\text{wt gain}}$$

$$\text{Feed efficiency (\%)} = \frac{\text{wt gain}}{\text{dry feed consumed}} \times 100$$

3. Digestibility of nutrients

$$\% \text{ Digestibility} = \frac{\text{nutrient absorbed}}{\text{nutrient consumed}} \times 100$$

4. Efficiency of protein utilization

$$\text{Protein Efficiency Ratio (PER)} = \frac{\text{wt gain}}{\text{protein intake}}$$

$$\text{Biological Value (BV)} = \frac{\text{N retained}}{\text{N absorbed}}$$

Net Protein Value (NPV) or Productive Protein Value (PPV)

$$= \frac{\text{N retained}}{\text{N consumed}} \quad \text{or} \quad = \text{BV x digestibility}$$

Net Protein Utilization (NPU)

$$= \frac{\text{nitrogen increase in fish fed the test protein diet} + \text{nitrogen decrease in fish fed the protein free diet}}{\text{nitrogen intake from the test protein diet}} \times 100$$

5. Survival rate

$$\% \text{ Survival} = \frac{\text{final count}}{\text{initial count}} \times 100$$

6. Proximate composition of fish samples (initial and final)

7. Biological parameters

a) amino acid and fatty acid composition
b) stored nutrient levels in tissues, serum, or plasma
c) enzyme activity
d) oxidation of radioactively-labelled nutrients

8. Histological changes in tissues (e.g. gills, skin, liver, muscle)

Summary

The choice of high quality feedstuffs for incorporation into aquafeeds is crucial to the success of an aquaculture venture. Proper methods of feedstuff and feed evaluation should be learned and applied to attain an effective feed. The systematic evaluation of feedstuffs and feed includes physical, chemical, and biological methods. In the application of these methods, standard sampling procedures should be followed in order to effectively carry out the evaluation process. Among these methods, the biological method which involves actual feeding experiments gives a more accurate estimate of feed utilization.

Guide Questions

1. What are the different methods of feed evaluation? Differentiate one from the other.
2. What does proximate analysis measure in feed evaluation? What are the limitations of this procedure?
3. What is the principle behind the "Kjeldahl method" of protein determination?
4. How is the protein quality evaluated?
5. Describe briefly how to calculate the essential amino acid index (EAAI) of a protein.

6. Explain the principle behind gas chromatography.
7. What are the tests used to detect lipid rancidity?
8. What are peroxides? Differentiate between peroxide value and thiobarbituric acid number.
9. What is the implication of having a high free fatty acid value in feed materials?
10. What is the significance of the thiobarbituric acid number or TBA number?
11. Explain the principle behind atomic emission spectroscopy?
12. What are the characteristics of a high quality feed?
13. How is aflatoxin produced in feed/feed ingredient?
14. What are the antinutritional factors that may be present in the feed ingredient and explain how they are minimized or removed from feedstuffs.

Suggested Readings

ADCP. 1980. Fish Feed Technology. Rome, FAO, ADCP/REP/80/11, 395 p.

AOAC (Association of Official Analytical Chemist). 1980. Official methods of analysis. (13th edition). Washington. 1108 p.

Asakawa T, Nomura Y, Matsushita S. 1975. A modified TBA test for the determination of lipid oxidation. Yakagaku 24:481-482.

Bligh EG, Dyer WJ. 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. 37:911-917.

Block RJ, Mitchell HH. 1946. The correlation of the amino acid composition of proteins with their nutritive value. Nutr. Abst. and Revs. XVI:249.

Cheeke PR. 1991. Applied animal nutrition: feeds and feeding. Department of Animal Science, Oregon State University. 504 p.

Chow KW. 1980. Quality control in fish feed manufacturing. IN: Fish Feed Technology, FAO/UNDP Training Course, College of Fisheries, University of Washington, Seattle, 9 October-15 December 1978. ADCP/Rep/80/11, p 369-385.

Church DC, Pond WG. 1974. Basic animal nutrition and feeding. O and B Books, 1215 NW Kline Place, Corvallis, Oregon 97330, United States of America. 300 p.

Cho CY, Cowey CB, Watanabe T. 1985. Finfish Nutrition in Asia: Methodological Approaches to Research and Development. Ottawa, Ontario. IDRC. 154 p.

CRC Handbook of Chromatography. CRC Press, Inc., Florida, USA. 2: 319-355.

Crampton EW, Harris LE. 1969. Applied animal nutrition; the use of feedstuffs in the formulation of livestock rations. W.H. Freeman, San Francisco, USA. 753 p.

Feed Development Section. 1994. Feeds and Feeding of Milkfish, Nile Tilapia, Asian Sea Bass, and Tiger Shrimp. SEAFDEC Aquaculture Department. Tigbauan, Iloilo, Philippines. 97 p.

Folch J, Lees M, Stanley GH. 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226:487-509.

Fish Nutrition and Mariculture. 1988. The General Aquaculture Course, A JICA Textbook. Watanabe T. (ed). Kanagawa International Cooperation Agency. 233 p.

Hastings WH. 1969. Nutritional Score. In: Neuhaus,O.W and J.E. Halver (eds). Fish in Research Academic Press, Inc. New York. p 263-292.

Hastings WH, Dickie LM. 1972. Feed formulation and evaluation. In: Halver, J.E. (ed). Fish Nutrition. Academic Press, Inc. New York. Chapter 7, p 327-370.

Jowaman, K, Duangsmorn, S, Ankana, H, Uthai, K. 1987. Manual of feed microscopy and quality control. American Soybean Association National Renderers Association and US Feed Grain Council. p 154-160.

Lovell RT. 1984. Microbial toxins in fish feeds. Aquaculture Magazine 10(6A):34-36.

Mangold HK, Zweig G, Sherma J. 1984. Lipids. CRC Handbook of Chromatography. CRC Press, Inc. Florida, USA.

Marinetti, GV. 1967. Lipid chromatographic analysis Volume 1. Marcel Dekker, Inc., New York. 537 p.

Marinetti, GV. 1967. Lipid chromatographic analysis Volume 2. Marcel Dekker, Inc., New York. 596 p.

Maynard LA, Loosli JK, Hintz HF, Warner RG. 1979. Animal Nutrition, 7th edn. New York, McGraw Hill.

Metcalfe LD, Schmitz AA, Pelka JR. 1966. The rapid preparation of fatty acid methyl esters from lipids for gas chromatographic analysis. Anal. Chem. 38:514.

Morrison WR, Smith LM. 1964. Preparation of fatty acid methyl esters and dimethylacetals from lipids with boron triflouride-methanol. J. Lipid Res. 5:600-608.

Oser BL. 1951. Method for integrating essential amino acid content in the nutritional evaluation of protein. J. Amer. Dietetic Assn. XXVII: 396.

Pierce JG. 1976. Feed microscopy in quality control. In: Feed Manufacturing technology. Feed Production Council, American Feed Manufacturers Association Inc., Arlington, Virginia, p 270-273.

Roach AG, Sanderson P, Williams DR. 1967. Comparison of methods for the determination of available lysine value in animal and vegetable protein sources. J. Sci. Food Agric. 18:274-278.

Simpson RJ, Neuberger MR, Liu TY. 1976. Complete amino acid analysis of proteins from a single hydrolysate. J. Biol. Chem. 251:1936-1940.

Tacon A. 1987. The nutrition and feeding of farmed fish and shrimp. A training manual. 2. Nutrient sources and composition. FAO Field Document, Project GCP/RLA/075/ITA, Field Document, No. 5, Brasilia, Brazil. p 95-117.

Yu TC, Sinnhuber RO. 1957. 2-Thiobarbituric acid method from the measurement of rancidity in fishery products. Food Tech. 11:104-108.