Systematic Approach for Evaluating Aquaculture Feed and Identifying Feed Related Problems

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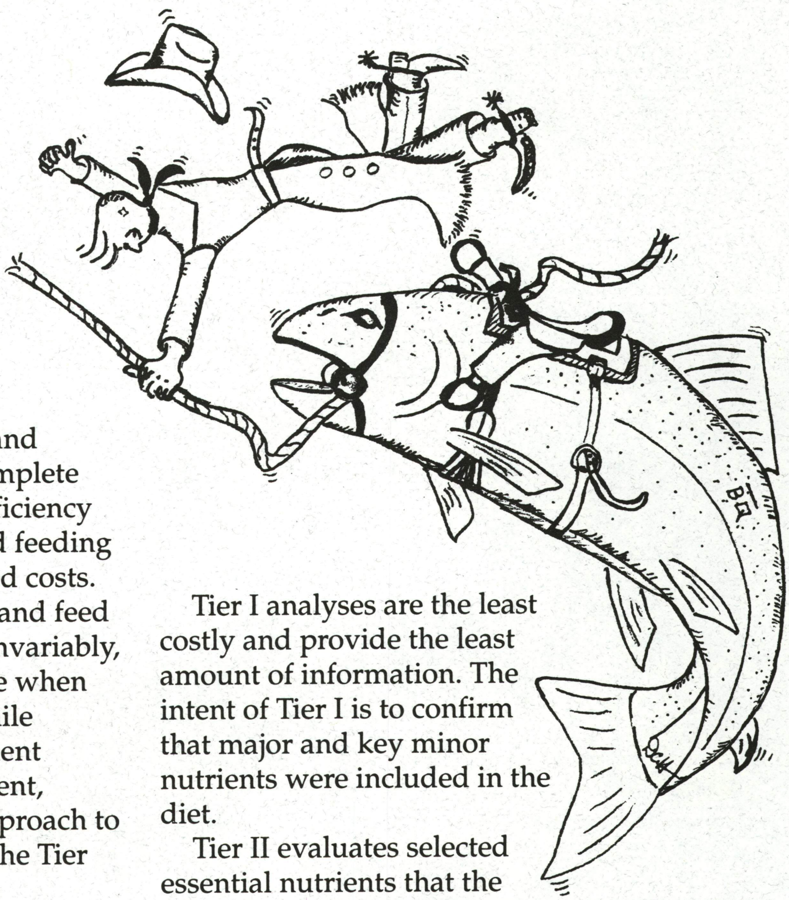

Feed is a major cost of aquaculture and livestock production. Nutritionally complete diets are necessary to optimize feed efficiency and growth. Sound nutritious feed and feeding programs can reduce disease-associated costs. Laboratory analysis can evaluate feed and feed ingredients for nutritional adequacy. Invariably, questions concerning feed quality arise when disease problems are encountered. While analyzing every nutrient and antinutrient known to be found in feed is not prudent, producers should take a systematic approach to feed analysis. This approach is called the Tier System.

The Tier System

The Tier System is divided into four levels or parts (table 1). The first three tiers build on the previous tier. Tier IV is a group of tests for specific feed ingredients. If a specific feed ingredient is in question aside from its nutrient content then jumping to Tier IV would be reasonable. In this publication, items are selected for analysis (table 1) based on relative importance and the analysis cost. Relative importance refers to the frequency and significance of a problem in relation to an animal's nutritional or disease state.

Tier I analyses are the least costly and provide the least amount of information. The intent of Tier I is to confirm that major and key minor nutrients were included in the diet.

Tier II evaluates selected essential nutrients that the animal requires. Also, Tier II is to test for oxidation (rancidity) and spoilage problems.

Tier III is the most expensive. Individual analysis of vitamins, minerals, mycotoxins, and pesticides are conducted in Tier III. In most cases, problems encountered with Tier III items are less frequent but are nevertheless extremely important considerations.

Tier IV is a separate group of analyses primarily for individual feed ingredients. You can use Tier IV analyses on individual feedstuffs or ingredients mixed in a complete feed to determine the presence or absence of a specified ingredient.

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Table 1. The four levels of the Tier System.

Tier I
Proximate analysis:
- Crude protein
- Fat
- Ash
- Dry matter
- Fiber
- Residual fraction

Vitamin A
Vitamin C
Zinc

Tier II
Amino acids
Fatty acids
Calcium and phosphorus
Measure of nutrient loss from oxidation
Measure of spoilage
Aflatoxin
Total plate count/coliform/salmonella

Tier III
Fat soluble vitamins: A, D, E, and K
Water soluble vitamins
Macro, trace, and heavy minerals
Botulism (mass mortality)
Type A *C. perfringes*
Mycotoxin screen
Pesticide screen

Tier IV
Feed microscopy
Cottonseed meal: gossypol
Soybean meal: trypsin inhibitor, urease
Canola or rapeseed meal: erucic acid, glucosinolates
Blood meal: hemoglobin
Crustacean meals: chitin
drugs/pigments/antioxidants, etc.
In vitro digestion of fish or meat and bone meal

Benefits of Using a Tier System

Errors occasionally occur in feed manufacturing. Some errors are easily identified, while others are more elusive. Major feed ingredients such as fish meal, soybean meal, and corn are mixed with minor components, such as vitamin and trace mineral premixes, salt, calcium, and phosphorus sources.

Tier I is primarily designed to indicate that all ingredients were included in the feed or that the feed was correctly formulated. Starting with proximate analysis, these tests help determine that the expected major ingredients are present in the feed. Next, the analysis of one vitamin and trace mineral normally present in the vitamin and trace mineral premixes is designed to indicate that the premixes were included in the feed and at the proper level.

Do not overlook the vital importance of vitamin C in aquaculture diets. Vitamin C is not part of the vitamin premix and needs analysis.

Nutrient absence in a feed may be from omission or by destruction from spoilage, oxidation, or both. Assuming that good quality ingredients were used and expected proximate analysis values were found, the probability of a good quality feed leaving the feed mill is more certain. Logically, with the assurance of the feed analysis, you can expect animals eating this feed to grow as expected. However, if problems arise, then Tier II is designed to evaluate the feed in more detail (fig. 1).

Tier I

Proximate analysis subdivides the sample into six major categories of general information about a feed. Tier I provides information to estimate whether the feed was prepared according to the feed tag description. This information is a broad general description.

√ **Crude protein**, for example, is the total amount of nitrogen converted to a protein equivalent. The nitrogen may come from a variety of substances in feed other than protein. Therefore, crude protein does not reveal the source of the nitrogen, the amino acid content (amino acids are building blocks of protein), or whether the protein used was heat damaged, poorly processed (i.e., soybean meal: elevated trypsin inhibitor, urease activity), or spoiled.

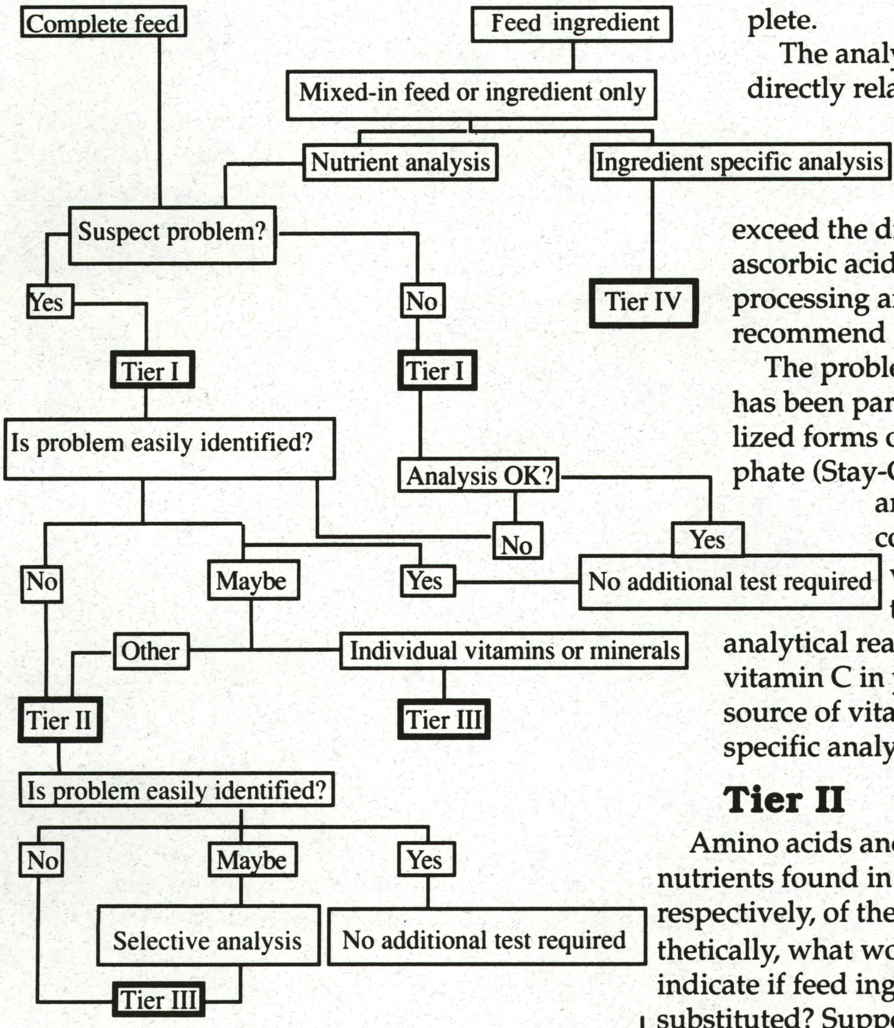
√ **The percentage fat** is the sum of all fat-like material in feed. This fraction contains triglycerides, essential and nonessential fatty acids, volatile fatty acids, fat soluble vitamins (A, D, E, and K), cutin, and waxes. The percentage fat does not indicate the state of rancidity.

√ **Ash is the sum of minerals** in a feed. Minerals may come from mineral supplements, premixes, bone, salt, sand, and minerals in the ingredients.

√ **Acid detergent fiber** is the measure of cellulose and lignin in a sample. Fiber is generally considered a bulking agent or an indigestible fraction in aquaculture diets. (For a complete discussion of the many aspects of fiber, see your Extension agricultural agent or librarian about nutritional aspects of the various fiber classes.)

√ **Water content** is the reciprocal of **dry matter** and a **residual fraction** remains after crude

Fig. 1. Tier System for feed and feed ingredient analysis.



protein, fat, ash, fiber, and water have been accounted for in a sample. The residual fraction is composed primarily of carbohydrates but other noncarbohydrate compounds also may be present. Historically, the residual fraction has been called nitrogen free extract (NFE), nonstructural carbohydrates, or nonfiber carbohydrates.

The analysis of vitamin A and zinc was included in Tier I to indicate that the vitamin and trace mineral premixes have been added to the feed. If the vitamin A concentration is low, two possible explanations come to mind: (1) the premix was not added, or (2) the feed was subjected to conditions that lead to oxidation. In either case, additional testing using Tiers II and III (fig. 1) is necessary to identify and confirm the cause of the problem. Zinc concentrations outside the expected level for the feed, for example, may indicate that the trace mineral premix was not properly added or that the mixing process was incomplete.

The analysis of vitamin C in Tier I is directly related to the importance of ascorbic acid in fish health and nutrition. Vitamin C concentrations must meet or exceed the dietary requirements. Free ascorbic acid is readily susceptible to processing and oxidation losses, and I recommend feed analysis of vitamin C.

The problem of ascorbic acid stability has been partially overcome by new stabilized forms of vitamin C. Ascorbyl-phosphate (Stay-C®), ascorbyl-sulfate (Astos®), and encapsulated ascorbate are commercial stabilized forms of vitamin C that are less susceptible to degradation. For analytical reasons, specify the type of vitamin C in your feed. Each commercial source of vitamin C requires a product-specific analysis technique.

Tier II

Amino acids and fatty acids are essential nutrients found in the protein and fat fraction respectively, of the proximate analysis. Hypothetically, what would the feed analysis data indicate if feed ingredients were incorrectly substituted? Suppose a mixture of corn and urea (a nitrogen containing compound) replaced a part of the fish meal in the feed. Proximate analysis would suggest that the amount of nitrogen or protein equivalent meets the feed tag guarantee. However, an amino acid analysis would show that the expected levels of amino acids were not present, because urea provides nitrogen but not amino acids.

Continuing the example with fat, where tallow was substituted in place of fish oil, proximate analysis again would suggest that the feed was within the specification listed on the feed tag for fat. However, Tier II fatty acid analysis would show that the expected levels of highly unsaturated fatty acids ($\underline{\omega}$-3 and $\underline{\omega}$-6) were limiting. Thus, Tier II analysis provides more in-depth information than Tier I alone can provide.

See the flow diagram in figure 1 to determine when and which analyses to conduct. In the previous example, the feed was analyzed prior to suspecting a problem. The combination of Tier I and Tier II analyses would have been sufficient to identify the problem(s). In the case where you suspect a problem with feed based on your knowledge of aquaculture and on indications from the animals, then the flow diagram in figure 1 also would lead us to Tier II and if necessary to Tier III.

Within Tier II, nutrient loss due to rancidity or oxidation of fat and oil and spoilage is considered. Rancid fat or oil in feed reduces the concentrations of essential fat soluble vitamins D, E, and K), folic acid, biotin, and essential unsaturated fatty acids. In addition, rancidity elevates concentration of peroxides, free fatty acids, and thiobarbituric acid reaction products.

Elevated total volatile nitrogen (TVN), trimethylamine, histamine, cadaverine, putrescine, aflatoxin, and microbial numbers indicate spoilage. In cases where raw fish spoiled prior to drying during fish meal production, the TVN value will be low due to the drying process but other indicators would suggest that the fish meal spoiled during production.

Aflatoxin is a product of aspergillus mold. Trout diets containing greater than 2 parts per billion (ppb) aflatoxin cause liver tumors. Thus, aflatoxin-free feed is critical for trout. The Food and Drug Administration allows aflatoxin concentrations in feed up to 20 parts per million (ppm). Although catfish have a higher tolerance level for aflatoxin, 20 ppm is clearly too high for most aquaculture species.

Total plate count of microbes greater than 100,000 colony forming units and the presence of any coliform or salmonella bacteria are not acceptable and are evidence that the spoilage process is active.

Tier III

Tier III is the complete analysis of individual vitamins, minerals, mycotoxins, and pesticides. Evaluating individual vitamins or minerals is useful for unanswered questions from Tier I and Tier II. For example, additional verification of the trace mineral premix addition may be required if the data for zinc in Tier I was not correct. In a second example, if vitamin A was found to be low (Tier I) and measures of oxidation (Tier II) were borderline, then quantifying additional vitamins would be necessary to determine if the premix indeed was added or whether oxidation was the problem. Thirdly, the wrong premix may have been used or the premix may not be correctly formulated by the premix manufacture.

Limited data are available for the toxicology of mycotoxins in aquatic animals. Until data are available, the relative toxicity of the different mycotoxins in mammals can serve as a guide or reference. Toxicology of pesticides in aquatic animals is available but most of the research has evaluated the lethal dose or lethal concentration (LD_{50} or LC_{50}) of pesticides in water. Therefore, in most cases, toxicity of dietary pesticides will be extrapolated from water-borne studies. Extrapolation of the data should provide a relative guideline in accessing the toxicity of dietary pesticides.

Tier IV

In Tier IV, feed microscopy can determine presence or absence of major ingredients and estimate the amount of filth and pest contamination. When a producer specifies that an ingredient be excluded or included in a feed formulation, analysis of an indicator specific for that feedstuff is used. For example, if cottonseed meal is not to be in a feed, you should request feed microscopy and gossypol analysis to determine the presence of cottonseed meal.

To determine the presence of soybean meal or to determine the quality of processed soybean meal, analysis of trypsin inhibitor or urease activity should be conducted. Poorly processed soybean meal will have high trypsin inhibitor and urease activity. Other examples of indicator specific test are listed in table 1.

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