
NUTRITION ADVISORY GROUP HANDBOOK



QUALITY CONTROL OF FEEDSTUFFS: NUTRIENT ANALYSES^a

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Nutrient analyses are integral features of a quality control program designed to ensure the nutritional value and monitor nutrient composition of diets used for captive animals. Other components of a good quality control program include issues such as the presence of toxins, including mycotoxins, microbial contamination, and organophosphate/pesticide contamination. These issues will not, however, be included in this discussion. A quality control program begins with identification of the items to be analyzed and the establishment of a regular schedule of sampling. Specific protocols should be used to ensure that sampling is representative. Selection of the nutrient analyses to be performed should be based on the item to be analyzed and potential nutritional or quality-related problems with that item. When choosing an analytical laboratory, evidence of competency, as well as analytical costs, should be considered. Details of an appropriate quality control program, using nutrient analyses, are presented below.

Purpose of Quality Control

If it looks good and the animals eat it, it must be okay, right? This may sound nonsensical, but all too often these criteria predominate when assessing the quality of feeds used in zoo animal diets. While the appearance and palatability of feeds are important, nutrient analyses will more accurately define nutritional value.

^a Adapted from Dempsey, J.L., and J.B. Bernard. 1999. Everything you never wanted to know, but should, about feedstuffs 'or' the importance of chemical analysis in feed quality control. Proceedings of the American Association of Zoo Veterinarians, Columbus, OH.

Nutrient analyses are most useful when there is a basis for comparison to typical or expected nutrient concentrations. Many zoos now use fixed formula feeds where both ingredients and nutrient concentrations are specified. In such cases, it is relatively easy to make comparisons of analytical results to specified standards. In other cases, however, “off the shelf” products are purchased so that the only basis for comparing analytical results may be the guaranteed analysis stated on the label. Fortunately, many feed manufacturers marketing to zoos will routinely provide calculated approximate nutrient composition data or typical laboratory analyses. Such information should be requested from those manufacturers that do not routinely provide it.

Feeds should be systematically and regularly analyzed for a number of reasons. Manufactured feeds may not meet specifications due to inattention to detail during mixing or due to mechanical problems during the manufacturing process. Although feeds may meet minimum nutrient specifications, some nutrients may be included at higher than appropriate levels. Sometimes, unauthorized ingredient substitutions are made. While nutrient analyses can detect some substitutions, identification of this latter problem is often best accomplished by an experienced feed microscopist. Individual feedstuffs, or foods such as frozen fish, should be analyzed to monitor the wide fluctuations in some nutrients (e.g., fat in fish) that may occur on a seasonal, regional, or species-specific basis. Fluctuations in nutrient composition also occur in forages based on season of harvest, geographic region, and species of plant, and in whole prey items such as rodents and insects due to variations in developmental stages or differences in diets fed to these prey items.

A quality control program is essential to ensure the nutritional quality of feeds and food items used in captive animal diets. Nutrient analyses are basic components of such a program. A schedule for sampling and analysis should be established for all items, especially those that have a major impact on animal welfare and represent a significant part of the zoo budget.

Identifying Feeds for Analysis

One of the first steps in instituting a quality control program is to identify primary feeds, i.e., feeds that are used in large quantity and fed to a number of different animals throughout the collection. For most zoos, these would include forages, fish, herbivore pellets, carnivore diets, and primate diets. Since the quality of primary feeds will have a great impact on a large proportion of the collection, these feeds should be analyzed more often than specialty feeds. Primary feeds should be analyzed at least four times per year. Specialty feeds, those that are fed to a small proportion of the collection, should be analyzed at least twice per year. If problems are encountered, analyses may be conducted more frequently.

In general, it is not economically feasible or necessary to set up a regular schedule of sampling and nutrient analysis for high-moisture produce items, such as fresh fruits and vegetables, since produce inventory typically has a rapid turnover, usually one week or less. In addition, produce items should contribute minimally to the dry matter in individual animal diets, with the majority of nutrients supplied by nutritionally complete feeds. As a consequence, the produce portion should not significantly impact overall nutrient composition of the diet, and slight variations in the nutrient content of produce are of less concern.

Choosing the Analyses To Be Performed

The analyses to be performed depend on the type of food item and the reasons for sampling. Typically, chemical analyses for the nutritional assessment of feeds should include proximate fractions (moisture [or conversely, dry matter], crude protein, ether extract, and ash) and fiber (neutral detergent fiber, acid detergent fiber, and acid lignin). Gross energy may also be included and is particularly useful if there are opportunities to estimate apparently digestible or metabolizable energy concentrations of the feed by also collecting and analyzing excreta. Analyses for major minerals (calcium, phosphorus, sodium, potassium and magnesium) as well as trace minerals (iron, copper, manganese, zinc, and selenium) are also important. For some feeds, it may be important to determine vitamin concentrations, but high cost may limit the number and frequency of analyses. Analyzing for other nutrients, elements, or compounds may be necessary if there is evidence of a specific health problem with a potential link to these items. Routine analyses that should be performed, based on type of feed, are listed below in order of priority:

Forages – proximate fractions, fiber fractions, major and trace minerals, gross energy.

Dry/semi-moist/moist feeds – proximate fractions, fiber fractions, gross energy, major and trace minerals.

Fish – proximate fractions, gross energy, vitamins A and E, major and trace minerals.

Meat-based foods – proximate fractions, gross energy, major and trace minerals, vitamins A and E.

Whole prey - proximate fractions, gross energy, major and trace minerals, vitamins A and E.

Selecting the Representative Sample

The goal, when sampling feeds for analysis, is to obtain a small portion that is representative of the entire lot (batch, catch, load). Obtaining a representative sample is critical because the initial sampling step frequently introduces the greatest variability and most affects the usefulness of the analytical result. Each product's manufacturer, lot number, receipt date, and feed tag should be recorded in a permanent file. Ideally, every new lot should be sampled and sent for analysis, and the larger the sample size, the more representative the results are likely to be. However, the constraints of time, cost, and facilities available for collecting and storing samples, and producing analytical results, seldom accommodate the ideal. Thus, it is important to establish a realistic plan for sampling and nutrient analyses that will support rational decision making. Well-defined statistical sampling procedures that consider the heterogeneity of foods and the analytical precision of selected assays are most appropriate. As an alternative to these mathematical procedures, the following protocols include proposed sample numbers and amounts based on circumstances typically found in zoos. If inappropriate in a particular situation, rational adjustments can be made.

Protocol for sampling forages for nutritional analysis

1. Hay should be sampled using a core forage sampler with a minimum cutting diameter of $\frac{1}{2}$ and a minimum sampling length of 12". (Examples: Multi-Forage Sampler™, Star Quality Samplers, 5719-114A St., Edmonton, Alberta T6H 3M8; Penn State Forage Sampler™, NASCO, 901 Janesville Ave., Fort Atkinson, WI 53538-0901; 414-563-2446)
2. Cores should be taken to full depth of the sampler from the center of the end of randomly selected rectangular bales or coincident with the radius of randomly selected round bales.
3. A composite (weighing about 500 g) of cores from 15-20 bales should be collected from each lot of each type of hay to be analyzed.
4. The composite should be placed in an airtight plastic bag, sealed, and labeled with the identity of the hay, the date the sample was taken, and the name of the contact person and collecting institution.
5. Composite samples should be stored in a cool, dry place until shipped to a certified laboratory for chemical analysis. Although sometimes used for forages, near infrared reflectance spectrophotometry (NIRS) is recommended only if the analytical laboratory can demonstrate that their NIRS instrument and analytical system has been calibrated with and is valid for the purchased hays. See **NAG Fact Sheet 001: Hay Quality Evaluation**.

Protocol for sampling dry/semi-moist/moist feeds for nutritional analysis

1. Samples should be taken from ≥ 10 containers (bags, boxes, cans). Samples consisting of ≥ 100 g should be collected from the center of each container holding 5 kg or more and combined. A 500 g mixed composite should be held for analysis and the remainder discarded. (Note: ingredients in dry ground feeds may segregate during shipment and handling, so extra care is required to ensure that samples are representative. A spiral probe from the Seedburo Equipment Co. [1022 W. Jackson, Blvd., Chicago, IL 60607; 800-284-5779] or a three-zone powder sampler from NASCO are particularly helpful in obtaining representative samples from the bottom to the top of dry-ingredient containers.)
2. The composite should be placed in a plastic bag (thick enough to limit moisture exchange), sealed, and labeled with the feed identity, manufacturer's name, date code or lot number, date the sample was taken, and the name of the contact person and collecting institution.
3. Dry feed samples should be stored in a cool, dry place. Samples of semi-moist/moist feeds should be placed immediately into freezer storage. When shipped, samples should be packed with a sufficient amount of dry ice to prevent thawing.
4. All opened dry/semi-moist feed containers, which do not require refrigeration or freezing after opening, should be closed and sealed. Open containers of semi-moist/moist feeds which require refrigeration or freezing should be used immediately or discarded.
5. For dry feeds delivered in bulk and placed in hoppers, random sampling should take place when the hopper is being filled, with due attention to the potential for separation of particles differing in size, shape, and density.

Protocol for sampling frozen fish and other frozen foods for nutritional analysis

Frozen fish

1. Samples should consist of ≥ 1 kg for each species.
2. For individually quick-frozen (IQF) fish, samples should be taken from at least 5 randomly selected cases from different pallets and different areas of the pallet, for each species of fish.
3. Bulk frozen fish should be sampled by cutting sections from blocks using a band saw. Samples should be taken from each of 5 randomly selected cases of fish from different pallets and different areas of the pallet. Cases should be opened and the block of fish cut into 2 approximately equal sections. One of the 2 sections should have a strip (approximately 5 cm wide) cut from the original outer side and from the inner, newly cut side. Total material obtained from the 5 cases should be at least 3 kg. This should be thoroughly mixed, a 1-kg sample held for analysis, and the remainder discarded.
4. Fish samples should be placed immediately into thick plastic bags that have been pre-labeled with species of fish, lot number and date of catch, the date the sample was taken, and the name of the contact person and collecting institution. The bags should be placed immediately into freezer storage. When shipped, samples should be packed with a sufficient amount of dry ice to prevent thawing.

Other frozen foods

1. Other frozen foods, such as bags of carnivore diet, should be sampled by cutting approximately $\frac{1}{4}$ kg off the end of each of 3 randomly selected tubes, and $\frac{1}{4}$ kg from the center of 3 randomly selected tubes, using a band saw.
2. Samples should be placed immediately into thick plastic bags that have been pre-labeled with type of feed, manufacturer's name, date code or lot number, the date the sample was taken, and the name of the contact person and collecting institution. The bags should be placed immediately into freezer storage. When shipped, samples should be packed with a sufficient amount of dry ice to prevent thawing.

Protocol for sampling whole prey for nutritional analysis

Vertebrate prey

1. Frozen or fresh vertebrate prey samples should consist of a minimum of 10 animals with a maximum of about 1 to 2 kg total weight for each species.
2. Prey samples should be placed into thick plastic bags that have been pre-labeled with species, the date prey were received, the date the sample was taken, and the name of the contact person and collecting institution. The bags should be placed immediately into freezer storage. When shipped, samples should be packed with a sufficient amount of dry ice to prevent thawing.

Invertebrate prey

1. Frozen or fresh invertebrate prey samples should consist (if feasible) of a minimum of 100 g for each species. The protocol for sampling marine invertebrates is the same as for fish. For small invertebrates (e.g., insects, annelids, arachnids), it may be necessary to

limit the analyses to the assays that are most important because of minimum analytical sample size requirements.

2. Prey samples should be placed into thick plastic bags that have been pre-labeled with species, the date prey were received, the date the sample was taken, and the name of the contact person and collecting institution. The bags should be placed immediately into freezer storage. When shipped, samples should be packed with a sufficient amount of dry ice to prevent thawing.

Choosing a Laboratory

Once appropriate samples have been collected and are ready for nutrient analysis, there are numerous laboratories from which to choose, including commercial, university, and hospital laboratories. There are even a few zoos with nutrition laboratories. However, all laboratories are not equal in their abilities or experience in analyzing different types of feeds and food items. For example, a laboratory specializing in hay analysis may not be familiar with procedures for sample preparation and analysis of fish or whole prey items.

Cost is certainly a consideration when choosing a laboratory, but it is important to note that inexpensive analyses are no bargain if the results produced are not reliable. Reliability of an analytical procedure depends upon its (1) *specificity*, (2) *accuracy*, (3) *precision*, and (4) *sensitivity*. Analytical *specificity* infers that the measured response is unique to the nutrient whose concentration is being determined and is not affected by interfering or related substances in the sample. An *accurate* result is a true estimate of the concentration of the nutrient, and is sometimes tested by adding a known amount of the nutrient to the sample and determining its recovery. A more reliable test of accuracy involves comparisons of the results of analyses of standard reference materials from the National Institute of Standards and Technology (Gaithersburg, MD 20899; 301-975-6776) with their certified concentrations. The reference material should have a matrix similar to that of the samples to be analyzed (i.e., it should be similar in its overall physical and chemical composition). A *precise* result is one that is highly repeatable. It should be noted that an analytical result can be very precise but inaccurate. Analytical *sensitivity* is an expression of the smallest difference in composition that can be measured between two samples.

Regulatory agencies are particularly concerned about assay reliability, and the AOAC (Association of Official Analytical Chemists International) characterizes assays with regard to the following:

1. Reproducibility – between-laboratory precision.
2. Repeatability – within-laboratory precision.
3. Systematic error or bias – deviation from the ‘true’ value.
4. Specificity – ability to measure what is intended to be measured.
5. Limit of reliable measurement – smallest increment that can be measured with confidence.

Prospective laboratories should be critically evaluated with respect to these issues before sending samples for analysis. Questions to ask include:

1. *Does the laboratory have experience performing the analyses requested on the type of feed to be analyzed?*
2. *Does the laboratory use AOAC-approved methods or methods which are proven/accepted and referenced in current literature?*
3. *Is the laboratory willing to provide detailed references for the methods used?*
4. *Is the laboratory familiar with required differences in methods of sample preparation depending on the type of food sample being analyzed?*
5. *Does the laboratory have a quality control program and use certified standard reference materials from the National Institute of Standards and Technology?*

Professionals in nutrition can be consulted for recommendations on laboratories and acceptable analytical methods. More specific information on sampling and analytical techniques can be found in the following references:

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