# Contemporary and Emerging Issues
## Analytical Method Challenges for Measuring Nutrients and Anti-Nutrients in Plants

**666 A survey of methods of analysis used for minerals in feedstuffs.** Milan Ihnat*, *Agiculture and Agri-Food Canada*.

A wide range of minerals occur in feedstuffs as naturally-occurring and purposely added elements as well as by adventitious contamination. These mineral elements can generally be classified as nutritionally-essential major elements such as Na, K, Mg, Ca, N, P; nutritionally essential minor and trace elements: B, Si, F, V, Cr, Mn, Fe, Co, Cu, Zn, As, Se, Br, Mo, Sn, I; and those regarded as toxic or with an essential/toxic duality: F, V, Cr, Mn, Co, Ni, Zn, As, Se, Mo, Pd, Cd, Sn, Hg, Tl, Pb. A survey is presented of the state of the art regarding methods used for the determination of major, minor and trace elements in feedstuffs and related biological materials. Challenges posed by analytical endeavours in general, as well as challenges posed by some difficult-to-measure elemental analytes will be discussed. Currently available methods for determination of elements in feedstuffs and related materials include: atomic absorption spectrometry, atomic emission spectrometry, mass spectrometry, neutron activation analysis, X-ray emission spectrometry, molecular light absorption spectrometry, molecular fluorometry, electrochemistry, Kjeldahl method for nitrogen, combustion elemental analysis, volumetry, ion chromatography and gravimetry. Attributes of currently available definitive, reference, routine, field, official, unofficial and recommended methods are reviewed as a basis for the formulation of recommendations of most suitable methods for feedstuffs. A summary is also presented of related work in progress to develop unified comprehensive, consolidated schemes of analysis utilizing widely utilized atomic absorption spectrometry and also the complimentary techniques of inductively coupled atomic emission and mass spectrometries for multielement measurement in biological materials.

**Key Words:** Minerals, Analytical methods, Feedstuffs

**667 Challenges with nonfiber carbohydrate methods.** and M. B. Hall*[1], [1]*Dept. of Animal Sciences, University of Florida*.

Nonfiber carbohydrates (NFC) not found in neutral detergent fiber (NDF) encompass a compositionally and nutritionally diverse group. They have often been described with a single value estimated by difference as 100% of dry matter # crude protein (CP) # NDF # NDFCP - ether extract # ash. A calculated value was used because of difficulties with the assays for individual NFC; it does not differentiate among nutritionally distinct NFC. Errors in NFC estimation can arise from not accounting for CP in NDF, and when multipliers other than 6.25 are appropriate to estimate CP. Analyses that begin to distinguish among NFC are those for starch, soluble fiber, and sugars (mono- and oligosaccharides). Many starch analyses quantitate alpha-glucans through specific hydrolysis of alpha (1->4) and (1->6) linkages in the glucan, and measurement of released glucose. Incomplete gelatinization and hydrolysis lead to underestimation of starch content. Use of enzymes preparations that hydrolyze carbohydrates other than alpha-glucan, measurement of all released monosaccharides without specificity for glucose, and failure to exclude free glucose present in the unhydrolyzed sample inflate starch values. Soluble fiber analyses can err in a fashion similar to NFC if correction for CP requires multipliers other than 6.25, or if contaminants such as CP and starch have not been properly accounted. #Sugars# have been defined as carbohydrates soluble in 78 # 80% ethanol, which separates them from the polysaccharides. They can be measured in extracts using broad spectrum colorimetric assays (e.g., phenol-sulfuric acid assay, and acid hydrolysis of the extract with reducing sugar analysis), or chromatographic methods. Colorimetric assays do not differentiate among mono- and oligosaccharides. The results of the phenol-sulfuric acid assay rely on selection of a sugar standard that reflects the predominant sugar in the sample. Reducing sugar analysis without acid hydrolysis measures only monosaccharides. HPIC can differentiate among various mono- and disaccharides; many larger oligosaccharides often lack appropriate carbohydrate standards. Current methods for NFC can separate nutritionally relevant fractions, but care needs to be taken to assure accurate measurement.

**Key Words:** NFC, carbohydrates, methods

**668 Challenges with insoluble fiber methods.** D.R. Mertens*[1], [1]*US Dairy Forage Research Center, Madison, WI*.

Insoluble fiber is an important criteria of nutritional quality because it is related to digestibility and energy value. The challenge with fiber methodology is to select those that are relevant and reproducible. Without relevance there is no purpose in measuring fiber, and without reproducibility there is no practical value in measuring it. For routine analysis, fiber methods must not only satisfy nutritional and analytical criteria, but also be convenient and economical. Fiber is unique among nutrients because it is defined in nutritional terms, but is determined using chemical, physical or enzymatic techniques. Laboratory analysis of insoluble fiber can be divided into: those that measure chemical entities, those that measure biological or enzymatic entities, and those that measure empirical entities. Crude fiber is a purely empirical method in which fiber is defined by the method. It fails to recover lignin and many components of fiber that affect nutritive value. Its status as a legal definition of fiber should be abolished. Dietary fiber that is measured by enzymatic or in vitro methods is applicable only for the animal system it was designed to mimic, and are typically difficult to reproduce among laboratories. Chemical methods of determining fiber vary considerably in approach. Some methods use extraction, hydrolysis and sugar residue analysis to quantify specific groups of insoluble polysaccharides. Others use detergents to measure cellulose, hemicellulose and lignin more directly using chemical solubility. The first approach provides more detailed and specific information, but requires more time and expense. The latter is rapid, but less specific. Both are somewhat empirical because the conditions of extraction, hydrolysis, and measurement must be followed exactly to obtain repeatable and reproducible results. Fiber analyses are of unequal value in providing useful nutritional information, and no single analysis can explain the entire complexity of nutritive value. Selection of appropriate insoluble fiber methods will always be a compromise between nutritional relevance and analytical convenience and reproducibility.

**Key Words:** fiber, feed analysis

**669 Challenges with fats and fatty acid methods.** D. L. Palmquist*[1] and T. C. Jenkins[2], [1]*Dept. of Animal Sciences, The Ohio State Univ., Wooster,* [2]*Dept. of Animal and Veterinary Sciences, Clemson Univ., Clemson, SC*.

The content and chemical nature of lipids in feedstuffs is heterogeneous. It has long been known that ether extraction by the Weende procedure inadequately characterizes fat content of feedstuffs, yet it remains the official method. Diethyl ether (or hexanes that are often used) extracts significant amounts of non-nutritive, non-saponifiable lipids from forages, and often incompletely extracts lipids of nutritional value, especially fatty acids present as salts of divalent cations. Pre-extraction hydrolysis of insoluble fatty acid salts with acid releases these fatty acids, and this step is included in the official procedure for certain feedstuffs in the UK. However, acid hydrolysis increases analysis time, decreases precision, and increases extraction of non-nutritive materials. Acid hydrolysis also causes confusion as to the proper definition of the fat content of feedstuffs. A preferred method of fat analysis determines the total fatty acid concentration in feed samples by converting fatty acid salts, as well as the acyl components in all lipid classes, such as triacyglycerols, phospholipids, and sphingolipids, to methyl esters using a simple, direct one-step esterification procedure. Fatty acid methyl esters are then quantified by gas-liquid chromatography, which provides information on both fatty acid quantity and profile in a single analysis. Adjustments in conditions and reagents may be necessary to overcome difficulty in quantitatively preparing esters from certain types of fatty acids and their derivatives in commercial fat supplements. After correction for glycerol content, analysis of oils by this procedure provides information on the content of non-saponifiable material, such as chlorophyll, waxes and indigestible polymers formed from heat- or oxidatively-damaged fats. The correct description of feedstuffs for nutritive value of fats is the content of total fatty acids.

**Key Words:** Analysis, Fat, Fatty acids

**670 Challenges and new opportunities in the analysis of raffinose oligosaccharides, phytate and glucosinolates.** D. Vinjamoori*, P. Das, and T. Hayes, *Monsanto Co., St. Louis, MO/USA*.

Oligosaccharides of the raffinose series are major components in many grain legumes and are implicated in causing flatulence and diarrhea for both humans and livestock, as they are not hydrolyzed in the upper gut due to the absence of alpha-galactosidase enzyme. Phytic acid has been identified, as an antinutritional factor of soybean since it can reduce the bioavailability of some essential metals and phosphorous because of the formation of insoluble chelates that cannot be absorbed by the intestine under normal physiological conditions. Phytic acid has also been shown to inhibit the action of some important proteins such as trypsin, alpha-amylase and pepsin during digestion. Glucosinolates derived from Brassica species have been clearly shown to have deleterious effects such as reduced fertility and induction of goitrogenic effects in live-stock, premature death in rats and damage to vital organs stemming from the interference with the thyroid

In this presentation we will review the current status of the analytical technologies for the assays of raffinose oligosaccahrides, phytic acid and glucosinolates in terms of selectivity, sensitivity and sample throughput. Implementation of innovative sample preparation schemes, use of novel separation approaches and alternate detector technologies will be presented. The challenges and opportunities posed by these assays will be highlighted along with the recommendations for best analytical practices.

**Key Words:** Raffinose, Phytate, Glucosinolates

**671 Challenges in measuring moisture content of feeds.** N. Thiex*[1] and C. R. Richardson[2], [1]*South Dakota State University, Brookings, SD,* [2]*Texas Tech University, Lubbock, TX*.

Accurate determination of the moisture (water) content in individual feed ingredients and mixed feeds is important, but often the analytical methods used differ greatly in effectiveness resulting in over or under evaluation. Bias in measuring the water content of feedstuffs directly effects accurate quantification and expression of other nutrient values and ratios. Factors affecting accurate determination include: range in moisture content, sampling of feedstuffs, transport and storage of laboratory samples, loss of volatiles other than water, and choice of analytical method. Several methods in use to determine apparent water content of feedstuffs are empirical, estimating water by loss of weight on drying, while other methods measure water directly. Poor agreement among laboratories and among methods is illustrated in results of moisture determinations reported to the American Association of Feed Control Officials Check Sample program and in the National Forage Testing Association Proficiency Testing program. Oven drying methods and a Karl Fisher method were compared in this study using forage and dried, ground animal feed. Forages tested included hay, haylage, and corn silage while feeds included various sources of mixed feed with and without urea. Oven drying of forages, compared to the Karl Fischer method, yielded recoveries for hay, haylage, and corn silage, respectively, as follows: 135° C for 2 h # 113%, 162%, and 133%; 104° C for 3 h # 96%, 122%, and 113%; 104° C for 6 h # 97%, 129%, and 117%. Mixed feeds yielded recoveries for non-urea and urea containing feed, respectively, as follows: 135° C for 2 h # 116%, and 2746%; 104° C for 3 h # 88%, and 239%; 95° C for 5 h under vacuum 83%, and 727%; 104° C for 6 h # 90%, and 427%; 110° C for 3 h # 94%, and 425%. NIR calibrations for water (moisture) based on the Karl Fischer method were ($r^2 = 0.98$; SEC = 0.20). In conclusion, a need to evaluate and improve moisture methods, and standardize practices in laboratories is apparent.

**Key Words:** Moisture, Oven Drying, Karl Fischer

## ARPAS-FASS Symposium
## ARPAS-FASS Symposium on Animal Care Training and Certification for Research Facilities and Commercial On-Farm Assessment Programs

**672 ARPAS Animal Care Certification Program.** J.C. Swanson*[1], [1]*Kansas State University*.

Research and teaching institutions are required to meet training mandates for animal care workers and professionals. The *Guide for the Care and Use of Agricultural Animals In Agricultural Research and Teaching* states "It is the responsibility of the institution to ensure that scientists, agricultural animal care staff, students, and other individuals who care for or use agricultural animals are qualified to do so through training or experience." Although the American Association for Laboratory Animal Science offers certification at the level of techinician and technologist for laboratory animal personnel, no program exists specific to agricultural animal care. The American Registry of Professional Animal Scientists (ARPAS) is developing a certification program specific to agricultural animal care at the professional and technician level. This program is being developed in conjunction with the Federation of Animal Science Societies' development of training modules for the different agricultural species.

**Key Words:** Animal care, Training, Certification

**673 The ARPAS - FASS - AAA Animal Care Project.** K.E. Olson*[1], B.R. Baumgardt[2], C.L. Sapp[3], and B.P. Glenn[3], [1]*KEO COnsulting,* [2]*American Registry of Professional Animal Scientist,* [3]*Federation of Animal Science Societies*.

Animal care is an issue of increasing importance to consumers and to retailers. Most species have developed quality assurance programs or best management practices that include animal care guidelines, but in most cases consumers are unaware of these efforts and their use is not documented. The Animal Agriculture Alliance (AAA) is a relatively new organization whose mission is to #support and promote animal agriculture practices that provide for farm animal well-being through sound science and public education.# It is recognized that for a program to be credible with the public it must be based on sound science and be verifiable.

AAA has identified six basic animal care principles felt to be critical in assuring animal well-being. They have contracted with the ARPAS and FASS to develop criteria and a process for evaluation of species specific farm-animal well-being guidelines to assess their compliance with these principles. Submitted programs that comply will be recognized. To the extent possible, quantifiable measures are used to assess compliance.
A two step process has been used in this project. Initially a steering committee, comprised of individuals with scientific backgrounds related to the species being evaluated, as well as others with expertise in animal behavior, veterinary medicine, engineering, transportation and handling, ethics, and consumer interests, developed an umbrella set of criteria for use with all species. Next, species specific subcommittees, comprised of individuals with expertise in each of the species, identified science based numeric ranges and other measures appropriate for assessing care within their species. The species reports are reviewed by the steering committee to provide the greatest consistency possible. Initial species included beef, broilers, dairy, layers, pork, sheep and turkeys. Species programs will be submitted for review and recognition of compliance.
This process assures consumers that all species are being evaluated in a similar manner, buyers that there will be consistency in assessments by different individuals, and producers that the evaluations are based on the best science available.

**Key Words:** Animal Care, Consumers, well-being