

# 2011 Annual Meeting Abstracts

## Agricultural Microscopy

### MONDAY

#### MORNING

---

#### AM 1: Agricultural Microscopy I

---

Chair(s): P. Ramsey, California Department of Food and Agriculture (Retired), USA; and G. Kobata, California Department of Food and Agriculture, USA

#### **Ethical Lapses and Ignorance in the Formulation, Labeling, and Marketing of Technical Nutritional Products.** L.D. Bunting, ADM Alliance Nutrition, Quincy, IL, USA

As livestock feeding programs strive to meet the highest standards for performance and optimal animal health nutrition programs are becoming both very technical and formulas highly complex. The number of specialty ingredient suppliers and products has increased in proportion to this increased sophistication. Both producer end-users and many working in the feed business lack sufficient technical depth to understand the nuances of how many nutritional products are best applied. Recently, the feed business has become fiercely competitive. Ultra-competitive environments always call for reinforcement of ethical standards relative to providing producers with nutritional products and programs with as-advertised performance at logical prices. Both ethical lapses and ignorance can lead to a number of questionable practices, such as including additives and other ingredients at below-recommend rates, window dressing labels, undisclosed substitution of brand-name ingredients, use of additives that are expired or of suspect activity and many forms of product misrepresentation. These practices are discussed relative to the importance of training and transparency throughout the supply chain. Also, the role of professional ethics within technical staff and other leaders within the feed businesses is discussed.

#### **Case Studies in Forensic Entomology.** Neal H. Haskell, Saint Joseph's College, Rensselaer, IN, USA

The following murder cases contained insect evidence and a forensic entomologist was consulted to establish a time of death estimation. The perpetrators were apprehended, charged with the murders and arraigned for trial. The previously determined entomological estimated PMI corresponded closely with the later known time of the murders as determined by other sources. The decomposed remains of a female were discovered in a wooded area in the vicinity of Funkhouser Quarry, York County, Pennsylvania on July 29, 2004 at 1030 hrs. The location was within 25 yards of the eastern edge of a cliff overlooking the quarry. There was marked decomposition with the ribs exposed and detachment of the left arm. The head was missing but skull fragments were lying between the outstretched arms on the ground above the shoulders. It appeared that she was shot in the back of the head and fallen backwards as she climbed up an incline. The primary indicator species was *Phormia regina* with the primary indicator life stage

being mature 3rd instar larvae. This species of blow fly is generally the most common blow fly in the northern latitudes of the U.S. during the summer season. The required energy units *Phormia regina* (the Black Blow Fly) needs to lay eggs and produce mature 3rd instar larvae ranges in excess of 1800 ADHs to as many as 2400 ADHs (From Kamal). The total ADH-B10 needed for *P. regina* to produce late 3rd instar larvae would have occurred, at the latest, sometime before sunset on July 24, 2004 to as early as prior to sunset on July 22, 2004. The species, *P. regina*, will usually arrive later at carrion with a 16 to 24 hour delay with maximum daily temperatures in the 70s and 80s.

### **Science in Murder: A Look at the Multiple Scientific Disciplines used When Conducting a Routine Forensic Entomology Analysis using the Life Cycles of Calliphorids.** Neal H.

Haskell, Saint Joseph's College, Rensselaer, IN, USA

A 10 year old boy went missing in the late afternoon of July 15. Six days later, July 21, the remains were discovered at the bottom of a large culvert. The area where the body was found was shaded by over-hanging trees and tall weeds. There was a small stream of water flowing under and around the remains (ca. 6 in. deep). Thousands of maggots were found on the remains, with the head completely skeletonized from the maggots. A variety of blow fly larvae were collected at the scene and autopsy and were identified as mature 3rd instar larvae of *Phormia regina* (as the oldest specimens). Based upon appropriate methods for assessing the PMI it was concluded that the minimum time colonization would have occurred was prior to sunset on July 16th. With the potential delay of *P. regina* of from 12 to 24 hours, death could be as early as prior to sunset on July 15th. To reach this conclusion, a number of scientific disciplines were used. **Insect biology, insect behavior, insect growth and development, climatology, statistics; and mathematics** were all used to facilitate the final postmortem interval determination. It should be noted that without the temperature calibration of the scene to the NWS, an error of ca. 48 hours would have ensued due to warmer temperatures at the NWS than were actually present at the bottom of the culvert. He had told his brother that he had killed the little boy prior to sunset on July 15th. The defendant was convicted of capital murder and has been executed in the Indiana State Penitentiary. This case resulted in the Zachary Law, Indiana's attempt to register all previous convicted child sex offenders with the communities in which they live. Even when conducting a simple, straight forward case analysis, there are many scientific disciplines used in formulating our forensic entomology conclusions.

### **Forensic Microscopy: The Science of Trace Evidence.** S. Palenik, Microtrace LLC, Elgin, IL, USA

The value of microscopy in the solution of analytical problems is not always appreciated by modern chemists whose education rarely includes any mention of this versatile technique. In this presentation, examples from the author's forensic casework will be used to illustrate the importance of the microscopical approach in solving difficult cases. After an initial introduction to the instruments and methods of the forensic microscopist, the contributions of microscopy to such cases as the Narita Airport Bombing (Tokyo), the Atlanta Child Murders, Assassination of Dr. Martin Luther King, Ivan the Terrible (Jerusalem), and the Green River Murders (Seattle), in addition to several other less well known forensic problems, will be described and illustrated.

**A Microscopy Staining Procedure to Assess Bran Removal Patterns.** D.F. Wood<sup>1</sup>, T.J. Siebenmorgen<sup>2</sup>, T.G. Williams<sup>1</sup>, W.J. Orts<sup>1</sup>, G.M. Glenn<sup>1</sup>, <sup>1</sup>U.S. Department of Agriculture, Agricultural Research Service, Western Regional Research Center, Albany, CA, USA, <sup>2</sup>University of Arkansas, Department of Food Science, Fayetteville, AR, USA

Color is conventionally used to measure rice milling quality. Pigmented bran and non-pigmented germ contain large quantities of lipid and are sequentially removed during milling. Starchy endosperm has small amounts of lipid. Thus, surface lipid content may more accurately measure milling quality than color. Surface lipid content includes bran and germ lipid and does not simply rely on color. Bran lies flat against the starchy endosperm so is more readily removed than the convex germ which extends into the starchy endosperm. Lipid-specific stains combined with microscopy provided a procedure to highlight milling characteristics and varietal differences in rice. A protocol for sectioning whole milled rice while preserving lipid was used and modified for this study. Intact rice grains were encased in paraffin, sectioned, picked up with clear packing tape and affixed to microscope slides. The sections were stained with Nile Blue A or Sudan Black B and documented with fluorescence or light microscopy, respectively. Intact rice kernels of pureline varieties and hybrids were milled for 0, 10, 20, 30 and 40 sec and treated to reveal lipids in whole grain sections to illustrate differences in lipid distribution and remaining lipid in rice.

## **AFTERNOON**

---

### **AM 2 / PRO 2.1: Food and Feed Safety**

---

Chair(s): G. Ideus, Archer Daniels Midland Co., USA; and G. Graul, Bunge Oils Inc., USA

**Salmonella Risk Assessment in Pet Food and Animal Feed Manufacturing - Factors to Consider.** D.A. Hill<sup>1</sup>, L.A. Carrasquillo<sup>2</sup>, F.T. Jones<sup>3</sup>, <sup>1</sup>ADM Alliance Nutrition, Inc, Quincy, IL, USA, <sup>2</sup>American Dehydrated Foods, Inc, Springfield, MO, USA, <sup>3</sup>Performance Poultry Consulting, LLC, Springdale, AR, USA

Producing safe food and safe feed is the constant objective of pet food and animal feed manufacturers. Pet foods and treats are often found in the home kitchen and in food preparation areas. Pet foods and certain animal feeds are often handled by children, elderly and others with immune system deficiencies. Food safety issues involving direct human contact with processed pet foods and animal feeds is a major regulatory focus by US FDA and the US Congress. Salmonella is capable of surviving for extended periods in a variety of environments on numerous materials. Complete elimination of pathogens is not realistic, but adherence to GMPs can help reduce/control pathogens and industry risk level. Some practices are easy to apply, others may require significant plant redesign to accomplish. This presentation focuses on risk considerations and scenarios, expectations, Salmonella kill steps, recall considerations, product sampling and chemical decontamination of facilities.

**Melamine in the Feed and Food Chain.** Christian W Cruywagen, Tanja Calitz, Stellenbosch University, Stellenbosch, South Africa

Melamine contains 667 g/kg N, which makes it an attractive protein adulterant, as it has the ability to inflate the crude protein content of feed- and foodstuffs artificially. Our research confirmed for the first time that a pathway exists for the transmission of melamine from feed to milk. Melamine appeared in the milk as soon as 8 h after first ingestion and reached a maximum concentration within 56 h after first ingestion. Upon melamine withdrawal, milk melamine concentration responded rapidly and dropped 85% within 32 h. Only after 152 h upon melamine withdrawal, melamine was non-detectable in the milk samples. Excretion via milk accounted for only 2% of the ingested melamine. An experiment with sheep showed that the apparent absorption rate of ingested melamine was 77%. Urine was the major excretion route at 53%, followed by faeces at 23%. Approximately 3.5% of the ingested melamine was deposited in muscle. Our research also confirmed that melamine is excreted in eggs as soon as one day after first melamine ingestion. Maximum concentrations were reached on day 3 of melamine ingestion and four days after melamine withdrawal, melamine disappeared from the eggs. A milk production study was also done where cows grazed pasture that was fertilized with a melamine contaminated fertilizer. Melamine was observed in the milk within 8 hours after cows grazed on the pasture.

**FDA, Center for Veterinary Medicine Update.** T. Schell, FDA, Center for Veterinary Medicine, USA

Topics of his talk will include, New Legislation, Salmonella CPG, Feed Contaminants, Reportable food Registration, Other 2011 Issues.

## TUESDAY

### AFTERNOON

---

#### AM 3: Agricultural Microscopy II

Chair(s): J. Makowski, Messiah College, USA; and K. Koch, Northern Crops Institute, North Dakota State University, USA

#### **Microscopic Identification and Application of Common Macro and Micro Minerals in the Animal Feed Industry.** E. Jacobsen, Prince Agri Products, Inc., Quincy, IL, USA

Macro and Micro minerals are commonly used in the animal feed industry to supplement or fortify feeds to meet the mineral requirements for the particular species being fed. Microscopy can be used to help identify and classify these minerals along with a general understanding of the source of each mineral type. Examples will be given to allow others to begin to recognize minerals as they may apply to their particular trade or profession.

**Using Microscopy to Identify Adulterated Protein Feedstuffs.** C.W. Cruywagen, T. Calitz, Stellenbosch University, Stellenbosch, South Africa

Protein feedstuffs that are used in animal feeds are expensive and are often subjected to

adulteration. The protein content of feeds is not determined directly, but is calculated from their N content, and thus commonly referred to as crude protein. Any nitrogen source can therefore artificially increase the apparent protein content when added to feeds or feed ingredients. Non-protein nitrogen sources, such as melamine and urea, have been used as adulterants in the past. The crude protein content ( $N \times 6.25$ ) of pure melamine would be 4167 g/kg, which is substantially higher than that of pure urea (2917 g/kg), which makes melamine especially attractive as a protein adulterant. A variety of other protein adulterants have, however, also been observed by the author during the last few years. Fish meal, especially, is an expensive protein source used in animal feeds. Adulterated fish meal, originating from various countries, has been observed and the most popular adulterants were blood meal, meat and bone meal and hydrolyzed feather meal or poultry by-product. In one case, sand and poultry manure was found in a fish meal sample. Wheat bran and corn gluten have also been observed in a few fish meal samples. Adulterated poultry by-product and meat and bone meal have also have been observed frequently. In these cases, the one is often contaminated or adulterated with the other.

**Defining and Characterizing Limits of Detection for Qualitative Results: A Realistic Challenge? Study Case in Feed Microscopy for PAPs Detection.** P. Veys<sup>1,2</sup>, C. Belinchón Crespo<sup>1,2</sup>, B. Baeten<sup>1,2</sup>, <sup>1</sup>European Reference Laboratory for Animal Proteins in Feedingstuffs, Gembloux, Namur, Belgium, <sup>2</sup>Walloon Agricultural Research Centre, Gembloux, Namur, Belgium

Qualitative methods used in feed and food safety deliver results in terms of presence or absence of analytes in products. These results are not numerical data that can be treated by classical statistics as recommended by usual standards. They are purely binary. Their expression is eventually independent of semi-quantitative parameters such as cut-offs used as threshold values around which decisions must be taken. Mislaying cut-offs or any other reference level for the presence of an analyte leads to a high risk of false positive results associated to a high alpha-error or false negative results associated to a high beta-error. Applying classical standard recommendations to the field of qualitative methods is unrealistic. Actually, there is an absence of any applicable guidelines for the determination of detection limits for qualitative methods. The lecture presents an original approach developed and tested for determining limits of detection in such situation. Methodology and model was applied to the reference microscopic method for the detection of processed animal proteins in the framework of TSE prevention in the EU. Results are examined within the issue on future application of the present total feed ban.

## WEDNESDAY

### MORNING

---

#### AM 4: Agricultural Microscopy III

---

Chair(s): M. McCutcheon, West Virginia Department of Agriculture, USA; and C. Rogers-Kelly, Mississippi State Chemical Lab, USA

**Zoo Nutrition and Its Complexities.** B. Henry, Cincinnati Zoo and Botanical Garden, Cincinnati, OH, USA

Formulation of diets for captive exotic animals must be performed systematically utilizing specific criteria. This criteria is based on foraging ecology, gut morphology, published information specific to the species in question, known nutrient requirement data on domestic species, physiological state of the animal, the nutrient content of foods available, management constraints, and the health status of the animal. These eight criteria are researched and considered when developing a target range appropriate for each animal. The approach to diet formulation must be in a team that encompasses all who care for the animal from keeper to veterinarian such that all have a full understanding otherwise the correct diet may not get offered. The last remaining factor to consider in this equation is consumption of the designated diet by the animal itself. The great varieties of species housed in zoos means a great variety of food items are necessary to provide proper nutrition. The number of species and the total quantity of animals held are reflected in the size of the food budget. Cincinnati Zoo & Botanical Garden hold approximately 530 different species and 2000 total animals. The cost of food for the last five years has ranged from 765K to 1M.

**Option Tour to Cincinnati Zoo Available - advance ticket purchase required.**