

DNA-marker tests for quantitative traits in beef cattle. If the scientific validation of this approach shows that it is able to consistently deliver accurate genetic merit estimates for young beef sires, producer education and integration of genomic data into national cattle evaluation will be requisite for the ultimate adoption of whole genome selection.

**Key Words:** beef cattle, genomic selection, industry structure

**202 Utilization of next generation sequencing technologies for development of a high-density pig SNP genotyping platform.** R. P. M. A. Crooijmans<sup>\*1</sup>, M. A. M. Groenen<sup>1</sup>, and L. B. Schook<sup>2</sup>, <sup>1</sup>Wageningen University, Wageningen, the Netherlands, <sup>2</sup>University of Illinois, Urbana.

The Illumina Genome Analyzer (Solexa) and Roche 454 FLX 'next generation' sequencing platforms were used to identify porcine SNPs from diverse commercial breeds. The combined approach permitted increased sequence depth and longer 454 reads to obtain adjacent SNP sequence information to design the primers for the genotyping assay. Considering that only approximately 70% of the pig genome had been sequenced at the time of SNP discovery and beadchip design, the utilization of the longer 454 reads further allowed coverage of the genome at the same SNP density as for the remaining 30% not sequenced or assembled. The only caveat in this approach is the fact that because no information was available regarding the position of these SNPs, it was not possible to predict even SNP distribution. Thus, to maximize SNP spacing, the position of such SNPs on the porcine genome was predicted based on the human-porcine comparative map and end sequences of BACs present on the highly robust BAC contig map. Mapping results on build 8 of the pig genome clearly underscored the success of this approach. The importance of this strategy can be extended to other species where the reference genome and the genomic resources are less developed and/or for which there is no genome sequence available. The number of new porcine SNPs identified in this study exceeded 375,000, which demonstrated that the identification of large numbers of novel SNPs is now feasible in a highly efficient manner. The overall confidence of the SNPs identified by this approach using the porcine SNP60 beadchip demonstrated > 95% of the predicted SNPs were validated. These SNPs were subsequently used to design an Illumina iSelect pig DNA chip (60,000 SNPs). This Illumina iSelect chip was used to SNP genotype global wild boar (35 samples from Europe and Asia) and ten European, North American and Asian domesticated breeds. The average heterozygosity and MAF ranged from 21.6 to 31 and 0.15 to 0.23 respectively for Western breeds, from 14.3 to 17.3 and 0.08 to 0.19 respectively for

Chinese breeds, and from 8 to 17.6 and 0.09 to 0.24 for global Wild boar populations. Considering that the breeds used for the discovery of these SNPs included the four main breeds used in pig production (Duroc, Pietrain, Landrace and Large White) as well as the wild boar, the ancestor of all modern pig breeds, it is anticipated that the porcine SNP60 beadchip will be highly efficient to be used for genomic selection by the pig breeding industry.

**203 Bioinformatics requirements to apply whole genome prediction in livestock.** D. Garrick<sup>\*</sup>, Iowa State University, Ames.

The traditional method of evaluation adjusts records for non-genetic effects then combines adjusted deviations on the individual of interest and its relatives. The emphasis attributed to various relatives is dictated by their covariance, derived from pedigree information based on expected identity by descent. The prediction has three potential sources, the collective knowledge of its parents genetic merit, the individuals own information relative to its contemporaries, and the average merit of any offspring, adjusted for the merit of mates. Individuals without their own or progeny information can only be estimated from the average merit of their parents and reliability ( $r^2$ ) cannot exceed 0.5. Whole genome prediction involves tracking inheritance of chromosome regions to infer identity by descent and thereby derive genetic covariances between members of the population. A consequence of this approach is that merits of relatives other than parents and progeny can directly influence the evaluation of a young selection candidate so that it can depart from parent average before individual or progeny information is collected, increasing reliability above the 0.5 threshold. The application of whole genome prediction requires a bioinformatics system to store, access and analyze genotypes, phenotypes and relationships. One computational approach directly uses genotypes to construct the variance-covariance matrix using knowledge of the appropriate emphasis that should be attributed to each chromosomal region. That information can be obtained from prior Bayesian analyses known as training, that estimates the genetic variance of each region, or equivalently, estimates the effect associated with each marker. In circumstances whereby the marked genomic regions collectively account for less than 100% additive variation, predictions can be improved by accounting for covariation between relatives due to residual polygenic effects, not captured by genomic relationships. Prediction of genomic and residual polygenic contributions can be undertaken jointly, or in separate analyses with results subsequently combined into single predictions of merit.

**Key Words:** genomic analysis, genetic evaluation, Bayesian analysis

## Companion Animals: Dietary Supplements in Companion & Exotic Animal Nutrition - Use, Regulations & Safety

**204 Navigating the FDA's regulation of animal feed "supplements".** J. B. Murphy<sup>\*</sup>, U.S. Food and Drug Administration's Center for Veterinary Medicine, Rockville, MD.

The Center for Veterinary Medicine (CVM) is the branch of the U.S. Food and Drug Administration (FDA) that is responsible for the regulation of products (food, drugs, and devices) intended for animals, which includes both livestock and pet animals. The use of food products for both humans and animals is governed by the provisions of the Federal Food, Drug and Cosmetic Act (FFDCA). CVM regulates two classes of orally ingested products intended for animals: food or drugs. The Dietary Supplement Health and Education Act (DSHEA) created a third

class of products for humans, the dietary supplements. In 1996, FDA determined that DSHEA does not apply to animals and that the use of the term "dietary supplement" and associated labeling practices are not permitted for animal products. Therefore, depending on the intended use, an animal "supplement" is considered either a food or drug under the FFDCA. The evolution of the pet product market over the past several years has led to an exponential increase in the number of pet supplements available for consumers. Many of these products contain ingredients, claims, and labeling practices that are associated with human dietary supplements that are not permitted for animal products. In addition, CVM is concerned about the inclusion of dietary supplement-type substances, such as herbs and their extracts in pet products. Many of

the substances that are suitable for human supplements are not permitted for use in pet food and may cause adverse effects in animals. Ingredients that are acceptable for use in an animal feed product are approved food additives (FA), substances that are generally recognized as safe (GRAS) for an intended use, and ingredients that are defined in the *Official Publication* (OP) of the Association of American Feed Control Officials (AAFCO). The process for establishing a new FA or GRAS substance is defined in federal regulations, and that for defining new AAFCO ingredients can be found in the AAFCO OP. CVM is responsible for reviewing FA and GRAS substances and serves as the scientific reviewer for AAFCO ingredients.

**Key Words:** regulation, pet food, supplement

**205 Safety of Dietary Supplements for Horses, Dogs and Cats – New NRC Publication.** G. L. Czarnecki-Maulden\*, *Nestle Purina Research, St Louis, MO.*

Evaluating the safety of inherently safe compounds and complex ingredients can be challenging, particularly in the absence of traditional toxicology studies. At the request of the FDA, the National Research Council (NRC) convened a committee to evaluate the safety of lutein, garlic and evening primrose oil for dogs, cats and horses. After compiling and studying the information available the committee's conclusions were published in a National Research Council publication (*Safety of Dietary Supplements for Horses, Dogs, and Cats, 2009*). One objective of the committee was to examine factors to consider when assessing safety of supplements. Extrinsic factors, such as growing and processing conditions, and intrinsic factors, such as presence of antinutritive factors, can affect dietary supplement safety. Supplements can be given in purified form. However, they are also fed as intact ingredients further complicating safety evaluation. Physiological differences between species must be considered when evaluating data obtained from other species. Likewise, physiological state can affect supplement safety. The committee assessed the strength of data obtained from various types of published and nonpublished studies and gave guidelines to follow when evaluating studies. Few traditional toxicology studies had been published for these three supplements. Therefore, data was also obtained from a number of non-traditional sources including efficacy studies, case reports, and historical use documentation. After evaluating all available information, the committee concluded that there was insufficient data to determine No Observed Adverse Effect Levels (NOAEL) or Safe Upper Limits (SUL) for the three supplements. The committee defined an alternate term, Presumed Safe Intake (PSI) as, "amounts that will not impair animal health or production efficiency". With the exception of garlic for cats, PSI levels were estimated for each of the supplements and animal species. The PSI's estimated by the committee for horses, dogs and cats, respectively were lutein: 8.3, 1.8 and 7.2, evening primrose oil: 400, 424 and 391, and garlic (as dried garlic powder): 90 and 56 mg/kg bw/day.

**Key Words:** safety, dog, cat

**206 The big "S" supplementation in exotic animal diets.** N. A. Irlbeck\*, *Colorado State University, Fort Collins, CO.*

Modern day zoos are living museums preserving animal species that would not otherwise survive in today's world. Many mammals, reptiles, birds, fishes and invertebrates no longer live in natural habitats and more are threatened with extinction. In order to maintain health and the ability

to propagate, dietary nutrient requirements must be provided. Until the world of exotic animal nutrition reaches the level of expertise found in domestic animals supplementation is dietary essential! But as in all supplementation issues, there is a more complex reality. Researchers are able to evaluate nutrient requirements in domestic animals for each physiological status with feeding trials. In exotic species this is seldom feasible due to limited animal numbers, management issues, lack of dietary information, and diversity of dietary strategies (herbivores, carnivores and omnivores). Public scrutiny often complicates the ability to feed efficiently because of viewing needs. Representative domestic animal models can be used as starting point for nutrient requirements for some exotics (i.e., horse for rhino, elephant and wild equids; goats for smaller antelope species). Evaluation of "natural" diets provides data to feed exotics. Domestic foodstuffs are used to mimic nutrients in wild diets. Development of exotic animal diets is in its infancy and often they are not available or are expensive. Ultimately a combination of feeds must be fed to meet dietary needs and supplementation becomes a reality. With the diversity of animal species and non-traditional diets, limitations of dietary foodstuffs must be considered. Frozen raw fish require thiamine supplementation. Feeding invertebrates like crickets and mealworms requires supplemental calcium. Animals from similar families but from different parts of the world must be fed differently. New world primates need vitamin D3 supplementation and old world primates do not. Browsers need low starch diets and higher vitamin E supplementation than grazers. Exotic animal nutrition is incredibly complex and each nutrition issue needs to be approached with a problem-solving attitude when determining which supplement is to be fed and when.

**Key Words:** exotics, supplementation, nutrition

**207 From arthritis to zinc deficiency, veterinarians are increasingly recommending pet supplements.** P. Brown\*, *Nutri-Vet LLC, Boise, ID.*

While consumers have always connected sound nutrition with the long-term health of their animals, pet owners are now going beyond the blind acceptance that pet foods provide "everything" needed for a full and long life and are seeking out supplements capable of optimizing their pets' life. As veterinarians have increased their appreciation of the roles that supplements play in certain physiological dysfunctions and come to better understand the potential negative side effects of drugs, the use of pet supplements in veterinary practices has increased dramatically. Pet owner demand for pet supplements is certainly growing. According to Packaged Facts, spending on supplements and functional treats is expected to hit \$1.7 billion by 2012. The National Animal Supplement Council (NASC) reports that pet supplements have grown sales 15 percent annually since 2000 and are now a \$1.3 billion business. Some manufacturers are seeing increases of 35-40% from the introduction of veterinary formulated "condition-specific" supplements. The Simmons Research Bureau reported that approximately 17% of dog or cat owners routinely give their pets supplements. The trend in pet dietary supplements is shifting away from general wellness formulations such as multi-vitamin and mineral formulations and toward age-related, condition-specific products that address health issues. Pet supplements that support joint health and skin and coat maintenance are most commonly recommended. Now there are products that address specific conditions such as cataracts, memory loss, liver health, and even behavioral disorders associated with household stress or separation anxiety. There are indeed thousands of circumstances where pet supplements are helpful to the veterinarian's clients. Most owners learn about supplements

through friends, retail stores, internet and media advertisements, but, unfortunately, this information may be incomplete or biased. The judicious use of pet supplements have provided veterinarians an emerging segment that helps address client demand and promote animal health and well being.

**Key Words:** pet supplements

**208 Who are we, what do we do and how can we help?** W. Bookout\*, *National Animal Supplement Council, Valley Center, CA.*

The National Animal Supplement Council was founded in 2002 in response to the lack of a legal category for dietary supplements for companion animals and initiatives to remove them from the US marketplace. Over 100 companies now belong to NASC. In 1994, the Dietary Supplement Health and Education Act created a specific legal category for these products under the Federal Food, Drug and Cosmetic Act that allowed their marketing for human use. The agency responsible for the regulation of animal food and drugs is the FDA's Center for Veterinary Medicine. CVM works closely with the states through regulatory associations like AAFCO. In 1996, CVM published

**209 *Clostridium difficile* in cattle and swine.** R. Harvey\*, *FFSRU, ARS, USDA, College Station, TX.*

There are some implications that human disease from *Clostridium difficile* (Cd) may originate from animals or meat. The objective of this study was to determine the prevalence of Cd among different age and production groups of swine in a vertically integrated swine operation in Texas in 2006, and to compare our isolates to those originating from humans, meat, and other animals. Cultivation of Cd was performed utilizing enrichment/concentration techniques and restrictive media. We recovered 131 Cd isolates from 1008 swine fecal samples with the majority (72%) of isolates occurring in nursing piglets. Decreased prevalence was observed in grower/finisher swine (11.5%) and pork trim (3.0%). Isolates were tested for resistance to 11 commonly used antibiotics. Molecular characterization demonstrated that 127/131 of the isolates were positive for toxins A and B genes, were positive for binary toxin, possessed a 39 bp gene deletion, and were of toxinotype V. These results compare favorably to our non-clinical human isolates (toxinotype V), but differ from clinical isolates of human hospitals in which most are the more virulent toxinotype III. Our swine isolates appear to be genetically similar to each other and have similar antibiotic resistance patterns to isolates from cattle which tend to be of toxinotype V. When sampling meat, we recovered 4 Cd isolates from pork trim, and 1 each from pork chorizo, ground turkey, and pork sausage, but not ground beef. All 7 were toxinotype V. This is in contrast to isolates from ground beef and veal in which the majority have been toxinotype III. Our isolates tended to be less resistant to antibiotics than human clinical isolates. In this study, Cd primarily originated from nursing piglets, but not in grower/finisher swine. If Cd were to be considered food related, then a relatively low prevalence in late production and the predominance of toxinotype V (a less virulent strain of Cd), suggest a low food safety risk. Our results do not appear to implicate Cd as food-vectored.

**Key Words:** *Clostridium difficile*, cattle, swine

a notice explaining why DSHEA does not apply to animals. This ruling gives products marketed for animals that are similar to human dietary supplements only two possible legal categories under US law: animal feed or drugs. If a product on the market is not approved as an animal drug or contains unapproved feed ingredients, it may be deemed an adulterated drug or feed, and subject to regulatory action. NASC had three options to address this issue: 1) file legal action challenging the ruling that DSHEA does not apply to animals; 2) introduce new legislation; or 3) engage regulatory agencies at the state and federal levels to identify key metrics for responsible industry conduct, thereby allowing products to be marketed under regulatory discretion in the near term while establishing the foundation for a long-term solution. We clearly believe the last approach is in the best interest of all stakeholders. To support this goal, NASC implemented: a comprehensive adverse event reporting system (NAERS); labeling guidelines; scientific review for ingredient risk; an independent mandatory audit program for member companies; guidance for labeling claims; and other requirements suggested by CVM and AAFCO including proposed cGMP guidelines. We have a productive working relationship with US regulatory agencies and are currently working with organizations in other countries as they address similar issues in their markets.

**Key Words:** companion animals, regulatory, dietary supplements

## Food Safety

**210 Optimising fluorescence of feces as a real-time solution for the detection of fecal contamination on carcasses.** M. R. F. Lee\*<sup>1</sup>, V. J. Theobald<sup>1</sup>, M. K. Theodorou<sup>1</sup>, A. Veberg Dahl<sup>2</sup>, F. Lundby<sup>2</sup>, and J.-P. Wold<sup>2</sup>, <sup>1</sup>*Aberystwyth University, Wales, UK,* <sup>2</sup>*Nofima Mat, Ås, Norway.*

In most abattoirs carcasses are checked by 'eye' and washed with chemical sprays or dissected to remove areas contaminated with feces or digesta contents. Unfortunately small areas of contamination are seldom visible to the naked eye and may harbour millions of potentially pathogenic bacteria. The 'VerifEYE'® system uses ultra-violet imaging to detect chlorophyll and its fluorescent degradation products in feces. Not surprisingly animals offered fresh forage have a greater concentration of fluorescent compounds in their feces than animals offered conserved forages and concentrate based diets. Consequently the accuracy of the 'VerifEYE'® detection system can vary as it depends on the nature of the animal's diet and this may explain the poor uptake of the technology by the industry. We have investigated the use of five different markers to be added to the diet in a pre-slaughter feed in an attempt to provide a stable level of fluorescence in the feces. Ten Cheviot sheep were offered a concentrate and barley straw diet and split into five treatment groups during a duplicate changeover 5 × 5 Latin square design where each period lasted 2 weeks. Four of the groups received a different marker at a rate of 1 g/d for the second week of each experimental period. The last group received no supplement and was used as the control. At the end of each period feces were collected and analysed for fluorescent compounds and intensity of the fluorescence. There were no differences in fecal concentration of the markers or their derivatives 3.1 ± 0.15 and 7.2 ± 0.37 mg/g DM, respectively. Each of the markers significantly increased the fluorescence intensity of the feces over the control. The use of markers in pre-slaughter diets would thus improve the accuracy of fecal detection as a result of greater fluorescence and pin pointing the excitation wavelengths of the marker to help with visualisation. Further work is being continued to identify the most suitable marker and feeding regime.

**Key Words:** fecal contamination, fluorescence markers, pathogenic bacteria