Nutritional Genomics: Implications for Companion Animals

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ABSTRACT This is an exciting time for biological scientists as the “omics” era continues to evolve and shape the way science is understood and conducted. As genome sequencing of the human comes to a close, other mammals are in line to be sequenced. Along with pigs and cows, dogs are now on the high priority list for sequencing, and cats may soon follow suit. Until sequence data are available, genetic maps may be used to reveal important physical characteristics of a genome. Genome mapping is important in identifying gene placement, but gives little information regarding function. Therefore, functional genomics, including the global analysis of RNA and protein expression, protein localization and protein-protein interactions will emerge as important areas of study. The major use of the dog and cat genome maps hitherto has been for the study of human and veterinary medicine. These powerful resources also can be applied to the field of nutritional genomics and proteomics, enhancing our understanding of metabolism and optimizing companion animal nutritional and health status. Genomics has begun to be applied to nutritional research, but issues specifically relevant to companion animals have not been elucidated thus far. The study of genomics and proteomics will be crucial in areas such as nutrient requirement determination, disease prevention and treatment, functional ingredient testing and others. Nutritional genomics and proteomics will definitively play a vital role in the future of pet foods. J. Nutr. 133: 3033–3040, 2003.

KEY WORDS: • cats • dogs • genome mapping • nutritional genomics

Genome sequencing and mapping

As the Human Genome Project approaches its goal of sequencing the entire human genome, very little understanding of gene function exists. Of the estimated 30,000–40,000 protein-encoding genes in the human genome (1), most have not been studied to any extent.

Even less is known about the placement and function of genes in the canine and feline genomes. It is estimated that whole genome sequencing of a mammalian genome containing ∼2.7–3.2 billion bases (similar to that of humans, dogs and cats) at 5× coverage costs ∼50 million U.S.$ (2). Therefore, only a few mammalian species have been selected for sequencing thus far (humans, mice and rats are completed or in progress) (3). In the past year, the National Human Genome Research Institute (NHGRI)3 added dogs, cows and pigs to the high priority list of model organisms and cats to the medium priority list to be considered for genome sequencing as capacity becomes available (http://genome.gov/page.cfm?pageID = 10002154). High priority does not automatically begin the sequencing process. Once capacity is available, the NHGRI determines the resources to be expended on that organism. Therefore, it may be several years before the canine and feline genomes are fully sequenced.

Because whole genome sequencing has not been plausible until recently, various genome-mapping strategies (genetic, physical and cytological maps) have been implemented to reveal the physical characteristics of a given genome (Fig. 1). “Genetic maps” describe the relative order of genetic markers in linkage groups in which the distance between markers is expressed as units of recombination (4). The centiMorgan (cM), equal to a recombination frequency of 0.01, is the standard unit of genetic distance. In humans, dogs and cats, 1 cM is equal to ∼1000 kb. Genetic mapping requires multigenerational reference populations and polymorphic markers. Genetic maps are commonly constructed by a technique called radiation hybrid (RH) mapping. Irradiation of fibroblast cells causes chromosomes to fragment. By irradiating fibroblast cells from an organism of interest, these fragments are incorporated into a panel of hamster fibroblast cell cultures (5). Species-specific PCR amplification then is used to ascertain which loci are present in each line, and the frequency at which markers cosegregate is an indicator of the physical distance between the markers (6). The power of RH mapping is that nonpolymorphic markers can be mapped and gene order can be achieved. RH mapping permits higher resolution mapping that would be difficult, if not impossible, for large populations. “Physical maps” assemble contiguous stretches of chromosomal DNA (referred to as contigs) in which the distance between markers is expressed in kb. Physical maps can be constructed by a technique called chromosome walking or by the alignment of randomly isolated clones based on shared chromosome fragment profiles. Bacterial artificial chromosomes (BAC) and yeast artificial chromosomes are commonly used with these procedures. Because physical maps provide a scaffold upon which anonymous polymorphic markers can be placed, they facilitate finer scale linkage mapping than genetic maps (4).

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3 Abbreviations used: 2-D PAGE, two-dimensional PAGE; BAC, bacterial artificial chromosome; NHGRI, National Human Genome Research Institute; RH, radiation hybrid.
“Cytological maps” portray the banding patterns observed through a microscope on stained chromosomes. These maps aid in the alignment of physical and genetic maps and can be used to differentiate chromosomes from one another. Finally, by using comparative mapping techniques, gene-rich species maps of humans, mice or rats can be used to develop maps of species about which little is known. Syntenic groups, conserved segments of two or more organisms, can be used as anchoring landmarks on the gene-poor species map. With the use of fluorescent dyes, a technique referred to as “chromosome painting” can be performed; this defines regions of synteny between two genomes. Table 1 lists common genomic terms and their definitions.

Canine and feline genomes

Compared with the human genome (haploid chromosome number = 23; size = ~3.3 billion nucleotides), that of the canine has a smaller overall size (~2.7 billion nucleotides) and is split into many more chromosomes (haploid chromosome number = 39) (7). Because of the high number of total chromosomes and the small size and similar banding patterns of many autosomes, the karyotype of the dog has been difficult to work with. In fact, an internationally accepted numbering system of all 38 canine autosomes was completed only in the last decade (8,9). The standardization of the sex chromosomes and the first 21 autosomes of the dog karyotype was achieved using conventional cytogenetics (10). However, molecular cytogenetic techniques (e.g., fluorescent in situ hybridization and DNA binding AT-specific 4’-6-diamidino-2-phenylindole banding) were required to reliably identify the remaining 17 autosome pairs.

Through incorporating new markers with those of other individual mapping efforts (11–16), Breen et al. (17) published the first fully integrated, comprehensive map of the canine genome. This 1800-marker map contained cytogenetic, RH and meiotic information covering >90% of the canine genome. On this map, each chromosome is identified by at least one meiotic linkage group and one RH group. The RH map consists of 320 type I (coding genes) and 1078 type 2 (microsatellites) markers. An RH map of the canine genome having a 1-Mb resolution was recently published by Guyon et al. (18). This RH map contained 3270 markers, including 1596 microsatellite-based markers, 900 cloned gene sequences and expressed sequence tags, 668 canine-specific BAC ends and 106 sequence-tagged sites (18).

Compared with dogs, the genome of cat is more similar to that of humans vis-à-vis size (~3.3 billion nucleotides) and chromosome number (haploid chromosome number = 19) (7). As of December 2002, the feline genome map contained 1881 total markers (including 784 type I coding genes and 1086 type II microsatellite loci) with an average marker density of 1.8 cM (2). The placement of genes in the dog and cat genome maps enhances the existing comparative mapping data between the two species and with humans, whereas microsatellite markers are important for genome scanning studies (17). More information regarding the canine and feline genome maps can be accessed through the websites listed in Table 2.

Current use of genome maps

As sequencing and mapping projects continue to generate information regarding genome structure, scientists must continue to develop biological techniques and bioinformatics programs capable of revealing gene function. Functional genomics may be defined as “the development and application of global experimental approaches to assay gene function by making use of the information and reagents provided by genome sequencing and mapping” (19). Global analysis of RNA and protein expression, protein localization, protein-protein interactions and chemical inhibition of pathways will be important areas of study (1).

The major use of dog and cat genome maps hitherto has been for the study of veterinary and human medicine. Medical surveillance of dogs and cats is second only to that of humans.

Table 1

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<th>Common genomic terms</th>
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<td>Allele: one of several forms that a gene for a trait can take.</td>
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<td>Expressed sequence tag (EST): a sequenced piece of a transcribed gene.</td>
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<td>Functional genomics: the study of the function of every gene that is encoded in a genome.</td>
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<td>Genomics: the study of genomes, including genome mapping and sequencing.</td>
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<td>Karyotype: the entire chromosomal complement of an organism (as visualized during mitosis).</td>
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<td>Metabolomics: the study of the metabolic profile found in a particular cell, tissue, or organism.</td>
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<td>Proteomics: the study of the full complement of proteins that are found in a particular cell, tissue, or organism.</td>
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<td>Single nucleotide polymorphism (SNP): a site in the genome at which a single nucleotide is found to have two or more states. These are responsible for most of the genetic variation between individuals.</td>
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At the time this paper was written, 263 feline and 451 canine genetic diseases had been described (20). For monogenic diseases, molecular biological techniques can be used to find the genetic cause of a disease. This information can then be used to prevent or treat the disease. The first canine disease-causing gene was cloned and described in 1989 (21). Since then, at least 20 canine disease genes have been cloned and characterized (22). Once DNA sequence data for a disease are known, mutation-based tests can be developed to test for diagnosis and carrier detection. Test results may be used to eliminate carriers from the breeding population to decrease or eliminate incidence of disease. Determination of genetic loci responsible for complex genetic diseases is a much more daunting task. However, this may be possible in the coming years with the use of single nucleotide polymorphism haplotype maps for linkage disequilibrium studies, which already have been performed in humans (23).

Dogs and cats also are excellent animal models for human genetic and nutritional diseases. The dog may be a very useful model because many of the most common diseases of purebred dogs are also major health concerns in humans. Dogs are well-suited animal models for arthritis, various cancers, deafness, heart disease, blindness, epilepsy, chronic metabolic diseases (e.g., obesity, diabetes and hypercholesterolemia), and several other diseases that afflict humans (24). Although a genetic component exists, nutrition plays a major role in the development and (or) treatment of many of these disease states.

Noteworthy parallels exist among dogs, cats and humans concerning changing lifestyles and the increase in the incidence of obesity and associated diseases in the past 50 y. Up to 40% of dogs presented to veterinarians in the United States are now overweight, which is significantly higher than just a few decades ago (25). The incidence of diabetes mellitus in dogs has also increased, from ~1.9 cases/1000 in 1970 to 6.2 cases/1000 in 1993 (26). Dogs have been crucial in our understanding of glucose metabolism, pancreatic function and diabetes research. In fact, dogs were the first animal to become diabetic experimentally, and the function of pancreas was determined in dogs by Mering and Minkowski (27). The cause of obesity in dogs is similar to that in humans, with inadequate daily exercise and excessive intake of high quality animal-based ingredients taking much of the responsibility (28). Many of the negative health outcomes of obesity observed in humans are also present in dogs. Weight gain in dogs, as in humans, is associated with increases in blood pressure, heart rate, plasma volume, cardiac output and fasting insulin concentration (29). Rocchini et al. (30) also reported a direct relationship between high fat diet–induced mean arterial pressure and the degree to which insulin resistance developed. Many of the complications associated with diabetes in humans, including hypertension (31), hypercholesterolemia (32), atherosclerosis (33) and retinopathy (34), are also present in canines. In fact, the dog is a popular model for ocular manifestations because diabetes causes cataracts and is the leading cause of blindness in dogs, as it is with humans (34). Finally, clinical signs of diabetes are similar to those of humans, with polydipsia and polyuria being the most common signs in newly diagnosed diabetic dogs (35).

The cat also is a popular human model for many diseases, including arthritis, Cushing disease, epilepsy, leukemia and several metabolic disorders (e.g., obesity, diabetes, glycerogen storage disease and hypertriglyceridemia). Of great importance is the use of the cat as a model for AIDS research because they are susceptible to their own version of the disease (36). Although feline immunodeficiency virus is able to infect cats, it is not lethal as HIV is in humans. Therefore, successful treatment for HIV may greatly benefit from this field of research. The incidence of obesity and diabetes in cats continues to increase (as much as 40% of the population) as they become accustomed to a sedentary lifestyle indoors (37). Therefore, cats comprise another naturally occurring model for obesity and diabetes research. Although some differences exist, many of the negative health outcomes of obesity observed in humans and cats also are present in cats. For example, feline obesity results in reduced insulin sensitivity, glucose intolerance and hypertriglyceridemia (38). As the feline and canine genome maps become more highly developed, comparative studies may be performed to identify and study loci affecting disease across species.

Because approximately half of the feline and canine genetic diseases are homologous to human genetic defects (2), dogs and cats are also good models for human genetic diseases.
Companion animal models of human genetics are important to identify disease genes in humans, test conservation of synteny between species, analyze protein function and aid in treatment of disease (39). Cats and dogs offer several advantages as animal models for humans. First, due to dog and cat breeding practices, disease heterogeneity can be avoided. In contrast to many human genetic diseases that are genetically complex, dogs and cats often have diseases due to the mutation of a single gene. In addition to having monogenic diseases, different breeds of dogs and cats having the same phenotype may have a different disease-causing mutation. In this case, all genotypes may be useful in determining the cause and treatment of the human form of the disease. Second, larger families in dogs and cats result in greater statistical power than can be achieved studying inherited disease in humans. Moreover, related dogs and cats can be mated to produce more informative families for genetic mapping. Third, although rodents are members of the same evolutionary clade as humans (clade III) and dogs and cats are not (both members of clade IV) (40), dogs and cats are more closely related to humans in terms of genome structure. To match the human genome, cats (13 translocation steps) and dogs (45 translocation steps) require fewer chromosomal rearrangements than the mouse genome (160 translocation steps) (7). Finally, dogs and cats are monitored and treated with a high standard of veterinary medicine, providing important clinical data on certain disease states.

Methodology used to study functional genomics

In addition to PCR techniques and Northern-blot analysis, several molecular biological techniques are used to study nutritional genomics and proteomics. Gene expression profiling may be performed with the use of microarray technology, which can monitor the expression of thousands of genes simultaneously (41). The two major applications of microarrays are to identify sequence variations (e.g., single nucleotide polymorphisms, gene mutations) and the determination of the expression level (abundance) of a set of mRNA molecules (42). Microarrays are powerful alternatives to conventional, classical techniques that have limited past experiments to measuring only a few genes at a time. Two-dimensional PAGE (2D-PAGE) is used for proteome analysis because this method provides the highest resolution available. This technique separates proteins according to charge (isoelectric point) by isoelectric focusing in the first dimension and according to size (molecular mass) by SDS-PAGE in the second dimension (42). Proteins of interest can be isolated from the gel and identified via MS. Matrix-assisted laser desorption/ionization time-of-flight MS also is being used for proteome analysis. After a sample is desolvated and ionized, it is ablated with a laser and analyzed by a mass spectrophotometer to measure time-of-flight, which can be converted to molecular mass of proteins in the sample. Data collected by the mass spectrophotometer then can be analyzed by computer software programs to identify the proteins present in the sample. Although these techniques remain problematic at times and come at a high cost, they are capable of providing scientists with crucial information that conventional techniques simply cannot.

Nutritional genomics

The driving force for genetically mapping cats and dogs has been for veterinary and human medicine research. However, genetic maps will also be very important for the field of nutritional genomics and proteomics, enhancing our understanding of metabolic pathways and optimizing nutritional and health status. Certain dietary constituents (e.g., vitamins A and D, zinc, fatty acids) are able to influence gene expression directly, whereas others (e.g., dietary fiber) can have an indirect effect through changes in hormonal signaling, mechanical stimuli or metabolites produced from gut microflora (43). By utilizing the powerful molecular biological techniques now available, scientists can measure the effects of a single nutrient on the gene expression profile of a cell or tissue.

In recent years, scientists have begun applying genomics to the field of nutrition. Changes in gene expression have been used to study a broad range of topics, including energy restriction (44), vitamin (45) and mineral deficiencies (46), glucose metabolism (47) and diseases affecting nutritional status (48). However, this field has not yet made its presence felt in companion animal nutrition. Although some advances have been made in the past 20 y, many of the nutritional requirements of cats and dogs are not known (49). In addition to knowing the expected concentration required to avoid deficiency, determination of optimal and toxic concentrations will be important. Nutritional effects on gene expression in different life stages and genotypes also must be a focus in companion animals. The importance of maternal nutrition during pregnancy on the gene expression and development of offspring was demonstrated in sheep (50). In pigs, nutrient excretion was shown to vary depending on breed, suggesting differences in metabolism due to genotype (51). Determination of nutrient requirements of dogs participating in different physical activities (e.g., dog sled racing, sprint racing or hunting/herding) also would be a worthy research venture.

Because of the vast improvements made in veterinary medicine and companion animal nutrition in recent years, dogs and cats are living longer than ever before. Now, instead of being troubled by intestinal parasites or succumbing to viral infections (e.g., canine distemper), many companion animals are living long enough to suffer from obesity and many of the same diseases that affect their human owners. Unlike diseases due to a mutation at a single gene locus, diabetes, cancers and heart disease are highly complex and involve several genes; they are also affected by lifestyle and environmental conditions. Renal disease, arthritis and several diseases associated with abnormal immune function (e.g., ulcerative colitis, atopic disease) also are becoming more prominent in cats and dogs. A great need for biomarkers capable of predicting disease incidence and longevity exists. Scientists studying the effects of energy restriction on aging already have begun this search (52). Once the genes responsible for developing a given disease are known, dietary intervention may be able to avoid or prolong its development. If dietary intervention is not the answer, the development of genotype-specific drug therapy that would be expected to have lower toxicity than current drugs may be an alternative approach. Several dozen human drug–metabolizing enzyme polymorphisms have been characterized (53). Because companion animals likely have similar polymorphisms, the type and dose of drug prescribed by veterinarians may soon be influenced by genotype. Although scientists have mixed views on the concept of personalized drug therapy, many drug companies have high expectations and are investing heavily in this area of research (54).

Our laboratory is currently conducting a long-term experiment to evaluate the effects of diet on gene expression in geriatric and weanling dogs. Our experimental design is presented in Figure 2. In this experiment, a diet containing primarily high quality animal-based ingredients is being compared with a diet containing mainly plant-based ingredients.
Blood and liver biopsy samples are collected over the course of the experiment and tissue samples are harvested at the end for RNA isolation. Ribonucleic acid samples are being analyzed using oligonucleotide microarrays to generate gene expression profiles (Swanson, K. S., Kuzmuk, K. N., Schook, L. B. & Fahey, G. C., Jr., unpublished data). We have designed an oligonucleotide microarray containing 384 genes with major emphasis placed on metabolic pathways and immune function (http://www.wesbarris.com/locg/). The basic methodology by which we designed and constructed our oligonucleotide microarray is presented in Figure 3 (55). As with humans, diabetes is highly prevalent in dogs and is positively correlated with age. Detection of biomarkers predictive of disease is needed and is a goal of this experiment. Therefore, genes associated with glucose metabolism and homologous to human diabetes were included on the microarray. In addition to nutritional genomics and proteomics, metabolomics, the study of metabolic pathways and immune function, the study of metabolic profiles in a cell, tissue or organism, will enable scientists to identify markers indicative of nutritional and (or) health status. The field of metabolomics is already being used to catalog and quantify metabolites to gain more information about specific biological pathways (56,57). Although much of the human and rodent metabolomic research will likely be applicable to companion animals, research using cats and dogs also will be needed to identify species-specific profiles. In our experiment, blood samples were used for analysis of complete blood count and serum chemistry profiles. Although researchers have identified serological indices associated with aging (58), the identification of biomarkers predictive of disease remains to be accomplished. For prediction of complex disease states, it will likely require the measurement of several biomarkers (using both serological indices and gene expression profiles). Statistical analysis of these biomarkers then can be used to predict likelihood of disease development. With data generated from this experiment, we intend to begin identifying biomarkers that can be used for this purpose. Feces and digesta were also collected for the measurement of total tract nutrient digestibility (59), populations of fecal microbes and concentrations of fermentative end products. Statistical analyses will detect important correlations present between gene expression and changes in diet and consequent metabolism. In addition to its effect on metabolism, aging has been shown to influence the expression of genes associated with stress response and biosynthesis (44). Therefore, additional oligonucleotide microarrays are being designed to also study genes of these functional categories. For more information on the design of our oligonucleotide microarrays, visit our website (http://www.wesbarris.com/locg/).

The recent influx of “functional” ingredients into human and pet foods has created another area of important research that may be best studied by gene expression profiling. A recent survey reported that 41% of pet owners had considered or tried alternative therapies, including nutritional supplements (29%), herbal remedies (7%) and homeopathy (4%) (60). Although the Developmental Therapeutics Program of the National Cancer Institute has screened >70,000 compounds, and the large number of approved drugs for human conditions, there is a need for new drugs, including those for veterinary medicine. To increase the number of drugs that can be considered for human use, both central nervous system and non-central nervous system drug targets will be identified. In addition, the recent inroads of functional genomics into human and rodent disease research, particularly as it relates to aging, has increased the validity of using small, model species to study complex conditions such as diabetes and cancer. In addition to the ability to screen a large number of compounds, small species are also amenable to the study of nutrition-related diseases, such as obesity. In our experiment, blood samples were used for analysis of complete blood count and serum chemistry profiles. Although researchers have identified serological indices associated with aging (58), the identification of biomarkers predictive of disease remains to be accomplished. For prediction of complex disease states, it will likely require the measurement of several biomarkers (using both serological indices and gene expression profiles). Statistical analysis of these biomarkers then can be used to predict likelihood of disease development. With data generated from this experiment, we intend to begin identifying biomarkers that can be used for this purpose. Feces and digesta were also collected for the measurement of total tract nutrient digestibility (59), populations of fecal microbes and concentrations of fermentative end products. Statistical analyses will detect important correlations present between gene expression...
including botanicals, with in vitro experiments using microarray technology (61), these should be examined using in vivo models. Mechanisms of action, optimal inclusion levels and toxicological and (or) harmful effects of most functional ingredients are not completely understood. Several functional ingredients are already making their way into pet foods, including those intended to improve joint health (e.g., glucosamine, chondroitin sulfate, green-lipped mussel), protect the body from free radical damage (e.g., vitamin E, β-carotene, selenium), and improve skin (e.g., n-3 fatty acids) and gut health (e.g., oligosaccharides, probiotics) (62). Although a few experiments have tested the effects of these ingredients on companion animal health, the authors are unaware of any experiments measuring gene expression as an end point. Herbs such as Ginkgo biloba, ginseng, and St. John’s wort may not be included in pet foods, but are likely being used by pet owners as well. Gohil and Packer (61) recently used gene expression to demonstrate the effects of a Ginkgo biloba extract on genes important in the regulation of neutrophil apoptosis and host defense. Although many functional ingredients may provide health benefits, more experiments similar to that conducted by Gohil and Packer (61) are needed to determine mechanisms of action and efficacious, yet safe, levels of consumption. If ignored, incidence of toxicosis resulting from herbal supplements, as reported by Ooms et al. (63), may continue to increase in companion animals.

Diets formulated for genotype—the future of pet foods?

Genomic technologies are powerful tools that will be applied to the pet food industry in the future to optimize nutritional and health status. This will be accomplished by determining minimal nutrient requirements and mechanisms by which “functional” ingredients act to prevent and treat diseases. Genotype-nutrient interactions will complicate matters even further. These interactions will have to be ascertained and considered when formulating diets for a given genotype. Several polymorphisms relating directly to nutritional and immunological status already have been identified in humans, dogs and cats (Table 3). This list will continue to grow as the field of genomics matures.

The availability of large-breed dog diets in the current market is a mere glimpse of what the future may hold in the pet food industry. Like personalized drug therapy, genomics will allow diets to be formulated according to genotype, which will be much more precise than some of the current dog diets marketed for large size or geriatric animals. Although skeletal diseases such as hip dysplasia, osteochondrosis and hypertrophic osteodystrophy are greatly influenced by diet, there is also a high heritability associated with them (64). Therefore, the benefits provided by a large-breed diet for one dog may not be the same for another. As we identify genetic polymorphisms affecting nutritional status and disease, and biomarkers used for their detection, diets may be formulated not only for the prevention of skeletal abnormalities, but also for more complex diseases such as diabetes, cancer and heart disease.

In conclusion, as biological scientists embrace the new technologies surrounding genomics, proteomics and metabolomics, scientific knowledge continues to progress at astonishing rates. Genome sequencing is the first of many steps required to fully understand the function of biological systems and how they can be manipulated to enhance health and prevent disease. Scientists specializing in “omics” fields of study will be relied upon to determine the answers to these important questions. Although many concepts may be applied across species, some questions can be answered only by performing research on the species in question. Therefore, the molecular biological techniques (e.g., microarray technologies) continually being developed and improved are powerful tools that must be incorporated into companion animal research. Nutritional genomics, proteomics and metabolomics will be important in the determination of nutrient requirements of dogs and cats at different life stages, the prevention and treatment of various disease states, and the testing of numerous functional ingredients and herbal supplements that are making their way into the pet food market.

LITERATURE CITED
