

Companion Animals

615 Use of genomic biology to study companion animal microbiota. K. S. Swanson,* *University of Illinois, Department of Animal Science, Urbana.*

The gastrointestinal (GI) tract of dogs and cats contains a complex and dense population of microbiota, including bacteria, archaea, fungi, protozoa, and viruses. Depending on its composition, the microbiota may be beneficial or harmful to the host. Commensal microbes may be a burden in some respects, but is outweighed by the role they play in GI immunity, resistance to pathogenic colonization, short-chain fatty acid production, and epithelial gene expression. The knowledge pertaining to GI microbiology and health has largely been obtained using traditional (i.e., plating) techniques over the past several decades. A hindrance to this field has been the inability to effectively identify and quantify microbial species. Molecular tools, such as quantitative polymerase chain reaction (qPCR), fluorescent in situ hybridization (FISH), denaturing gradient gel electrophoresis (DGGE), quantitative dot blot hybridization, restriction fragment length polymorphism (RFLP), and high-throughput DNA sequencing, have dramatically changed the research landscape. Our lab has recently used such techniques to characterize the phylogeny and functional aspects of canine and feline GI microbiota and identify the effects of diet and age on these communities. Several hundred phylotypes, predominated by members of *Firmicutes*, *Fusobacteria*, *Proteobacteria*, *Bacteroidetes*, and *Actinobacteria*, inhabit the dog and cat gut. Although these populations are quite stable over time, our data have demonstrated that dietary macronutrient profile (e.g., dietary protein: carbohydrate ratio), fiber amount and type, and form of food (e.g., raw vs. extruded) may affect the number and/or metabolism of the colonic microbiota. Continued use of DNA-based techniques to characterize microbial phylogeny and metabolic capacity, along with other technologies to analyze microbial RNA, protein, and metabolite profiles, will increase our understanding of host-microbe relationships. Despite the challenges that surround this field, these powerful tools will enhance our ability to understand GI health and diseases in pets on a molecular level, leading to improved disease diagnosis and treatment in coming years.

616 Pheromones and interomones that change heart rate and behavior of anxious dogs. G. Thompson* and J. J. McGlone, *Texas Tech University, Lubbock.*

Pheromones are species-specific odors used in communication. Interomones are pheromones in one species, but have diverse effects on other species. The objective of this study was to assess efficacy of pheromones/interomones to modulate heart rate and behavior in adult anxious dogs (trembling, cowering, shy). The dogs (8.1 ± 0.18 kg; estimated 5–12 yr intact males) were obtained from a local research facility. Body weights and feed intake were recorded. Each dog was housed in a separately ventilated room with a minimum of 12 m² of floor space. Heart rate (HR) and surface temperature was measured using a telemetry system (Data Science International, St. Paul, MN). Behavior was recorded on a DVR and later reviewed by a trained individual. A scan sample was used with a recording interval of 5 min over 24 h. At the end of 24 h with a given pheromone collar, each dog was startled with a 110 db foghorn 12 cm from the dog's head while behavior and heart rate were recorded. Each dog received each treatment in a Latin

square design with repeated measures over time. This model allowed evaluation of effects of treatment, dog, treatment by dog, time, treatment by time and dog by time. Treatments were given in the form of a collar containing each pheromone/interomone and included placebo (collar without pheromone), Sergeant's (SERG), 2-methylbut-2-enal-Rabbit Pheromone (RP), or a SERG+RP combined collar. Baseline HR did not differ among treatments (avg = 110.1 ± 13.3 bpm). After startle, the RP lowered ($P < 0.01$) HR compared with placebo (124.5 ± 7.2 vs. 157.8 ± 7.2 bpm); however, the treatment by dog interaction ($P < 0.01$) indicated certain dogs were more responsive than others. Dogs with RP collars spent more time lying down (87.4 ± 5.4 vs. $63.2 \pm 4.8\%$ of time, $P = 0.01$) and less time pacing (3.0 ± 0.2 vs. $3.8 \pm 0.02\%$, $P < 0.05$) than placebo-treated dogs. Pace/walk changed differentially among treatments and dogs (dog by RP and SERG effects for pace/walk, $P = 0.05$). In summary, RP lowered HR while SERG and SERG+RP did not change dog HR. However, pheromone/interomone treatments had differential effects on individual dog behavior and HR. Pheromones/interomones can cause meaningful changes in dog behavior and HR among certain anxious dogs.

Key Words: pheromone, behavior, dogs

617 Genome-wide linkage scan for loci associated with canine hypoadrenocorticism. A. M. Oberbauer* and J. M. Belanger, *University of California-Davis, Davis.*

Canine hypoadrenocorticism (Addison's disease) results from reduced adrenal production of corticosteroids and mineralocorticoids. Within the dog population, the disease is relatively uncommon but some breeds, bearded collie, great danes, Leonbergers, Portuguese water dogs, standard poodle, and west highland white terriers are at increased risk for developing hypoadrenocorticism. This disease can be lethal or require lifelong hormonal therapy. Prior studies have indicated a genetic component to the expression of the disease in these breeds. The aim of this study was to employ a genome-wide linkage scan to interrogate the canine genome and identify genomic regions associated with hypoadrenocorticism in the dog. Extracted DNA from bearded collies (43), great danes (11), Leonbergers (4), Portuguese water dogs (11), standard poodles (91), and west highland white terriers (11) were genotyped using the newly developed high-density genotyping array consisting of over 170,000 evenly spaced single nucleotide polymorphisms (SNP). Association analyses were conducted using the software package PLINK. Dogs were classified as having a positive diagnosis of hypoadrenocorticism if they failed to respond to an adrenocorticotropic hormone stimulation test and unaffected controls if they were over 7 years of age and healthy. The analysis included 96 confirmed cases and 75 control dogs. When considered in this manner, based on 50,000 permutations, 4 chromosomal regions reached genome wide significance: chromosomes 3 ($P < 0.006$), 6 ($P < 0.008$), 16 ($P < 0.04$), and 30 ($P < 0.04$). Chromosome 3 appears to be associated with a protective allele while 6, 16, and 30 confer a 3- to 4-fold risk. Each chromosomal region encompasses several genes that will require additional fine mapping to resolve the region that contributes to expression of the disease. Identifying the underlying causal mutations and implementing genetic testing will reduce the incidence of hypoadrenocorticism in dogs.

Key Words: hypoadrenocorticism, genome scan, dog

618 Effects of dietary macronutrient composition on postprandial endocrine response in domestic cats. P. Deng^{*1}, T. K. Ridge², T. K. Graves², J. K. Spears⁴, and K. S. Swanson^{1,3}, ¹*Department of Animal Sciences, University of Illinois, Urbana*, ²*Department of Veterinary Clinical Medicine, University of Illinois, Urbana*, ³*Division of Nutritional Sciences, University of Illinois, Urbana*, ⁴*Nestlé Purina PetCare, St. Louis, MO*.

Obesity is now the most common metabolic disease in cats, having adverse effects on quality of life. Identifying dietary effects on appetite-regulating hormones in cats may enhance our understanding of appetite control and obesity development in this species. The objective of current study was to investigate the effects of dietary macronutrient composition on appetite-regulating hormone release. Twelve neutered male adult domestic shorthair healthy cats were fed 4 nutritionally complete diets [control, high-fat (HF), high-protein (HP), high-carbohydrate (HC)] at amounts to maintain ideal BW and body condition score. The control diet provided 33% kcal from each macronutrient, while the other provided 50% of kcal from the macronutrient highlighted. Cats were randomly allotted to diets in a 4 × 4 Latin square design experiment for a total of 64 d (four 16-d periods). After a 15-d adaptation phase, blood samples were collected before (0 min) and 10, 20, 30, 60, 90, 120, 150, 180, 240, 300, 360, 720 min after the morning meal was consumed. Glucose, insulin, total ghrelin, and leptin concentrations were measured. Data were analyzed by comparing change from baseline and postprandial incremental area under the curve (IAUC) among treatments. Baseline concentrations of glucose, insulin, leptin, and ghrelin were not different among treatments. However, glucose IAUC_{0-6 h} and IAUC_{0-12 h} were higher ($P = 0.028$) in cats fed control and HC compared with those fed HF and HP. IAUC_{0-6 h} and IAUC_{0-12 h} of insulin, leptin, and ghrelin were not different among treatments. In conclusion, while diets containing higher carbohydrate content increased blood glucose, they did not appear to affect appetite-regulating hormone concentrations. More studies on macronutrients are required to identify any effect they may have on appetite.

Key Words: appetite-regulating hormones, macronutrients, cat

619 Digestibility of day-old, whole ground, extruded, and canned chicken-based diets in African wildcats. K. R. Kerr^{*1}, C. L. Morris³, S. L. Burke³, L. M. Garner¹, and K. S. Swanson^{1,2}, ¹*Division of Nutritional Sciences, University of Illinois, Urbana*, ²*Department of Animal Sciences, University of Illinois, Urbana*, ³*Henry Doorly Zoo, Omaha, NE*.

Small captive exotic cats are commonly fed whole prey to supplement traditional diets, such as raw-meat based, canned, and extruded diets. Data regarding the digestibility of these diet types is lacking. The objective of this study was to compare apparent total tract macronutrient digestibility differences of whole chicks [WHO; 24% DM, 76% crude protein (CP)], ground chicken (GRO; 31% DM, 43%CP), chicken-based canned diet (CAN; 40%DM, 47% CP), and chicken-based extruded (EXT; 92%DM, 36% CP) diets fed to captive African wildcats. Four animals were utilized in a crossover design. Apparent DM digestibility (81 to 85%) was not affected by treatment; however, apparent organic matter (OM) digestibility was higher ($P < 0.05$) in cats fed GRO (94%) compared with those fed WHO, CAN, and EXT (85 to 87%). Apparent CP digestibility was higher ($P < 0.05$) in cats fed GRO (91%) compared with those fed EXT (80%). Because the GRO had high digestibility and low levels of fiber or bulking materials (i.e., feathers), cats fed this diet had high fecal DM (68%), which was reflected in the fecal scores (1 out of 5; 1 = hard, dry pellets). Cats fed the other diets had fecal scores close ideal (2.6 to 3.6; 3 = ideal). In conclusion, all diets had high

digestibility (>85% OM digestibility), maintained BW and health short-term. Inclusion of whole-prey (WHO and GRO) as dietary items for AWC is appropriate; however, if fed as the sole dietary item additional nutrient supplementation, including fiber or fiber-like material, may be necessary. Further research is needed on the ability of whole prey to meet the nutritional needs of small exotic cats, including compositional analysis, bioavailability, and research including more species.

Key Words: digestibility, feline, whole prey

620 Dietary protein:carbohydrate ratio alters kitten fecal microbiota as analyzed by 454 pyrosequencing. S. Hooda^{*1}, B. M. Vester Boler¹, K. R. Kerr¹, S. E. Dowd², and K. S. Swanson¹, ¹*University of Illinois, Department of Animal Sciences, Urbana*, ²*MR DNA Molecular Research LP, Shallowater, TX*.

The dietary protein: carbohydrate ratio may affect the gut microbiota of growing kittens, but this aspect of the diet has not been well studied. This study was conducted to test the effects of moderate and high protein intake on the composition of the gut microbiota of growing kittens using 454 pyrosequencing. Male domestic shorthair kittens were raised by mothers fed moderate-protein, moderate-carbohydrate (MPMC; n = 7) or high-protein, low-carbohydrate (HPLC; n = 7) diets, then weaned at 8 wk of age onto the same diet. Fresh fecal samples were collected at 8, 12 and 16 wk of age; DNA was extracted, followed by amplification of the V4-V6 region of the 16S rRNA gene using barcoded primers for pyrosequencing. Pyrosequencing of barcoded amplicons resulted in a total of 384,588 sequences, with an average of 9,374 sequences per sample. The difference in protein:carbohydrate ratio resulted in dramatic differences in fecal microbiota, primarily in the *Actinobacteria* and *Fusobacteria* phyla; the abundance of fecal *Actinobacteria* being greater ($P < 0.05$) in MPMC-fed kittens and increased with age ($P < 0.05$). In contrast, the proportion of fecal *Fusobacteria* was greater in HPLC-fed kittens ($P < 0.05$). Among the well-characterized genera *Clostridium*, *Fecalibacterium*, *Ruminococcus*, *Blautia*, and *Eubacterium* predominated ($P < 0.05$) fecal samples of HPLC-fed kittens. In contrast *Dialister*, *Acidaminococcus*, *Bifidobacterium*, *Megasphaera*, *Mitsuokella*, and *Subdoligranulum*, were greater ($P < 0.05$) in MPMC-fed kittens. Kittens fed MPMC also had higher ($P < 0.05$) fecal *Lactobacillus* at 8 wk of age and then decreased ($P < 0.05$) at 12 and 16 wk of age. Further research is required to identify the physiological relevance of these diet-induced changes in gut microbiota during the nursing and weaning period, and test how important the weaning diet is on long-term microbiota populations and gut health-related outcomes.

Key Words: protein carbohydrate ratio, gut microbiome, 454 pyrosequencing

621 Influence of indigestible starch content in dry expanded diets on stool characteristics of dogs differing in body size. R. Goudez^{*1,2}, M. Weber², L. Martin¹, V. Leray¹, V. Biourge², H. Dumon¹, and P. Nguyen¹, ¹*LUNAM University, Oniris, National College of Veterinary Medicine, Food and Science and Engineering, Nutrition and Endocrinology Unit, Nantes, France*, ²*Royal Canin Research Center, Aimargues, France*.

Large breed dogs are known to be more sensitive to diet than smaller ones, and consequently to produce feces of poorer quality. This could be due to larger amounts of indigestible residues reaching the large bowel and leading to increased fermentative activity. The aim of this study was 1/ to determine whether variation in dietary indigestible starch level could affect fecal characteristics in small and large dogs and 2/ to assess

if large dogs have a higher digestive sensitivity to indigestible starch than small ones. Five German shepherds (GS), 5 beagles (BE), and 5 miniature schnauzers (MS) were used in the study. Two dry expanded diets were tested in a crossover design. They only differed in their source of corn starch: purified (CS) or high-amylose (HA), and contained respectively 0.4 and 3.6% of indigestible starch (i.e., resistant starch). Fecal quality, nutrient digestibility and fermentation by-products (SCFA and lactate) were measured. Effects of diet or breed on fecal parameters were statistically tested (Wilcoxon and Kruskal-Wallis tests). Contrary to CS diet, HA diet tended to modify fecal characteristics in the 3 tested breeds ($P < 0.059$). When HA diet was fed, higher fecal SCFA (GS, BE) or lactate (GS, BE, MS) concentrations, lower pH (GS), and poorer fecal quality (GS) were observed, and animals presented a lower apparent digestibility of starch. Compared with small ones, large dogs fed with the HA diet presented stools with lower consistency ($P < 0.003$) and higher moisture ($P < 0.003$). Fecal SCFA and lactate concentrations were higher ($P < 0.003$ and $P < 0.014$ respectively), and stool pH tended to be lower pH ($P < 0.055$). Starch apparent digestibility was higher in GS ($P < 0.01$). Those results show that indigestible starch level tends to increase colonic fermentative activity in all dogs whatever their size. However, small and medium dogs showed less sensitivity to indigestible starch based on fecal scores, while large dogs were quite responsive. These results suggest that indigestible starch content (strongly affected by source and processing) is a critical step for petfood manufacturer, especially regarding diets for large breed.

Key Words: dog, starch, fermentation

622 Influence of fresh citrus pulp and apple pomace on the digestibility of nutrients in dogs. S. Brambillasca,* C. Deluca, A. Britos, and C. Cajarville, *Departamento de Nutrición Animal, Facultad de Veterinaria, Universidad de la República, Montevideo, Uruguay.*

Six adult cocker spaniel dogs were used to evaluate whether the dietary inclusion of fresh citrus pulp and apple pomace affect the digestibility

of nutrients. Dogs (3 female, 3 male; BW: 12.7 ± 0.7 kg) were assigned randomly to treatments in a double 3×3 Latin square design (3 treatments, 3 experimental periods). Treatments consisted in a commercial dog food (CON) with or without the addition of 70 g/kg of either fresh citrus pulp (CIT) or fresh apple pomace (APP). Each experimental period consisted in a 5-d diet adaptation phase and 3-d for fecal collection. Diets and feces were analyzed for DM, OM, CP, NDF and ADF, and digestibility of each fraction calculated. Data was analyzed by PROC MIXED considering the treatment and period effects and means were separated by orthogonal contrasts. Digestibilities of nutrients were higher when dogs received CON than when fed diets containing both citrus and apple. Dogs fed CIT presented higher digestibility values than dogs fed APP for most of the nutrients analyzed. Despite there was a reduction in digestibility values with the inclusion of fiber in the diets, overall digestion was widely acceptable.

Table 1. Effect of diets on coefficients of total tract apparent digestibility of nutrients

	Treatment				Contrast (P -value) ¹	
	CON	CIT	APP	SEM	Fiber	Source
DM	0.850	0.811	0.804	0.011	0.005	0.020
OM	0.888	0.845	0.841	0.012	<0.001	0.003
CP	0.863	0.835	0.817	0.011	0.012	0.081
NDF	0.777	0.680	0.649	0.022	<0.001	0.001
ADF	0.575	0.383	0.340	0.030	<0.001	<0.001

¹Fiber = effect of fiber inclusion (CON vs CIT+APP); Source = effect of fiber source (CIT vs APP).

Key Words: fiber, canine, digestion