**T92** Effects of diet and age on metabolic characteristics and gene expression profile in the dog. Part 1: Metabolic characteristics. K. S. Swanson<sup>\*</sup>, K. N. Kuzmuk, L. B. Schook, and G. C. Fahey, Jr., *University of Illinois, Urbana, IL*.

The objectives of this experiment were to determine the effects of diet on metabolic characteristics and gene expression profile in old and young dogs. Old (ave. age = 11.1 0.6 yr; 6 M, 6 F) and weanling (age = 8 wk old; 6 M, 6 F) Beagles were used. Three of each gender and age were randomly assigned to one of two dietary treatments. Diet A was primarily composed of high quality, animal-derived ingredients and was formulated to contain 30% crude protein and 20% fat. Diet B was primarily composed of plant-derived ingredients and was formulated to contain 22% crude protein and 8% fat. Old dogs were fed to maintain bodyweight while weanling puppies were fed ad libitum. Blood samples were collected via jugular puncture at baseline and after 3, 6, and 9 months for analysis of complete blood count (CBC) and serum chemistry profile. A 4-day total fecal collection was performed to determine total tract macronutrient digestibilities after 3 months on the experiment. Data were analyzed using the GLM procedure of SAS. As expected, dry matter (DM) and organic matter (OM) digestibilities were greater (P <0.05) for dogs fed Diet A. Dry matter (P = 0.06) and OM (P < 0.05) digestibilities also were greater in old dogs. Dogs fed Diet B had lower (P < 0.05) fecal DM% and greater (P < 0.05) fecal output (when expressed either on as-is or DM basis) and fecal output (as-is): food intake (DM) ratio. Age had a major impact on CBC and serum chemistry profile. At baseline, young dogs had greater (P < 0.05) total white blood cell, neutrophil, lymphocyte, and blood glucose concentrations. Old dogs had greater (P < 0.05) red blood cell, hemoglobin, hematocrit, albumin, creatinine, blood urea nitrogen and total protein concentrations at baseline. In conclusion, diet and age had dramatic effects on nutrient digestibility, fecal characteristics, CBC, and serum chemistry profile.

Key Words: Canine, Nutritional genomics, Nutrient digestibility

**T93** Effects of diet and age on metabolic characteristics and gene expression profile in the dog. Part 2: Gene expression profiling. K. S. Swanson\*, K. N. Kuzmuk, L. B. Schook, and G. C. Fahey, Jr., University of Illinois, Urbana, IL.

Detecting dietary effects on gene expression profile in geriatric and weanling dogs was the objective of this experiment. Blood samples were collected via jugular puncture at baseline and after 3, 6, and 9 months for RNA isolation. Liver biopsies also were collected after 6 and 9  $\,$ months for RNA isolation. Isolated RNA samples can be hybridized with an oligonucleotide microarray to generate a gene expression profile. Oligonucleotide microarrays monitor the expression of hundreds or thousands of genes simultaneously, making them a powerful alternative to conventional techniques that limit experiments to measuring only a few genes at a time. We designed an oligonucleotide microarray containing 384 genes with major emphasis placed on metabolic pathways and immune function. With the use of Vector NTI (Informax Inc., Frederick, MD), a bioinformatics tool used for sequence analysis and molecular biology data management, canine expressed sequence tags (ESTs) and gene sequences were identified from the public domain. Genes of interest were analyzed to determine unique oligonucleotide sequences (70-mers) that could be used as a probe on the microarray. Selected sequences were then synthesized and printed on microarray slides. As with humans, diabetes is highly prevalent in dogs and is positively correlated with age. Identification of biomarkers predictive of disease is needed and is a goal of this experiment. Therefore, genes associated with glucose metabolism and homologous to human genes demonstrated to contribute to the development of diabetes were included on the microarray. To conclude, a 384-gene oligonucleotide microarray has been developed to study metabolic pathways and immune function in dogs with a strong emphasis on glucose metabolism and diabetes. This microarray will be used to generate gene expression profiles of dogs in the current experiment and those in future experiments.

Key Words: Canine, Nutritional genomics, Oligonucleotide microarray

**T94** Case study of preparing a submission for regulatory clearance of a new ingredient. L. B. Deffenbaugh\*, *Kemin Nutrisurance, Inc.* 

New ingredients for companion animal diets become available only occasionally because of the rigorous approval process. Regulatory options for clearance of a new ingredient for petfoods include: Food Additive Petition (US), Letter of Non-Objection (US), GRAS declaration (US) or an Assessment of Additives in Animal Nutrition (EU). The key objectives of the regulatory clearance processes are purpose (utility) and safety. Rosemary extract is a natural botanical for which antioxidant properties have been widely reported for decades. The US Food and Drug Administration allows for the use of rosemary extract as a flavor or spice, but not as an antioxidant, in animal feeds under 21 CFR 582.20, Substances Generally Recognized as Safe (GRAS). Kemin Nutrisurance, Inc. has prepared a Letter of Non-Objection submission to extend clearance of rosemary extract to include use as an antioxidant in animal feeds. While the data gathered for the submission appears replete and voluminous, the process to collect the information was quite straightforward once a clear outline was prepared. The data for the rosemary extract submission will be described in such a way to provide a template for readers who are considering preparation of an LNO submission for a new ingredient. The process was found to be valuable as the requirements for the submission fulfill many of the steps required to develop and launch a new ingredient.

**Key Words:** Regulatory clearance, Letter of non-objection, Rosemary extract

**T95** Effects of spray-dried animal plasma on apparent digestibility, intake and fecal consistency in adult Beagles. J. D. Quigley, III\*, K. Dahm, and T. A. Wolfe, *APC*, *Inc.*, *Ames*, *IA*.

Effects of spray-dried animal plasma (SDAP) on intake, fecal output, fecal scores and apparent total tract digestibility were determined using 14 adult Beagle dogs (BW = 13.3 kg). The SDAP (Endure, APC, Inc.) was coated on the exterior of extruded dry dog food kibbles at 2% of weight. Coated kibbles (27% CP and 13 to 16% ether extract) were fed to dogs in two experiments (n = 6 and 8 in experiments 1 and 2, respectively) in a switchback design using two 15-d periods. The final 5 d of each period were used for feed and fecal collections. In experiment 1, kibbles were coated with 5% tallow, 2% commercial flavor and 0 or 2% SDAP. In experiment 2, commercially available dry dog food, previously coated with fat and flavor were coated with 0 or 2% SDAP by mixing in a cement mixer. Intake, fecal consistency and apparent digestibility of nutrients were determined. Addition of SDAP did not affect chemical composition of diets or intake of most nutrients. Fecal scores were unaffected but total feces excreted was reduced by 13.1 and 21.8% in experiments 1 and 2, respectively. Addition of SDAP improved digestibility of DM, ash, crude fiber, and fat in experiment 1 and DM, organic matter, ash, and crude fiber in experiment 2. Digestion of crude fiber was improved from 1.8 to 20.3% in Experiment 1 and 5.4 to 29.1%in Experiment 2 with addition of SDAP. Digestion of ash was improved from 32.5 to 42.8% in Experiment 1 and 37.4 to 44.7% in Experiment 2. Changes in digestion that occurred with addition of SDAP suggested improved digestive capacity in dogs.

		Expt 1 $$				Expt $2$		
Item	CON	SDAP	SEM	$\mathbf{P}^*$	CON	SDAP	SEM	$\mathbf{P}^*$
DM intake, g/d	221	217	6	NS	262	246	9	NS
Wet feces, g/d	137	119	5	0.08	119	93	4	0.01
Fecal score <sup>**</sup>	3.82	3.80	0.10	NS	3.85	3.72	0.10	NS
Digestibility, %								
DM	78.3	80.1	0.4	0.04	83.1	86.2	0.6	0.01
Organic matter	84.4	85.2	0.3	NS	86.6	89.3	0.5	0.01
Crude protein	83.8	84.3	0.9	NS	85.8	89.4	1.1	0.07
Fat	93.2	94.2	0.2	0.03	93.5	94.5	0.4	NS
Ash	32.5	42.8	1.8	0.02	37.4	44.7	1.5	0.01
Crude fiber	1.8	20.3	3.0	0.01	5.4	29.1	2.3	0.01

\*Probability of a treatment effect; NS = P > 0.10.

\*\*Fecal score on scale of 1 = watery diarrhea to 5 = hard, dry, crumbly.

Key Words: Canine, Digestibility, Spray-dried animal plasma

**T96** Effects of supplemental spray dried plasma on food intake, nutrient digestibility, and gastrointestinal microflora in healthy adult dogs. J. M. Dust<sup>\*1</sup>, G. C. Liu<sup>1</sup>, C. M. Grieshop<sup>1</sup>, N. R. Merchen<sup>1</sup>, J. D. Quigley, III<sup>2</sup>, and G. C. Fahey, Jr.<sup>1</sup>, <sup>1</sup>University of Illinois, Urbana, IL, <sup>2</sup>APC, Inc., Ames, IA.

Four purpose-bred ileally cannulated adult female dogs with hound bloodlines were used to evaluate the effects of supplemental spray dried plasma (SDP) on food intake, nutrient digestibility, and gastrointestinal microflora using a 4 x 4 Latin square design. Spray dried plasma was solubilized in poultry fat and applied to the exterior of extruded kibbles at 0, 0.5, 1, or 2%. Dietary treatments were fed for a 10-d adaptation phase followed by a 4-d sample collection phase. Inclusion of SDP did not affect (P > 0.05) food intake during the collection period. Wet fecal output, fecal score, and fecal dry matter percentage also were not affected (P > 0.05) by SDP supplementation. Ileal digestibility of dry matter, organic matter, crude protein, and fat were increased in dogs fed diets containing 0.5 and 1.0% SDP but decreased in dogs fed diets containing 2.0% SDP resulting in a significant (P < 0.05) quadratic effect of SDP inclusion on ileal digestibility of these nutrients. In contrast, dietary inclusion of SDP did not affect (P > 0.50) total tract digestibility of dry matter, organic matter, crude protein, nor fat. Spray dried plasma supplementation reduced (P < 0.05) ileal concentrations of total anaerobic and total aerobic bacteria, lactobacilli, and Clostridium perfringens, but did not affect (P > 0.05) ileal concentration of bifidobacteria. In contrast, only fecal lactobacilli concentrations were reduced (P < 0.05) by SDP supplementation. From these data, low levels of SDP inclusion in diet do not adversely affect nutrient digestibility or fecal characteristics of healthy adult dogs.

Key Words: Spray dried plasma, Dog, Digestibility

**T97** Corn hybrid impacts ileal and total tract nutrient digestibility by dogs. A. M. Gajda, E. A. Flickinger\*, C. M. Grieshop, N. R. Merchen, and G. C. Fahey, Jr., *University of Illinois, Urbana, IL U.S.A.*.

Corn is a commonly used ingredient in dry pet foods because there is a stable supply and it is a relatively inexpensive source of nutrients. Corn hybrids are available that are higher in crude protein and amylose concentrations and lower in phytate concentration than conventional hybrids. Ileal and total tract digestibilities of high protein (HP), highprotein, low-phytate (HPLP), and high amylose (HA) corns were compared to those of a conventional (CONV) corn and amylomaize starch (AM). Five ileal cannulated dogs were fed each corn hybrid at approximately 31% of the diet in a 5 x 5 Latin square design. High protein corn-containing diets had higher (P < 0.05) ileal organic matter (OM) digestibility (70.3%) and tended to have higher (P < 0.10) DM digestibility (66.5%). Ileal starch digestibilities were lower (P < 0.05) for dogs fed HA (64.0%) and AM (63.0%). Ileal digestibilities of essential (66%) and non-essential amino acids (62%) were higher (P < 0.05) for HP diets, and tended to be higher (P = 0.09) for total amino acids, compared to CONV. Total tract DM, OM, CP, and gross energy digestibilities (76, 82, 77, 84%, on average, respectively) were higher (P < 0.05) for dogs fed CONV, HP, and HPLP compared to AM (66.9, 71.6, 72.6, 76.5%) and HA (60.6, 65.7, 69.7, 71.5%). Total tract fat digestibilities were lower (P < 0.05) for dogs fed HA diets (86.6%) compared to all other treatments (91.0%, on average). Total tract starch digestibilities were higher (P <0.05) for dogs fed CONV, HP, and HPLP (98%, on average) compared to HA (72.8%) and AM (76.5%). As-is fecal output and fecal DM output were higher (P < 0.05) for the HA treatment (218 and 72.6 g/d, respectively). Fecal scores were similar among treatments. No differences were detected among treatments in bifidobacteria, lactobacilli, or Clostridium perfringens concentrations. The experiment demonstrated that HP and HPLP corns had similar ileal and total tract nutrient digestibilities as CONV corn, while HA resulted in similar responses to AM, a well established resistant starch ingredient.

Key Words: Corn, Dog, Digestibility

**T98** Evaluation of low-oligosaccharide low-phytate whole soybeans and soybean meal in canine foods. R. M. Yamka<sup>\*1</sup>, B. M. Hetzler<sup>1</sup>, and D. L. Harmon<sup>1</sup>, <sup>1</sup>Department of Animal Sciences, University of Kentucky, Lexington, KY 40546.

Eight mature dogs (19.3  $\pm$  0.1 kg) were used in a replicated 4X4 Latin square design experiment to determine the consequences of feeding lowoligosaccharide, low-phytate soy on nutrient availability in complete foods fed to dogs. All foods were isonitrogenous (20% CP) and contained low-oligosaccharide, low-phytate soybean meal (LLM), conventional soybean meal (SBM), low-oligosaccharide, low-phytate whole soybeans (LLB), or conventional whole soybeans (WSB) as the protein source. Daily DMI averaged 287  $\pm$  4 g/d. The LLB and WSB foods had the highest fecal outputs averaging 48.2 g DM/d. Small intestinal DM digestibility was highest for LLM (80.9%) and lowest for LLB (74.0%). Large intestinal DM digestibility was not affected by oligosaccharides (P > 0.65). Total tract DM digestibility was highest for LLM (87.0%) and lowest for WSB and LLB (averaging 83.3%). Fecal N excretion was lowest for LLM and SBM (1.5 and 1.6 g N/d, respectively) and highest for LLB and WSB (2.3 and 2.0 g N/d, respectively). No differences in N balance, small intestinal N digestibility or large intestinal N digestibility were observed due to the presence of oligosaccharides (P >0.67, P > 0.21 and P > 0.12, respectively). Tryptophan digestibility was higher for SBM when compared to LLM (P < 0.04) and higher for WSB than LLB (P < 0.001). Histidine digestibility was higher in WSB when compared to LLB (P < 0.05). No differences in nonessential AA digestibility were observed when comparing soy-based foods. The results of this study demonstrate that LLM, SBM, LLB and WSB can be good sources of protein for canine foods and have a high digestibility. Statistical differences in small intestinal digestibility were not observed (with the exception of tryptophan and histidine) when comparing SBM to LLM and WSB to LLB indicating that oligosaccharide and phytate content did not affect digestibility in the present study.

Key Words: Dogs, Soybeans, Digestibility

**T99** Accounting for the proportion of alpha-amino nitrogen in crude protein improves metabolizable energy prediction in dry extruded dog foods. R. M. Yamka\*<sup>1</sup>, K. R. Mcleod<sup>1</sup>, D. L. Harmon<sup>1</sup>, H. C. Freetly<sup>2</sup>, and W. D. Schoenherr<sup>3</sup>, <sup>1</sup>Department of Animal Sciences, University of Kentucky, Lexington, KY 40546, <sup>2</sup>USDA, ARS, U.S. Meat Animal Research Center, Clay Center, NE 68933, <sup>3</sup>Hill's Pet Nutrition, Topeka, KS 66617.

The modified Atwater's equation [metabolizable energy (ME) kcal/kg  $= 3.5^{*}$ crude protein (CP)  $+ 8.5^{*}$ ether extract (EE)  $+ 3.5^{*}$ nitrogen free extract] is currently used to predict the metabolizable energy (ME) content of dog foods. However, we found that the equation consistently under predicted ME compared to the observed ME in 55 balance trials. It was our objective to use these balance trials to develop an equation based on chemical composition of the diet to predict ME content of the diets. Eight diets that varied in ME content (3,463 to 4,233kcal/kg) and were fed at maintenance and used in the analysis. The diets varied in protein source, with the major protein sources being lowoligosaccharide whole soybeans, low-oligosaccharide low-phytate whole soybeans (2 sources), conventional soybean meal (2 sources), low-ash poultry meal, low-oligosaccharide low-phytate soybean meal or conventional whole soybeans. A multivariate regression analysis was used to predict ME content based on chemical composition. Two initial models were fit to the data. Model 1 included CP, EE and crude fiber (CF). Model 2 replaced the CF term with acid detergent fiber (ADF) and neutral detergent fiber (NDF), which resulted in a model that contained CP, EE, NDF, and ADF. Because the diets varied in protein sources the ratio of alpha-amino N (AAN) to non-alpha-amino N (NAAN) ranged from 3.5 to 14.4, therefore we hypothesized that accounting for the proportion of AAN in CP would improve the fit of the models. Model 1 had an  $r^2$  of 0.46 and when AAN and NAAN were substituted for CP, the model had an  $r^2$  of 0.79. Similarly, Model 2 had an  $r^2$  of 0.43 and when AAN and NAAN were substituted for CP the model had an  $r^2$  of 0.82. Residual analysis suggests that by replacing the CF term in Model 1 with ADF and NDF in Model 2 there was an improvement in prediction of ME content. By splitting CP into an AAN and NAAN fractions we have further defined the chemical composition of the diet. These data suggest that defining protein quality improves the ability to predict ME content of dog foods.

Key Words: Dogs, Metabolizable energy, Alpha-amino nitrogen

**T100** Estimation of the proportion of bacterial nitrogen in canine feces using diaminopimelic acid as an internal bacterial marker. L. K. Karr-Lilienthal<sup>1</sup>, C. M. Grieshop<sup>1</sup>, J. K. Spears<sup>1</sup>, A. Patil<sup>2</sup>, N. M. Merchen<sup>1</sup>, and G. C. Fahey, Jr.<sup>1</sup>, <sup>1</sup>Unversity of Illinois at Urbana-Champaign, IL USA, <sup>2</sup>Nestle Purina Research, St Joseph, MO USA.

Approximately 50% of the mass of dog feces may be of bacterial origin, but this number is based on human data. A bacterial marker can be used to determine the portion of fecal N that is of bacterial origin as well as the effect of dietary ingredients on the bacterial N content found in feces of the dog. Two experiments were conducted to determine the efficacy of diaminopimelic acid (DAPA) and purines as bacterial markers in dogs. In experiment 1, five adult, female dogs were fed the same commercial diet. In experiment 2, fifty dogs were fed one of four test diets: a prebiotic-free control, or a diet containing either 1% chicory, 1% mannanoligosaccharide (MOS), or 1% chicory plus 1% MOS. Fresh feces were collected in both experiments and used to isolate a bacterial rich sample (BRS) by differential centrifugation. In experiment 1, the BRS had a 0.66 N:purine ratio and an 18.9 N:DAPA ratio. The coefficient of variation for the N:purine ratio was much higher than that for the N:DAPA ratio, indicating that DAPA would provide a less variable estimate of fecal bacterial N. Using either marker, approximately 50%of the fecal N was estimated to be of bacterial origin. In experiment 2, the N:DAPA ratio of the BRS was not different (P > 0.05) among treatments. Dogs fed prebiotic-containing diets had N:DAPA ratios ranging from 17.5 to 18.2, while dogs fed the control were lower at 15.9. For dogs fed prebiotic-containing diets, approximately 48% of the fecal N was of bacterial origin compared with 45% for dogs fed the control. When calculating fecal bacterial concentration using the average N:DAPA ratio for all dogs, little difference existed in the estimation compared to using the individual values. However, for dogs fed the control, the value using the average ratio was approximately 18% higher than when using the individual ratios. This is due to the lower N:DAPA ratio for dogs fed the control compared with dogs fed the other treatments. Based on the consistency of the N:DAPA ratio of the BRS, DAPA appears to be a valuable tool for estimation of bacterial N in feces.

Key Words: Diaminopimelic acid, Dog, Marker

## **T101** The effect of preservation time length and thawing on *Lactobacillus* population from fecal material. C. J. Fu and M. S. Kerley, *University of Missouri-Columbia*.

A study was conducted to determine the effect of preservation time length on Lactobacillus population in dog feces. Lactobacillus is typically used as an indicator of dietary-induced bacteria population change, because its presence is important to normal bowel function. Also, reviewers have questioned the validity of using preserved digesta/fecal material for enumeration bacterial populations. The objective of this research was to determine fecal storage effects on Lactobacillus population number. The treatment conditions were: 1) Maintained in buffered (pH = 7) anaerobic dilution solution for 2, 4, 7, or 14 days at 4 °C; 2) Frozen (-80  $^o\mathrm{C})$  overnight (12 h) in the same dilution solution, thaved at 4  $^o\mathrm{C}$  for 2 h, and then put in cold tap water for 30 min; and 3) Repeat of the freeze-thaw procedure on the thawed samples generated in treatment 2. Media used to plate the Lactobacillus were MRS broth (Difco, #288130, Sparks, MD, USA, 55 g/L) and agar (20 g/L) with 20 mg/L vancomycin supplement. Incubation time was 48 h in an anaerobic chamber at 37 °C. The population count was compared to the population count of fresh material. The Lactobacillus population decreased (P < 0.05) after 2, 4, 7, and 14 days storage at 4 oC with 92%, 83% 81%, and 68% of the initial population enumerated, respectively. The Lactobacillus population from treatment 2 and treatment 3 was decreased  $(P{<}\;0.05)$  by 57% and by 89% compared to fresh material, respectively. We concluded from this study that statistical differences in *Lactobacillus* population could occur due to preservation conditions. However, population differences deemed of biological significance (10-fold reduction in population count) would only have occurred when fecal material was refrozen. Caution should be exercised when comparing Lactobacillus population counts across different preservation regimens.

Key Words: Feces, Lactobacillus, Preservation

**T102** Effect of dietary antioxidants on immune system parameters in dogs and cats. D. Jewell<sup>\*1</sup>, K. Friesen<sup>1</sup>, L. Larson<sup>2</sup>, T. Sharp<sup>2</sup>, and R. Schultz<sup>2</sup>, <sup>1</sup>*Hill's Pet Nutrition, Inc,* <sup>2</sup>*The University of Wisconsin - Madison.* 

Forty dogs and forty cats were used to evaluate the effect of increasing dietary antioxidants on delayed type hypersensitivity and circulating antibody response to vaccination. The experimental protocol was reviewed and approved by the Institutional Animal Care and Use Committee. Subjects were assigned to dietary treatment groups and fed the treatment foods for 84 days. Dietary treatments consisted of a control food meeting all requirements for adult maintenance and three treatment groups with incrementally increased antioxidant supplementation. The foods with increased antioxidant supplementation all received similar amounts of vitamin C and  $\beta$ -carotene with varying levels of increased vitamin E. Dietary vitamin E was increased by 500, 1000, and 1500 IU/kg in the supplemented foods. Circulating vitamin E increased in a linear (P<0.01) and quadratic (P<0.05) fashion with antioxidant supplementation in dogs and cats. The standard intradermal skin test (delayed type hypersensitivity) increased in response to increasing dietary antioxidants in dogs (P<0.05) and numerically increased in cats. A standard vaccine for four strains of Leptospirosis was used in the dog with the stimulated antibody response measured by specific serovar titer. The sum of the titers from each strain, when compared between the control and the dietary treatment groups had a quadratic trend (P < 0.1) with the maximum response occurring at 1000 IU Vitamin E/kg. Rabies serology was used to determine an antibody response in cats. A quadratic response (P<0.05) was demonstrated in cats with the treatment groups of the first two levels of antioxidant increase having increased titers and the highest antioxidant level supplementation group having a decreased titer when compared to the control. In conclusion, immune function benefits of increased response to a vaccine and increased immune response in an intradermal skin test, were demonstrated in both dogs and cats when antioxidants were added to the food. Both dogs and cats had a maximal benefit by the 1000  $\mathrm{IU/kg}$ addition of vitamin E to foods.

Key Words: Antioxidants, Canine, Feline

**T103** Evaluation of delta-6 desaturase kinetics in canine liver microsomes for alpha-linolenic acid in the presence of competitive amounts of linoleic acid. J. E. Bauer\* and B. L. Dunbar, <sup>1</sup> Texas A&M University.

The rate-limiting step in the conversion of essential fatty acids to long chain derivatives is controlled by delta-6 (d-6) desaturase. This enzyme competitively utilizes both linoleic (LA) and alpha-linolenic acids (ALA) as substrates. Efforts to characterize the kinetics of unpurified enzyme for ALA are confounded by the presence of endogenous LA present in the source material. A technique to correct this problem was developed and used to more accurately estimate the Km and Vmax of d-6 desauturase for ALA in the presence of these inhibitory amounts of LA. Microsomes were prepared from fresh canine liver tissue from normally fed adult, mixed breed dogs, and incubated with 14C labeled LA or ALA substrates under standardized conditions. Lipids were extracted, saponified, and the resultant fatty acids phenacylated. Fatty acid phenacyl esters were separated by HPLC and counted by liquid scintillation counting. LA and ALA contents of the microsomes were determined using internal standardization and gas chromatography. This data was then used to construct a graphical correction for the presence of varying and competitive amounts of LA in canine hepatic microsomal enzyme preparations. A similar correction for LA activity was unnecessary due to insignificant amounts of ALA in the microsome preparations. Delta-6 desaturase activities (Vmax) of 62.4 and 5.4 pmol/min mg protein with ALA and LA respectively were thus found with apparent Km values of 12.4 and 41.8 mM, respectively. These data show that dog liver microsomes have EFA desaturation capabilities and that ALA is preferred due to its higher Vmax and lower Km compared to LA. In spite of this preference it was found that liver concentration of ALA averaged only 2.4 mM. Thus, tissue ALA concentrations his may never exceed the Km for desaturation unless high dietary ALA is provided. Hence conversion of ALA may be slow. By contrast, LA is readily converted because its microsomal content (64.4 mM) exceeds its Km. These characteristics may explain low in vivo ALA conversion rates in dogs and other species. The data further suggest that high levels of dietary ALA may be needed to exceed the Km for d-6 desaturase.

Key Words: Delta 6 desaturase, Kinetics, Alpha linolenic acid

**T104** The effect of dietary fat on the fatty acid composition of olfactory mucosal tissues in young adult dogs. C. T. Middendorf, K. A. Cummins\*, E. A. Altom, and M. Craig-Schmidt, *Auburn University, AL*.

Previous studies have indicated that dogs fed a diet high in saturated fat had a decrease in olfactory acuity. A study was designed to determine the influence of dietary fat source on the phospholipid fatty acid composition of olfactory and respiratory mucosa in young-adult dogs. Fifteen young-adult female beagles (average age = 2 yr, body weight average = 9.69 kg) were randomly assigned to receive one of three diets varying in the amount and source of fat. These were Diet A, with 12% crude fat; Diet B, containing 16% crude fat formulated by the addition of 4%corn oil to the maintenance diet; and Diet C, containing 16% crude fat formulated by the addition of 4% coconut oil. Dogs were fed the diets for a period of sixty days, euthanized, and then samples were collected from the olfactory turbinates and nasal passage. Fatty acid compositions of phosphatidylcholine (PC) and phosphatidyle thanolamine (PE)  $\,$ were analyzed by capillary gas chromatography. The amount of 16:0 in respiratory PC was greatest from dogs fed Diet B (P<0.10), while the amount of 20:4n-6 incorporated into respiratory PC was less (P<0.10). No differences were reported for the 20:4n-6 content in PC of olfactory mucosa (P>0.10). The amount of 18:2n-6 in PC from both mucosal tissues was greater in dogs fed Diet B than in dogs fed Diets A or C (P < 0.10). Despite increased amounts of 18:2n-6 in Diet B, there were no differences among treatments in the amount of 18:2n-6 or 20:4n-6 incorporated into PE (P>0.10) of either tissue. No differences were observed in the ratio of unsaturated to saturated fatty acid incorporation into phospholipids (P>0.10), or in the mean chain lengths (P>0.10). No differences were observed in the unsaturation index for the PE fractions and the olfactory PC (P>0.10). However, the unsaturation index was lower in dogs fed Diet B in respiratory mucosal PC (P < 0.10). Results from this study do not fully explain the differences observed in olfactory acuity.

Key Words: Canine, Nutrition, Lipids

**T105** Heritability of hypoadrenocorticism in the Portuguese Water Dog and the Leonberger. A. M. Oberbauer\*, K. N. Simpson, J. M. Belanger, and T. R. Famula, *University of California, Davis, CA*.

Hypoadrenocorticism (Addison's) is a recognized late onset disorder in the dog. Symptoms are diffuse and a result of deterioration of the adrenal cortex with its subsequent reduction in the capacity to synthesize and secrete glucocorticoids and mineralocorticoids. Diagnosis of Addison's is by ACTH stimulation challenge. Some breeds have a higher than expected incidence of Addison's suggesting a genetic component to the disorder. We have recently reported that Addison's has a genetic basis in the Bearded Collie and Standard Poodle although the mode of inheritance differed. Here we compare the heritabilities and mode of inheritance for the disorder in two additional but related breeds, the Leonberger and the Portuguese Water Dog (PWD). Owners were requested to submit data on the Addisonian status for the above-mentioned breeds along with pedigree, gender, and DNA. The binary nature of the data required a threshold model; a mixed Bayesian analysis model was used to arrive at heritability estimates. Complex segregation analyses were employed to characterize mode of inheritance. The heritability estimates for Addison's disease in the Leonberger (n=294 dogs) and PWD (n=504)were 0.62 and 0.57, respectively. In contrast to the findings in Standard Poodles, the Leonberger and PWD data do not support a single locus of large effect influencing the transmission of Addison's disease. However, when the Leonberger data is corrected for ascertainment bias, the major gene model becomes significant. Although these findings may reflect limited sample sizes, the possibility that different though related breeds have unique patterns of inheritance for Addison's disease may affect the search for genes causal in the expression of canine Addison's.

Key Words: Hypoadrenocorticism, Dog, Heritability

## Horse

**T106** Use of ass's milk for novel probiotic beverages. E. Salimei\*<sup>1</sup>, E. Sorrentino<sup>2</sup>, M. Succi<sup>2</sup>, F. Fantuz<sup>3</sup>, G. Varisco<sup>4</sup>, and R. Coppola<sup>2</sup>, <sup>1</sup>Dept. SAVA, Univ. of Molise, CB Italy, <sup>2</sup>Dept. STAAM, Univ. of Molise, CB Italy, <sup>3</sup>Dept. Sci. Vet., Univ. of Camerino, MC Italy, <sup>4</sup>Ist. Sperim. Zooprofilattico, Brescia Italy.

Nutritional and therapeutic properties of ass's milk are known since ancientry, and recent clinical studies confirm its efficacy in the treatment of most complicated cases of infants' multiple food intolerance. In order to deepen the knowledge on ass's milk and its feasible production, 6 asses were studied over two consecutive lactations (150 days/lactation). During the experimental period asses, machine milked, produced in average 740 mL milk/milking; milk yield was higher in the second lactation. Results on ass's milk chemical composition confirm the relative dilution of this product characterized by low fat (averaging 0.38%) and protein  $(1.72\%,\,\mathrm{in}~\mathrm{average})$  contents but with a high lactose content (mean value 6.88%). Protein fraction of ass's milk showed a low protein allergenic content along with a higher content of lysozyme (1.5 g/L). Moreover, due to its high lactose content, ass's milk could be placed amongst the new generation of milk beverages making it possible to effectively combine the advantageous properties of the raw ingredient with lactic acid bacteria. The high levels of lysozyme detected in raw and heat-treated ass's milk could explain the observed low bacterial concentration  $(4x10^4)$ CFU/mL) of raw milk. Besides this, pH values of control milk (heattreated and uninoculated) were stable throughout the experiment (15d), confirming the possible role of lysozyme. Ass's milk revealed to be a good growth medium for potentially probiotic lactobacilli strains since pH ranged between 3.67 and 3.85 for all the tested strains after a 48 hour period of incubation. Values were stable up to the end of the experiment. Results evidenced that ass's milk can be adopted as a substrate of probiotic and therapeutic beverages.

Key Words: Ass's milk, Probiotic beverages, Hypoallergenic food

**T107** The influence of training on flat walking temporal variables of Tennessee Walikng Horse yearlings. K. M. Holt\* and M. C. Nicodemus, *Mississippi State University, Mississippi State, MS*.

Tennessee Walking Horse (TWH) gaits are often described as learned, placing great importance on the training of young horses. To determine the impact of training on yearling TWH gaits, temporal variables were measured before and after a 60-day training period. 60 Hz frame-byframe analysis was done before and after training to measure temporal variables and kinematic analysis determined velocity and wither height. Five strides of a consistent flat walk with a velocity of 1.7 (0.2) m/s were measured for 4 yearlings. Means (SD) were determined with temporal variables given as % of stride and paired t-tests (P=0.05) were used to determine differences before (T1) and after (T2) the training period (Table 1). The flat walk was determined to be a symmetrical, 4-beat gait with an irregular rhythm and lateral couplets. The stride alternated between periods of bipedal and tripedal support in which the lateral bipedal support was longer than the diagonal. The majority of the stride was spent in a stance phase with a similar percent of stance for both the fore and hind limbs. These characteristics did not significantly change after the training period. Flat walking weanlings were found to have similar characteristics as the yearlings, except for some of the weanlings demonstrated regularity of rhythm with an equal percentage of lateral and diagonal bipedal support. This may indicate more of a relationship between growth and temporal variables, rather than training. Understanding the influence of such variables as growth and training on TWH gaits will assist in both clinical and performance applications.