
BREEDING AND GENETICS: COMPANION ANIMAL NUTRITION

0969 (T055) Influence of velocity on Weimaraner trotting stride mechanics. L. Carlisle¹, M. C. Nicodemus^{*1}, and K. Slater², ¹Mississippi State University, Starkville, ²Banfield Pet Hospital, Houston, TX.

The large size of the Weimaraner assists in the breed performing the tasks of a sporting dog. However, the size may also make the breed more susceptible to hip dysplasia. Nevertheless, the Weimaraner compared to other large dog breeds demonstrates a lower rate of dysplasia, which may be due to the preferred slower speed of this breed and resulting stride mechanics. Although kinematic research has been done on other large breeds, gait analysis of the Weimaraner is lacking, and thus, the objective of this study was to determine the influence of velocity on the trotting stride mechanics of the Weimaraner. Six American Kennel Club (AKC) registered Weimaraner dogs were led by the same handler at a slow (st velocity: 1.2–1.7 m/s) and fast (ft velocity: 1.9–2.3 m/s) trot on even, natural footing. Strides ($n = 10$) were selected for each dog for each trot based on soundness and correctness of gait, consistency of speed, and noticeable foot placement and lift-off. Stride variables were determined by frame-by-frame analysis with video frames of foot placement and lift-off documented. Stride variables were given as a % of stride. Means (SD) were determined for stride variables and t tests ($P < 0.05$) were performed between velocities. Both trots were produced with a diagonal footfall sequence and a similar stride duration (st- 0.53 ± 0.10 msec, ft- 0.47 ± 0.07 msec) and frequency (st- 1.94 ± 0.13 str/sec, ft- 2.12 ± 0.09 str/sec), alternating between periods of diagonal bipedal support (st- $88 \pm 2\%$, ft- $75 \pm 5\%$) and suspension (st- $12 \pm 1\%$, ft- $25 \pm 3\%$) with less than half of the stride cycle of each limb spent in stance (Fore: st- $44 \pm 4\%$, ft- $38 \pm 2\%$; Hind: st- $44 \pm 5\%$, ft- $38 \pm 4\%$). Although stride length significantly increased with speed (st- 0.90 ± 0.02 m, ft- 0.99 ± 0.04 m; $P < 0.05$), the diagonal limbs remained paired on contact (diagonal advanced placement: st- $0 \pm 0\%$, ft- $0 \pm 0\%$). The use of suspension at all velocities and the dependence of stride lengthening to increase velocity are distinguishable characteristics of the Weimaraner from other large breeds studied including the Labrador Retriever and German Shepherd. Understanding of canine locomotion through the analysis of the stride mechanics as was done in this study will assist in the clinical examination of gait and the assessment of veterinary locomotive rehabilitation.

Key Words: kinematics, trot, Weimaraner

0970 (T056) Effects of dietary resistant starch on the fasted plasma metabolome of healthy adult dogs. A. N. Beloshapka^{*1}, K. L. Pappan², and K. S. Swanson¹, ¹Dep. of Animal Sciences, University of Illinois, Urbana, ²Metabolon, Inc., Durham, NC.

Fermentable carbohydrates alter gut microbial activity and metabolite production, with many entering the bloodstream and consequently impacting host physiology. The effects of feeding fermentable carbohydrates, such as resistant starch (RS), on the canine plasma metabolome have not been well-studied. The objective of this study was to use a high-throughput metabolomics platform to identify differences in the fasted plasma metabolome of dogs fed increasing RS concentrations. Seven dogs (mean age = 5.3 yr; mean BW = 20 kg) were randomly allotted to one of three treatments (0%, 2%, or 4% high-amylose maize cornstarch (HI-MAIZE260) in an incomplete Latin square design. Treatments were formulated to be iso-energetic and consisted of graded amounts of 100% amylopectin cornstarch, RS, and cellulose, and fed as a top dressing on the food each day. All dogs were fed the same amount of a basal diet throughout the study and fresh water was offered ad libitum. Blood samples were collected after an overnight fast via jugular venipuncture on the last day of each treatment period (d 21) and were subjected to liquid/gas chromatography and mass spectrometry. A total of 288 named biochemicals were identified in plasma, but few statistical differences were noted among treatments. Compared to the controls, RS consumption appeared to alter amino acid metabolism, marked by increased ($P < 0.10$) plasma N-acetylglutamine, 3-hydroxy-3-phenylpropionate, and α -hydroxyisovalerate concentrations and decreased ($P < 0.05$) plasma kynurenine and kynurenate concentrations. Compared to controls, RS consumption also appeared to alter fatty acid and bile acid metabolism, marked by increased ($P < 0.05$) plasma 2-hydroxyglutarate concentrations and decreased ($P < 0.05$) plasma stearamide, cholate, and 1-pentadecanoylglycerophosphocholine concentrations. While RS supplementation produced some changes on the canine plasma metabolome, large, consistent changes were not observed.

Key Words: canine, plasma metabolome, resistant starch

0971 (T057) In vitro effect of diets added with fructooligosaccharides and differing in their protein content and digestibility on dog fecal microbiota. G. Biagi^{*}, M. Grandi, and C. Pinna, Dep. of Veterinary Medical Sciences, University of Bologna, Ozzano Emilia, Italy.

Feeding dogs with diets containing high amounts of low-digestible protein may negatively affect the animal intestinal ecosystem, increasing the presence of undesired proteolytic

bacteria. On the other hand, the administration of prebiotics can enhance the activity of beneficial bacteria residing in the canine intestine. The objective of the present study was to evaluate in vitro the effect of diets differing in their protein content and digestibility, and containing or not fructo-oligosaccharides (FOS), on canine fecal microbiota. There were six treatments: 1) Low-protein diet (LPHD, 23% CP); 2) High-protein diet (HPHD, 30% CP); 3) High-protein low-digestible diet (HPLD, 30% CP); 4) Diet 1 + 1.5% FOS (LPHD+FOS); 5) Diet 2 + 1.5% FOS (HPHD+FOS); 6) Diet 3 + 1.5% FOS (HPLD+FOS). After enzymatically digestion, the undigested fraction of the diets was added to a canine fecal suspension and incubated for 24 h in an anaerobic cabinet (five bottles per diet). From each bottle, a sample of fermentation fluid was collected at 6 and 24 h of fermentation for microbial counts (by FISH) and chemical analyses. Data were analyzed by three-way ANOVA, with protein level and digestibility and FOS as the main effects. At 24 h, FOS resulted in lower ammonia (36.4 vs. 40.3 mmol/l; $P < 0.001$) and iso-valeric acid (0.52 vs. 1.44% of total volatile fatty acids (VFA); $P < 0.05$) and higher total VFA concentrations (47.1 vs. 32.9 mmol/l; $P < 0.001$). Conversely, LD diets resulted in lower VFA (33.9 vs. 43.1 mmol/l; $P < 0.001$). The presence of FOS induced a shift in VFA production, reducing acetic acid (57.1 vs. 73.8%; $P < 0.001$) and increasing propionic and *n*-butyric acids proportions (20.9 vs. 16.4% and 21.0 vs. 7.5%, respectively; $P < 0.001$). With regard to biogenic amines, putrescine concentrations were increased at 6 and 24 h of fermentation by LD diets (+21 and 22%, respectively; $P < 0.05$) and FOS (+18 and 24%, respectively; $P < 0.01$). After 24 h of fermentation, HP diets resulted in lower counts of lactobacilli and enterococci (-0.5 and -0.7 log cells/ml, respectively; $P < 0.05$), whereas LD diets tended to increase counts of *C. perfringens* (+ 0.2 log cells/ml; $P = 0.07$). Results from the present study showed that diets rich in low-digestible protein may exert a negative influence on the canine intestinal ecosystem, increasing the presence of proteolytic compounds and reducing counts of beneficial bacteria. Conversely, administration of FOS may improve canine intestinal health, reducing proteolysis and enhancing VFA production.

Key Words: dogs, fructo-oligosaccharides, dietary protein

0972 (T058) The modified Atwater equation does not accurately predict diet ME value of premium food in adult cats.

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The diet ME value of pet foods is commonly predicted using the Atwater equation, because routine ME measurements are not practical or not feasible ethically or financially. As such, AAFCO recommends the use of the modified Atwater equation [$ME = 3.5 \times CP (\%) + 3.5 \times N\text{-free extract (NFE, \%)} + 8.5 \times \text{crude fat} (\%)$] to predict metabolizable energy (ME) of

dog and cat foods, which is then used to calculate daily feeding allotment. Previously, the modified Atwater equation underestimated the measured ME value of dog diets; hence, we decided to study this equation in cats. Twelve domestic shorthair cats were used in a quadruple 3×3 Latin square. Initial BW averaged 4.4 ± 0.8 kg and ranged from 3.0 to 5.7 kg. Three premium cat diets varying in predicted glycemic index (GI) based on ingredient composition and starch content (36.8, 30.7, and 23.6% for high, medium, and low GI, respectively) were fed for 10 d, with feces and urine collected quantitatively for the last 5 d. Diet, feces and urine were analyzed for GE to measure diet ME value. Predicted GI and ME value of the three diets were inversely related. The greater ME value of the low GI diet ($P < 0.001$) was associated with its greater fat content (22.9, 22.2, and 15.7% ether extract for low, medium and high GI diet, respectively) and energy digestibility (95, 95.4, and 92.9% for low, medium and high GI, respectively). The modified Atwater equation underestimated measured diet ME values by 12% (11.9, 10.8, and 13.6% for high, medium, and low GI, respectively). The traditional Atwater equation [$ME = 4 \times CP (\%) + 4 \times N\text{-free extract (NFE, \%)} + 9 \times \text{crude fat} (\%)$] did predict diet ME values accurately (underestimation of 2.0, 1.5, and 4.6% for high, medium, and low GI, respectively). In conclusion, the modified Atwater equation did not accurately predict diet ME value of diets fed to adult cats. The traditional Atwater equation should be used for premium cat diets.

Key Words: metabolizable energy, Atwater, cat

0973 (T059) Association of idiopathic epilepsy with a novel locus in the Belgian Shepherd.

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Idiopathic epilepsy in the Belgian shepherd dog is complexly inherited. The objective was to identify novel genomic loci associated with the expression of generalized seizures in the Belgian Tervuren and Sheepdog. Dogs were classified as cases if they met specific seizure criteria and controls if they were over 7 yr of age and had no other health disorder. DNA from cases ($n = 35$) and controls ($n = 58$) from dogs predominantly unrelated at the grandparent level, were subjected to a high-density genotyping array consisting of more than 170,000 evenly spaced single nucleotide polymorphisms (SNPs). Association analyses were conducted using the software package PLINK. Based on 100,000 permutations and removal of non-informative markers and markers with low call rate, five chromosomal regions reached genome wide significance: chromosomes 5 ($P < .034$), 7 ($P < .015$), 24 ($P < .0003$), 29 ($P < .020$) and 37 ($P < .05$). Chromosome 7 showed four significant SNPs between 46,094,658 and 49,666,725 bp (CanFam 3.1). A Sequenom iPlex analysis was conducted using 34 dogs representing 17 cases and 17 controls. Sequenom Assay Design Suite was used to multiplex 65 SNPs covering the region between 45.03 and 48.06 MB. Three SNPs demon-

strated genome wide significance in three genes: two were intronic SNPs ($P = 0.00001$ and $P = 0.04548$) and the third was a missense SNP ($P = 0.0061$) in a novel gene. The mutation in the novel gene changes the codon from proline to arginine. For the novel gene, a 344-bp region was targeted for resequencing uncovering a second missense SNP within the novel gene. The genome wide analysis confirmed the previously characterized locus found on chromosome 37 and revealed a second locus segregating for epilepsy on chromosome 7. The missense mutation in a novel gene validates the model of a multifactorial genetic regulation of idiopathic epilepsy for the Belgian shepherd, while also suggesting the existence of a previously unidentified neurological regulatory gene. Taken together, the data support the application of genetic selection to reduce the prevalence of this debilitating disorder.

Key Words: idiopathic epilepsy, canine, genome wide association

0974 (T060) Amino acid and mineral concentrations of whole grains and grain byproducts used in pet foods.

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Whole grains may be valuable components of canine diets, due to the functional ingredients, such as dietary fiber and β -glucans, or the amino acids (AA) and minerals they provide; however, the use of whole grains in pet food has not been thoroughly evaluated. Our objective was to measure the AA and mineral concentrations of various whole grains, processed grains, and grain byproducts that may be incorporated into dog food. Thirty-one grain samples, including: rice samples (brown rice, rice, rice bran, and rice flour); barley samples (barley flake, cut barley, ground pearled barley, malted barley, whole pearled barley, pearled barley flakes, and steamed rolled barley); oat samples (groats, ground oatmeal, ground steamed groats, instant oats, oat bran #1, oat bran #2, oat fiber, oat flour, quick oats, regular rolled oats, steamed rolled oat groats, and steel cut groats); and miscellaneous cereals and pseudocereals (conventional hulled millet, conventional whole millet, conventional quinoa, organic spelt hull pellets, potato flake, sorghum, whole wheat, and whole yellow corn) were analyzed. Total essential (0.65 to 7.51% DMB) and individual AA concentrations were highly variable among ingredients tested: Arginine (0.01 to 1.38% DMB), Histidine (0.02 to 0.47% DMB), Isoleucine (0.07 to 0.62% DMB), Leucine (0.15 to 1.49% DMB), Lysine (0.06–to 0.88% DMB), Methionine (0.03 to 0.36% DMB), Phenylalanine (0.08 to 0.77% DMB), Threonine (0.05 to 0.66% DMB), Tryptophan (< 0.04 to 0.20% DMB), and Valine (0.09 to 0.96% DMB). Of the ingredients tested, oat fiber had the lowest concentrations of most essential AA and rice bran had the highest concentrations of most essential AA. Calcium, phosphorus, and magnesium concentrations ranged from 0.0 to 2.22% DMB, 0.04 to 2.03% DMB, and 0.03

to 0.88% DMB, respectively, with rice bran having the highest concentrations of all three minerals. Based on our compositional analysis, whole grains and grain byproducts vary greatly in AA and mineral content. Although these ingredients are commonly used in canine diets, more research is needed to test the effects of feeding whole grains to dogs.

Key Words: whole grains, amino acids, minerals

0975 (T061) Metabolic phenotyping using mass spectrometry-based metabolomics: A cross-sectional pilot study of lean and overweight domestic cats.

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Overnutrition and sedentary behavior increases adiposity in domestic cats, a consequence of positive energy balance that can be detrimental to health. Adiposity is a risk factor for feline diabetes mellitus, characterized by insulin resistance. Chromatography and mass spectrometry (MS) technologies in the field termed “Metabolomics” may be a means to identify biomarkers for predicting pre-onset insulin resistance. The objective was to evaluate MS, coupled with gas chromatography (GC) or liquid chromatography (LC), techniques to perform metabolic phenotyping in lean and overweight domestic cats in preparation for a large-scale study aimed at identifying predictive biomarkers for feline insulin resistance. To evaluate methods, lean ($n = 6$; ≤ 4.5 kg) and overweight ($n = 6$; ≥ 6.5 kg), adult (2 to 10 y of age), neutered, client-owned male domestic short-hair cats were enrolled in a cross-sectional pilot study. Cats were not diagnosed with diabetes. Plasma samples were collected by a veterinarian following an overnight fast. For GC/MS, plasma metabolites were methanol extracted, derivatized, and analyzed by a GC tandem MS system. For LC/MS, plasma metabolites were chloroform-methanol extracted and analyzed by high pressure LC coupled to an electrospray ionization source of a triple quadrupole tandem MS system. Normalized data were analyzed using Student’s t test. For metabolomics data, statistics were performed on the log of the normalized, median-scaled data. To validate analytical methods, total free fatty acids (FA) and glucose were determined by colorimetry. Mean weights and ages for lean and overweight cats were 3.9 ± 0.5 and 7.1 ± 1.0 kg ($P < 0.05$), and 4.2 ± 2.6 and 6.8 ± 2.4 y of age ($P > 0.10$), respectively. Untargeted GC/MS analysis detected 40 metabolites including lactate, urea, glycerol, amino acids, citrate, saccharides, saturated and unsaturated free FA, uric acid, vitamin E, and cholesterol. Overweight cats had a nearly two-fold increase in palmitic acid, stearic acid, and oleic acid ($P < 0.05$), results validated by an 83% increase in total free FA ($P < 0.05$). In overweight cats, glucose levels were increased by 38% (MS; $P < 0.05$) and a similar nonsignificant numerical increase when determined by colorimetry. Cholesterol, vitamin E, and glycerol were elevated in

overweight cats ($P < 0.05$). Targeted LC/MS analysis detected 28 sphingolipids including ceramides, monohexylceramides, and dihexylceramides. Of interest, palmitoyl-linked ceramide level was increased 80% in overweight cats ($P < 0.05$); elevated plasma ceramides are associated with insulin resistance progression in humans. In overweight cats, GC/MS and LC/MS methodologies revealed metabolite phenotypes similar to patterns observed in diabetic humans.

Key Words: feline diabetes mellitus, metabolomics, obesity

0976 (T062) Effects of dietary energy restriction on the hunting behavior and home-range size of free-ranging domestic cats. A. N. DeGrave*, S. K. Carignan, and S. E. Kitts-Morgan, *Berry College, Mount Berry, GA.*

Free-ranging cats pose concern for native small mammals and birds due to their reputation as instinctual predators. Hunting and prey consumption may be altered by food amounts received from humans. This study's objective was to determine the effects of dietary energy restriction on hunting behavior and home-range size of free-ranging cats. Eight free-ranging cats (five female, three male) residing at the Berry College Equine Center were blocked by weight and assigned randomly to receive daily ME requirements for adult cats at maintenance (control; $n = 4$) or 80% of daily ME requirements (restricted; $n = 4$). For 11 mo, cats were individually offered commercial cat food daily with additional colored glitter to identify each cat's feces. Cats were fitted with harnesses and GPS units to construct home ranges. Feline feces were dried at 60°C and analyzed for prey teeth, total hair, and cat vs. non-cat hair. No differences in BW ($P = 0.99$) or DMI ($P = 0.66$) occurred throughout the experiment. Home ranges were constructed using 95% kernel density estimation and analyzed by season. While no differences in home-range size occurred during spring or summer, fall home-range was larger ($P = 0.04$) for control cats (7.35 ha; 95% CI 4.72 to 11.43 ha) compared to restricted cats (3.91 ha; 95% CI 2.67 to 5.73 ha). Likewise, winter home-range was larger ($P = 0.04$) for control cats (6.91 ha; 95% CI 4.71 to 10.12 ha) compared to restricted cats (3.92 ha; 95% CI 2.68 to 5.75 ha). Of 306 fecal samples identified by cat, a similar ($P = 0.83$) percentage of feces from control vs. restricted cats contained hair (92.05% vs. 92.9%). There was a tendency ($P = 0.11$) for total hair weight in control-cat feces (0.55 ± 0.058 g) to be greater than total hair weight in restricted-cat feces (0.42 ± 0.057 g). However, restricted-cat feces had a lower percentage (18.1%; $P = 0.003$) of non-cat hair compared to the percentage (33.1%) of non-cat hair in control-cat feces. The percentage (21.85%) of control-cat feces containing prey bones/teeth was greater ($P < 0.0001$) compared to the percentage (6.45%) of restricted-cat feces containing prey bones/teeth. However, there was no difference ($P = 0.82$) in total bone/teeth weight for control-cat feces (0.061

± 0.008 g) compared to restricted-cat feces (0.065 ± 0.015 g). These results suggest that cats will not expand home-range or increase prey consumption when energy-restricted. It may be possible that further restriction of energy intake might affect home-range size and hunting of free-ranging cats.

Key Words: cats, hunting, energy

0977 (T063) Differences in the cerebral cortex metabolome of young adult and geriatric dogs.

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Aging is responsible for chemical and morphological alterations in the brain (e.g., decreased brain mass, increased ventricular size, demyelination, neuroaxonal degeneration, decreased cholinergic activity, etc.) that lead to cognitive decline and neurodegenerative diseases. The longer lifespan of the canine pet population has increased the prevalence of cognitive dysfunction. A better understanding of the brain aging process would aid in preventing or reversing the progression of cognitive decline and improve the quality of life of geriatric dogs. Therefore, the objective of this experiment was to use untargeted GC-LC-MS to assess the impact of age on the metabolome of the cerebral cortex of dogs. Cerebral cortex samples were collected from 12 geriatric (12-yr-old) and 12 young adult (1-yr-old) beagles and stored at -80°C until GC-MS and LC-MS/MS analyses. A total of 239 named biochemicals were identified, with 101 being altered ($P < 0.05$) geriatric and young adult dogs. Prior transcriptomics analysis of these samples showed that 963 genes were altered due to age, with old dogs having increased expression of genes associated with inflammation, stress response, and calcium homeostasis and decreased expression of genes associated with neuropeptide signaling and synaptic transmission. In the current study, the cerebral cortex of geriatric dogs had a relative deficiency of excitatory amino acids, such as glutamate and γ -aminobutyrate (GABA), compared to young adult dogs. In addition, geriatric dogs had increased concentrations of palmitoylethanolamide (PEA), suggesting greater levels of oxidative stress or inflammation in the brain of these animals. Glutathione metabolism also differed ($P < 0.05$) between geriatric and young adult dogs, with the former having greater concentrations of reduced glutathione. A relative hyperglycemia in the cerebral cortex of geriatric dogs seemed to drive elevations in glycolytic pathway intermediates; lactate and sorbitol which, over long periods of time, could drive undesirable pathological events such as protein glycation and aggregation. Overall, the data suggest that the brains of geriatric dogs have reduced neurotransmitter metabolism and increased inflammation, possibly contributing to the altered neural functional capacity and health status of these animals.

Futures studies should investigate whether a metabolic signature of the aging brain may be detected in the plasma or serum of geriatric dogs. Identification of circulating metabolic biomarkers would allow for more frequent sampling using a non-invasive method and be useful in disease diagnosis and the development of nutritional interventions.

Key Words: age, dog, metabolome

0978 (T064) Use of gelatin as a strengthening agent in dry extruded pet food. A. Simmons^{*1}, C. G. Aldrich¹, T. Zhou¹, M. Remund¹, T. Putarov², S. Alavi¹, E. Maichel¹, and C. K. Jones¹, ¹*Kansas State University, Manhattan*, ²*Sao Paulo State University, Sao Jose do Rio Preto, Brazil*.

Recent pet food innovations have increased protein and decreased cereal inclusions in diets, which has diminished product durability and negatively changed texture. Low-bloom gelatin is a pure protein that is used to improve some pelleted feeds. The objective of this project was to determine the effect of low bloom gelatin (Pro-Bind Plus 100) on kibble physical properties. Two experiments were conducted on a Wenger X-20 single screw extruder. In Exp. 1, a total of six treatments were extruded: 0, 5, 10, or 15% gelatin inclusion at 400 RPM and 15% gelatin inclusion at 300 or 500 RPM. Chicken by-product meal was removed to add gelatin and maintain an iso-starch formulation. In Exp. 2, a total of six treatments were extruded: 0 vs. 10% gelatin inclusion; 300 vs. 500 RPM; and 15 vs. 30% hydration ratio, meaning the ratio of water

added in the extruder vs. the preconditioner. Extrudates were analyzed for moisture, expansion ratio, specific length, piece density, hardness (TA-XT2, Stable Micro Systems), and pellet durability index (PDI; Holmen NHP 100, Tekpro). Results were analyzed using the GLIMMIX procedure of SAS. Product hardness showed a good positive correlation with PDI. Hardness and PDI improved with gelatin inclusion ($P < 0.05$; 5.15 vs. 9.35 kg; 64.5 vs. 96.9%). It was surmised that the increase in kibble strength and durability was caused partially by the strengthening effect of gelatin on the solid matrix. Lower product expansion also had an important role in increasing hardness and durability, as radial expansion ratio increased at 10% gelatin inclusion ($P < 0.05$; 4.27 vs. 6.65 mm²/mm²) but decreased at 5 and 15% gelatin inclusion ($P < 0.05$; 4.27 vs. 3.31 or 2.40 mm²/mm², respectively). Increase in screw speed from 300 to 400 rpm and decrease in hydration ratio from 30 to 15% (implying greater degree of pre-conditioning) led to a slight increase in expansion ratio, suggesting that altering processing parameters may overcome the negative impact of gelatin on radial expansion. Additionally, specific length (a measure of longitudinal expansion) increased at 15% gelatin inclusion ($P < 0.05$; 42.11 vs. 48.30 mm/g), and as less of the total water content was added in the extruder ($P < 0.05$; 37.62 mm/g vs. 41.16). These results suggest that gelatin had a binding effect on finished product. This binding may be helpful in high protein formulation, especially those reducing the use of functional cereal grain starches.

Key Words: gelatin, pet food, extrusion