

Zignature® Original Turkey Formula for dogs AAFCO Feeding Trial for Assessing the Nutritional Adequacy and Overall Health of Dogs on this Diet

Purpose

Long term feeding studies are generally accepted as the best way to ensure nutritional performance of pet diets. However, feeding studies are not required for companies to manufacture and market pet food with a complete and balanced claim. It has been determined that that the AAFCO (American Association of Feed Control Officials) six month feeding study protocol can serve as a template for a more comprehensive health assessment of pets eating a specific diet over time. The value of doing feeding studies for a brand is many fold. They can give the pet parent confidence that a pet food manufacturer validates how a diet performs when fed. These feeding studies may also help gain recommendation of diets from veterinary and pet nutrition professionals. But most importantly, brands that do feeding studies with their diets show the commitment they have to claims' substantiation when performing long term feeding studies. Historically, companies have not done long term feeding studies because of the time involved, associated costs with feeding studies, and that there is no requirement for such by regulatory agencies. Some pet food brand may not do feeding studies because typically they are typically done with purpose-bred dogs. Long term feeding studies should be performed by pet food companies and using the AAFCO feeding study substantiation protocol as a basis, a much more comprehensive and rigorous feeding study design can be achieved.

The AAFCO official publication allows for formulation substantiation to meet the nutritional requirements of dogs. This is how most pet foods substantiate nutritional adequacy. In the alternative, an AAFCO feeding study may be used to validate a complete and balanced claim. The standard AAFCO feeding study protocol requires eight dogs and is six months long.

The standard AAFCO protocol requires the measurement of only four blood parameters including hemoglobin, packed cell volume, albumin, and serum alkaline phosphatase at the end of the study. Another requirement of an AAFCO study is weekly body weights taken of the dogs on the study in which no dog may lose more than 15% of their body weight from the beginning to end of the study. In addition an average weight loss of all the dogs on the study cannot exceed greater than 10% of the body weights from when the study began. There is no requirement as to how much of the diet is fed to each dog on the study. Body condition score, activity level, environmental conditions and neuter status are not considered when determining the amount of diet each individual dog should be offered. This potentially could lead to under or over feeding dogs on the study.

Pets Global, Inc. has performed a comprehensive long term feeding trial with Zignature® Original Turkey Formula for dogs. The study was designed by using a six month long AAFCO feeding study as the basis and adding many additional measurements as health indicators. Privately owned sporting dogs that resided in their own residence were the participants in this feeding study. In addition to the standard AAFCO study protocol other health assessments were performed prior to, at the midway point and at the end of the study. These included complete blood counts, serum chemistry panels, plasma amino acid profile, whole blood taurine, cardiac biomarker testing, body condition scores, and muscle condition scores. In addition a licensed veterinarian performed complete physical exams on all dogs at the same three timepoints of the study. Echocardiograms were also performed on all dogs prior to study start and at the completion of the study. Weekly stool quality scores and body weights were recorded by veterinary professionals and the amount of diet offered each dog was evaluated weekly to maintain ideal body weights based on history and activity level of each dog

Scope

The timeframe of this study was from May 2022 through November 2022. The study participants consisted of six English Pointers (5 males and 1 male) as well as two female Brittanys. The average age of all the dogs on this study was 4 years and average weight of all the dogs was 23 kg. All of the dogs on the study were from the same household and were current on vaccines, heartworm preventative and flea and tick preventative. A complete veterinary examination at the beginning of the study yielded results of good health for all the dogs. On a weekly basis body weight, stool quality checks and food intake evaluations were performed by veterinary professionals. If necessary, food intake adjustment was made with the goal to keep each dog at their ideal body weight. Prior to study start, midway and at the end of the study complete blood counts, serum chemistry panels, plasma amino acid profiles, whole blood taurine levels, and cardiac biomarkers were performed. Echocardiograms were performed prior to study start and at study completion to evaluate cardiac health as well.

Diet

Prior to the study, all of the dogs had been eating a commercial diet that met AAFCO requirements for a complete and balanced claim for adult maintenance. The study diet, Zignature® Original Turkey Formula for dogs is formulated to meet AAFCO requirements for all life stages of the dog. Turkey is the primary protein in this diet and it also contains chickpeas, peas flaxseed, turkey fat and is fortified with essential vitamins, minerals and probiotics. An AAFCO nutrient profile was performed on the same production lot of food as was fed throughout the entire study. The test diet met all AAFCO requirements for an all life stage diet.

The Zignature® Original Turkey Formula diet was the sole source of nutrition for all dogs throughout the study, Clean, fresh tap water was available *ad libitum* throughout the study. Each individual dog was fed based on its metabolic energy requirements which considers each dog's ideal body weight, each dog's body condition scores, neuter status as well as activity level. The dogs were fed twice per day as this was their usual feeding routine in this household. Daily caloric intake was calculated: $(BW \text{ (kg)})^{0.75} \times 70$. Due to their high activity level a daily energy requirement factor of 3.5 was used. The average kcal consumption was 2,296 kcal/day/dog. The test diet contained 3,600 kcal/kg.

Analysis

Blood Collections

Prior to the start of the study, at the midway point and at the end of the study fasted blood samples were collected via cephalic venipuncture using a sterile syringe. The blood volume of twelve milliliters (mL) collected at each timepoint and from each dog and was placed into five sterile blood tubes. Whole blood and serum for complete blood count, serum chemistry panel, heartworm antigen test, and cardiac troponin I were shipped chilled overnight to Idexx (Memphis, TN). Whole blood and plasma was shipped frozen to the University of California at Davis, Veterinary Medicine, Molecular Bio Sciences (Davis, CA) for whole blood and plasma taurine and plasma amino acid profiles. Frozen plasma was overnight shipped to the University of California at San Diego, Biomedical Genetics Laboratory for quantitative carnitine analysis.

Complete blood count assays included white and red blood cell counts, hemoglobin (Hgb), hematocrit, mean corpuscular value, mean corpuscular Hgb concentration, mean concentration Hgb, red cell distribution width percentage, reticulocyte count, reticulocyte Hgb,

platelet count, and complete white blood cell differential. The serum chemistry profiles that were performed included symmetric dimethylarginine (SDMA), total thyroxine (T4), glucose, total protein, alkaline phosphatase, cholesterol, calcium, potassium, blood urea nitrogen (BUN)/creatinine ratio, BUN, creatinine, albumin, alanine aminotransferase, phosphorus, chloride, bicarbonate, anion gap, globulin, total bilirubin, bilirubin unconjugated, bilirubin conjugated, aspartate transaminase, creatine kinase, sodium, sodium/potassium ratio, albumin/globulin ratio, gamma-glutamyltransferase, lipase, amylase, and lipemia and hemolysis index).

Physical Examinations and Observations

Complete veterinary exams performed by a licensed veterinarian were conducted at the beginning, middle and end of the study on all dogs. In addition, weekly body condition scores and stool quality scores were also performed by veterinary professionals. No abnormalities were noted on any of the examinations throughout the study. All dogs were observed by their pet guardians a minimum of twice daily for any signs of pain, stress, or distress. As this feeding study was performed with active sporting dogs each dog participated in their usual training and activities throughout the study period. All dogs performed well in their training and activity as attested by their pet guardian.

Summary

Complete Blood Count

Complete blood counts evaluate both red and white blood cell parameters. Each dog's complete blood count remained within the reference ranges throughout the study for all three bloodwork timepoints during this feeding study. No dogs participating in this study displayed symptoms of illness or abnormalities during this feeding study.

Serum Chemistries

At the same three timepoints serum chemistry evaluations were performed on all dogs. Serum chemistry profiles can help evaluate functions of liver, kidney and pancreas as well as serum electrolytes. A few dogs exhibited transient values outside of the reference range however no values were consistently outside of the reference range on multiple timepoints. This is not an uncommon finding in even clinically healthy dogs. Each dog had a negative heartworm antigen test prior to the beginning of the study.

Cardiac and Thyroid Biomarkers

Prior to starting the study, in the middle and at the end of the study serum was evaluated for the cardiac biomarker troponin I and it remained normal at each timepoint on each dog through the study. Troponin I is a cardiac muscle specific protein that can be an indication of myocardial damage if it is increased above the normal value of the reference laboratory. The thyroid hormone thyroxine (T4) was measured. All dogs at all three timepoints had T4 levels that were within the reference range of the laboratory.

Plasma Amino Acids

At the beginning of the study whole blood taurine was within the normal reference range for all dogs and was above the reference range in all but two dogs at two different timepoints which were within the reference range. Plasma taurine on all dogs was within the reference range at the beginning of the study for all dogs and above the reference range for all dogs at the other two timepoints. The plasma levels of the essential amino acids methionine, threonine, valine, leucine, isoleucine, phenylalanine, lysine, histidine, tryptophan and arginine remained consistent over the three timepoints taken in this study as there is not specific reference range for the dog.

Plasma carnitine levels were evaluated at the same three timepoints and no significant changes were noted on all dogs throughout the three timepoints during the study. Carnitine can help cardiac muscle utilize fat as energy. Carnitine levels may also indicate bioavailability of the amino acids lysine and methionine as well as other nutrients that are used to biosynthesize carnitine in dogs.

Echocardiograms

Echocardiograms are the best diagnostic test to evaluate cardiac function in a long term feeding study. Echocardiograms were performed by a licensed veterinary ultrasonographer and then evaluated by a board-certified veterinary cardiologist prior to the study start and at the end of the study. All dogs on this study had no significant changes in cardiac chamber size or in the cardiac functions measured from the beginning to the end of the study suggesting each dog's heart function remained normal during the six months on the diet. study period

Physical Examination

Physicals exams performed by a licensed veterinarian on all dogs prior to study start, at the halfway point and the end of the study indicated no abnormalities. Body condition scores, muscle condition scores and body weights remained ideal for each individual dog, as measured throughout the study. Each dog's body condition score started and remained a 5 on a 9 point scale, which would be considered ideal. Each dog's stool quality score remained good throughout the entire study with no incidence of loose or excessively hard stools. The activity level of each dog remained excellent throughout the study as attested by the dogs' pet guardian.

Conclusion

Pets Global, Inc has completed a long term feeding study with the Zignature® Original Turkey Formula for dogs and this satisfies the requirements of an AAFCO feeding study in adult dogs. This comprehensive feeding study monitored many health parameters to demonstrate that all dogs excelled on this diet throughout the study period. It should be noted that all dogs that participated in this study were athletic sporting dogs which is an excellent model to measure overall health of dogs eating this diet. This feeding study protocol conducted in privately owned dogs demonstrates the nutritional benefits of this diet. In home feeding studies such as this can be a valuable tool to objectively evaluate how dogs will perform when eating a diet during long term time periods. These types of feeding studies should lend confidence to pet guardians who choose to feed diets to their pets that have undergone studies such as this and achieved excellent results.

To conclude, a 26 week long-term feeding study with Zignature® Original Turkey has been successfully completed. This study not only satisfies the requirements for an AAFCO nutritional adequacy study but has shown results in these athletic sporting dogs that indicate that the diet delivered excellent nutrition under real world conditions.

Study coordinated and conducted under the guidance of:

Bradley Quest, DVM. Principal Veterinarian, BSM Partners, LLC

Stephanie Clark, PhD, CVT, PAS, CFS, Dpl. ACAS-Nutrition. Manager-Special Services, BSM Partners, LLC