

Zignature Select Cuts Trout & Salmon Meal Formula for dogs AAFCO Feeding Trial for Assessing the Nutritional Adequacy and Overall Health of Dogs on this Diet

Purpose

AAFCO (American Association of Feed Control Officials) six month feeding study protocol has been regarded by many pet nutrition experts as necessary to demonstrate a pet food manufacturer's commitment to good nutrition. However, other experts in the pet health industry believe that a standard AAFCO feeding study does not measure enough health parameters in pets to truly give an indication of the quality of the diet fed. Many brands choose not to do long term feeding studies. This may be due to AAFCO allowing nutritional substantiation through formulation and diet analysis therefore studies are not required, long term feeding studies come with high cost or that a company may recognize that the standard AAFCO feeding study protocol may be lacking enough health measurements to be meaningful. Other pet food companies may choose to not perform these feeding studies because they are generally done with purpose bred dogs. While a standard AAFCO feeding study can serve as good template, there is a need for a more comprehensive study protocol that examines many additional health parameters of dogs participating in the study.

AAFCO defines that nutritional substantiation may be validated by formulation and analysis or in the alternative a nutritional adequacy feeding study may be successfully completed. To do both would demonstrate the commitment that a pet food brand has to its customers. A standard AAFCO study is six months long, is done with eight healthy dogs at the beginning of the study. Most AAFCO feeding studies are performed with purpose bred dogs, however they may be performed with privately housed dogs as well. Feeding studies done with privately homed dogs which can indicate how dogs eating the diet perform in a home

environment. Standard AAFCO studies measure four blood health parameters: hemoglobin, packed cell volume, albumin, and serum alkaline phosphatase. The dogs are weighed weekly and their weights are recorded. The AAFCO protocol states that no individual dog may lose more than 15% of their body weight. An average weight loss of all dogs on the study must not exceed 10% of their initial body weight at the start of the study. There is no requirement as to how much food is fed to the dogs. However, best study practices would feed dogs an amount calculated for each dog's metabolic requirement. This requirement would be based on body condition score, muscle condition score, sex, age, neuter status and activity level.

A comprehensive long term feeding trial was performed with Zignature® Select Cuts Trout & Salmon Meal Formula for dogs. The study was commissioned by Pets Global, Inc. and was performed with privately housed sporting dogs. The long term feeding study used the AAFCO protocol as a basis but included many additional health parameters measured at the beginning, midway and end of the six month study. Included in this study were complete blood counts, serum chemistry panels, three plasma amino acid profile, whole blood taurine, cardiac biomarker testing, three complete physical examinations by a licensed veterinarian, body condition scores, muscle condition scores and echocardiograms (performed at the beginning and end of the study). A licensed veterinarian performed weekly weighs, health evaluations, stool quality scores and diet adjustment if needed. Any diet amount adjustment was based on body weight, body condition score and activity level at the time.

Scope

There were eight dogs participating in this feeding study from January 2023 through July 2023 and included six English Pointers (3 males and 3 females), 1 female Brittany and 1 male Brittany. The average age of the dogs on this study was 6.5 years and average weight of all the

dogs was 22 kg. All eight dogs were housed in the same residence. All dogs were current on vaccines, heartworm preventative and flea and tick preventative. A complete physical examination was performed by a licensed veterinarian at the study beginning and all dogs were in good health. Weekly body weights, stool quality scores, health observations were performed by a licensed veterinarian. Bloodwork was performed on day 0, 90, and 180 including complete blood counts, serum chemistry panels, plasma amino acid profiles, whole blood taurine levels, and cardiac biomarkers. Echocardiograms were done at the study beginning and also at the study end. Diet intake adjustments were performed if needed each week. Two dogs were removed from the study for non-nutritional health reasons.

Diet

All dogs had been eating the same diet before the study period. The previous diet was stated to be complete and balanced for adult maintenance per AAFCO guidelines. Zignature® Select Cuts Trout & Salmon Meal Formula for dogs is formulated to meet AAFCO guidelines for all life stages of the dog. The primary protein sources are salmon and trout and it also contains oats, millet and flaxseed. To validate nutritional adequacy prior to the study start, a complete AAFCO nutrient profile was performed at an external reference lab on the same production lot of food that all dogs consumed throughout the entire six month study and was the only source of nutrition throughout the study period. Dogs were fed according to their daily energy requirements. These energy requirements were calculated and took into account body weight, the dogs' activity level and neuter status. Food was offered once per day in the morning. Daily caloric intake was calculated: $(BW (kg))^{0.75} \times 70$. The eight dogs' activity level was high so a daily energy requirement factor of 3 was used. The average kcal consumption was 2,133 kcal/day/dog. The test diet contained 3672 kcal/kg.

Analysis

Blood Collections

At the beginning, middle and end of the blood samples were collected via cephalic venipuncture using a sterile syringe. Twelve milliliters (mL) of blood was collected at each of the three timepoints on 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.

The complete blood counts include (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 profile 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 indexes).

Physical Examinations and Observations

A licensed veterinarian performed physical examinations on all dogs at the beginning, midway, and the end of the feeding study as well as body condition and muscle condition scoring. A licensed veterinarian performed weekly body weights and stool quality evaluations. Physical examinations included evaluations of the eyes, ears, skin/hair coat condition, abdominal and lymph node palpation, evaluation of gait and general nerve reflexes. Heart and lungs were auscultated and no abnormalities were noted during each examination. Throughout the study dogs were observed throughout the day by their pet guardian for any signs of pain, stress, or distress. However, since all dogs continued to live in their own home environment this would be unexpected. All dogs on the study were active sporting dogs and continued their high activity throughout the entire study. Each dog performed well physically in their activities as reported by their pet guardian.

Summary

Complete Blood Count

Complete blood counts (CBC) evaluate red and white blood cells both quantitatively and qualitatively. A CBS can also indicate healthy function of blood cells in the parameters measured. No dog had any CBC parameters consistently outside of the reference range throughout the study. It is not uncommon to have even clinically healthy dogs experience slight deviations from the reference range occasionally on CBC measured blood cell levels. All samples at all time points indicated healthy dogs during the study.

Serum Chemistries

Internal organ function such as liver, kidneys, and pancreas were within the reference lab's reference range throughout the study. This indicates overall health of the dogs eating this diet. Each dog had a negative heartworm antigen test at the beginning of the study.

Cardiac and Thyroid Biomarkers

For the beginning, middle and end of the study each dog had serum levels for the cardiac biomarker troponin I evaluated. At all timepoints troponin I remained within the reference lab's reference range. Troponin I is a cardiac muscle specific protein that can be an indication of myocardial damage if elevated outside of the reference range. Thyroxine (T4) was also analyzed at the three blood collection timepoints and all dogs were within normal reference range throughout the study.

Plasma Amino Acids

Whole blood taurine as well as plasma taurine was within or above the laboratory reference range for all dogs at the throughout the three blood collection timepoints. Many dogs blood levels of taurine increased throughout the study. Cystine and methionine, which are sulfur containing amino acids used to biosynthesize taurine remained at consistent levels throughout the study. In addition, other essential amino acids such as threonine, valine, isoleucine, phenylalanine, lysine, histidine, tryptophan and arginine all remained consistent throughout the study.

Plasma carnitine levels were also measured and all dogs exhibited a steady value at all timepoints and in most dogs' plasma carnitine increased as the study progressed. Zignature® Select Cuts Trout & Salmon Meal Formula is fortified with both taurine and carnitine.

Echocardiograms

Echocardiograms were performed by a licensed veterinary ultra-sonographer at the beginning and end of the study. These echocardiograms were evaluated by a board-certified veterinary cardiologist. No dogs showed significant changes in measured cardiac parameters from the beginning to the end of this feeding study. Evaluations included measurements of chamber size and cardiac function.

Physical Examination

Physical examinations on dogs in this study were all within normal findings. These exams included evaluating the dogs as they did their sporting activities. Body weights, body condition scores, and muscle condition scores were all relatively unchanged during the study. Stool quality scores remained good throughout the study as reported by the pet guardian and observed by the attending veterinarian. The average body condition score on a nine point scale, as five which would be considered ideal. Eyes, ears, skin and haircoat remained healthy throughout. Activity levels remained at a high level in all dogs while eating this diet.

Conclusion

Pets Global, Inc has successfully completed a comprehensive long term feeding study with Zignature® Select Cuts Trout & Salmon Meal Formula for dogs. This long term feeding

satisfies the requirements for an AAFCO feeding study. However, many more health parameters were measured to be a true evaluation of the health of dogs eating this diet. Study protocols as was performed in this feeding study can evaluate how well active real world dogs will do when eating a tested diet. Feeding studies such as has been described here should be performed more often and done by more pet food companies. Companies that commit to doing feeding studies with their diets should be commended as they are not required. All physical exam criteria, bloodwork measured and cardiac evaluations performed throughout the study indicate that active dogs eating this diet will perform exceptionally.

Stool quality scores remained ideal which is a good indicator of how well a diet may be digested but is equally important for the pet guardian's perception of the diet quality as well. CBC, serum chemistry, cardiac biomarkers, carnitine, taurine and other amino acids all remained within the reference range or above throughout. Cardiac measurements performed at the beginning and end of the study showed no significant changes in cardiac function or size

In conclusion, Zignature® Select Cuts Trout & Salmon Meal Formula for dogs has successfully completed a long term 26-week feeding study that will satisfy the requirements for an adult dog nutritional adequacy AAFCO feeding study and additionally measured numerous other health parameters to indicate the high quality of this diet when fed to active sporting dog..

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