

ELISA Testing for Soy Antigens in Dry Dog Foods Used in Dietary Elimination Trials

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ABSTRACT

The use of elimination diet trials is necessary in the diagnosis of food allergies and intolerances. The objective of this study was to determine in vitro if four over-the-counter (OTC) dry dog foods carrying a “no soy” claim and seven veterinary therapeutic dry dog foods designed for food elimination trials were suitable for a soybean elimination trial. A 100 g sample of each diet plus one soy positive and one soy negative control diet were submitted for enzyme-linked immunosorbent assay testing to an independent food laboratory. The positive control diet contained >25 ppm soy protein antigens and the negative control contained <2.5 ppm. Three of the four OTC “no soy” claiming diets were positive for soy antigen. Two of the three soy-containing diets had >25 ppm. Three veterinary therapeutic diets had less than the lowest detectable limit of soy protein and four were positive (>2.5 ppm). OTC dog food diets that claim to contain “no soy” may contain high concentrations of soy protein and, therefore, should not be used in soy elimination trials in suspect food allergic dogs. The veterinary therapeutic diet selected for a soy elimination trial needs to be carefully chosen based on diet history. (*J Am Anim Hosp Assoc* 2014; 50:383–389. DOI 10.5326/JAAHA-MS-6063)

Introduction

Elimination diet trials are used to diagnose food allergies and intolerances. In an elimination trial it is imperative to know all the sources of protein in the diet. The consumption of either known or unknown suspected food allergen(s) during an elimination trial would either nullify or confound the results and prevent an accurate diagnosis.¹ Known consumption occurs when an owner gives unapproved treats, supplements, or medications during an elimination trial. A previous study demonstrated that three over-the-counter (OTC) venison and sweet potato dry canine diets, despite having no soy listed in the ingredient list, were positive for soy protein using enzyme-linked immunosorbent assay (ELISA).² That occurs when a dog food contains ingredient residues from other products (i.e., ingredient cross contamination during manufacturing). Therefore, one should not assume that an OTC diet is “soy free” based on the product name or ingredient list. That could be extrapolated to other potential antigen sources such as corn, rice, etc.³

Oral tolerance is both a local and systemic immunologic unresponsiveness to antigens routinely present in the gastrointestinal tract. A food allergy, as proposed by Verlinden et al. (2006) occurs when oral tolerance to a common protein in the gastrointestinal tract either fails to develop normally or changes such that a common protein is now antigenic.⁴ It is suspected that the immunologic reaction develops after a food-specific immunoglobulin E antibody on a mast cell binds with the food antigen, initiating the release of inflammatory mediators.⁵ Currently, it is believed that most food allergies are type I or immunoglobulin E-mediated but some evidence suggests type III and type IV hypersensitivities may play a role.⁴ There is no laboratory test available that accurately and consistently diagnoses food allergy.⁶ For that reason, veterinary practitioners, internists, nutritionists, and dermatologists use dietary elimination trials to diagnose food allergies in dogs.^{7,8} A typical food trial lasts 3–13 wk depending on the clinical signs (gastrointestinal versus dermatological) and

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AAFCO, Association of American Feed Control Officials; ELISA, enzyme-linked immunosorbent assay; OTC, over-the-counter

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requires a clients' strict adherence to the prescribed food.^{1,4} In the US, several pet food manufacturers market dietary products to veterinarians using novel or hydrolyzed proteins sources. The principle behind a novel diet is that the primary sources of protein (e.g., rabbit, kangaroo) in the product is one that the canine patient has not ever consumed previously; hence, the protein is novel and does not elicit a response from the dog's immune system.

The hydrolyzed proteins are positioned to be low molecular weight peptides (<18,000 daltons) having a very low antigenic potential because they are less likely to be detected and less likely to elicit a response from the dog's immune system. The premise is that digestion of an initially intact food protein results in free amino acids and small peptides in those products that are too small to bind with immunoglobulins.⁹ More specifically, immunoglobulin E binding to hydrolyzed soy protein was significantly less than that to native soy protein in dogs.¹⁰ Although it is not known what protein concentration, size, or structure is required to elicit an immunologic reaction in a food allergic dog, both types of therapeutic diets (novel and hydrolyzed) marketed for canine food allergy testing have had some, but variable, clinical success.^{7,8,11} An immune response may result as a consequence of either larger fragment sizes due to variability in the process of hydrolyzation, total concentration of detectable antigen, or alteration of the structural conformation from processing methods.

There has also been a growing interest by pet owners to use OTC commercial diets for diagnostic dietary trials due to cost and convenience.³ General practitioners and some veterinary specialists have conceded to those client requests to use OTC diets when attempting to diagnose food allergy. Such OTC diets are selected based on the product name (i.e., salmon and sweet potato), which, based on regulations, only ensures that the product contains at least 3% (not 100%) of those ingredients.¹³ Increasingly so, pet food manufacturers have taken the next step and are labeling products as "not containing" specific ingredients (e.g., "contains no corn, soy, or wheat"). Pet owners and veterinarians have come to base their diet selections on those label claims.

Food allergies to soy have been reported in dogs in several studies.^{1,8,14,15} The objective of this study was to determine in vitro the suitability of feeding OTC diets labeled as containing "no soy" versus veterinary therapeutic diets to dogs suspected for having soy allergies. More specifically, the purposes were to test for the presence of soy protein in OTC pet foods marketed with a "no soy" claim and to test the presence of soy protein in seven veterinary therapeutic diets marketed for food elimination trials.

Materials and Methods

The ingredient list for two control diets (positive and negative for soy), four OTC canine diets marketing a "no soy" claim, and seven veterinary therapeutic diets marketed for food elimination trials were reviewed for any potential sources of soy protein (**Table 1**). All diets were dry products appropriate for feeding adult dogs at maintenance, and sold as complete and balanced according to the Association of American Feed Control Officials (AAFCO) regulations regarding product label statements.

The positive control in this study was the same OTC product used in a previous study with soy clearly identified as soybean meal in the ingredient list and having previously ELISA tested >25 ppm soy protein. The negative control in this study was also the same veterinary therapeutic novel protein product used in a previous study having no identifiable soy protein in the ingredient list and having ELISA tested <2.5 ppm soy protein.² Both positive and negative control products were obtained from the hospital pharmacy stock in March 2010.

The OTC diets were selected based on finding the first four products that: 1) were canine dry maintenance diets according to AAFCO standards, 2) stated the product contained "no soy" either on the product label or company website, and 3) did not list any soy products in the ingredient list using internet searches or a visit to local retail stores. All the OTC adult canine dry diets were then purchased in March 2010 from one online food distribution company and shipped directly to the investigator.

The veterinary therapeutic diets were selected from each of the four different manufacturers marketing diets for food elimination trials within the US. Four diets used novel (i.e., fish, venison, kangaroo) proteins and three diets used hydrolyzed (i.e., soy, chicken liver) protein diets. Sample bags of each veterinary therapeutic diet dated well within their "use by" or "before by" time limits were obtained directly from the hospital pharmacy stock at the same time as the OTC products and control diets. None of the selected veterinary therapeutic diets made a claim of "no soy," but in this veterinary therapeutic diet group, two diets used a hydrolyzed soy ingredient and two diets contained soybean oil (**Table 1**).

One 100 g sample of each dry diet was sent to an independent laboratory to test for the presence of soy antigens.¹ The laboratory was blinded to the product name and ingredient lists of the diets. This laboratory was accredited (ISO17025^a) in allergen testing and routinely performed ELISA testing in quadruplicate for the presence of antigens in food products, feeds, drugs, food additives, and unknown biologic samples. The test was approved by the US Department of Agriculture and was an amplified, double-sandwich type ELISA^b that used specific anti-soy trypsin

TABLE 1

Control and Test Canine Dry Dog Food Product Ingredients Lists

Diet	Purpose	Ingredient List as Appearing on the Product ^c
Control Diets		
1	Negative control ^d	Dehydrated potato, duck by-product meal, natural flavors, potato protein, coprah oil, duck, anchovy oil (source of EPA/DHA), soya oil, L-tyrosine, DL-methionine, salt, taurine, vitamins (DL- α tocopherol acetate [source of vitamin E], inositol, niacin supplement, L-ascorbyl-2-polyphosphate (source of vitamin C), D-calcium pantothenate, biotin, pyridoxine hydrochloride (vitamin B ₆), riboflavin supplement (vitamin B ₂), thiamine mononitrate (vitamin B ₁), vitamin A acetate, folic acid, vitamin B ₁₂ supplement, vitamin D ₃ supplement], choline chloride, trace minerals (zinc proteinate, zinc oxide, ferrous sulfate, manganese proteinate, copper proteinate, copper sulfate, manganous oxide, calcium iodate, sodium selenite), rosemary extract, preserved with natural mixed tocopherols (source of vitamin E), and citric acid
2	Positive control ^e	Chicken, ground whole grain corn, ground whole grain sorghum, ground whole grain wheat, chicken by-product meal, soybean meal, corn gluten meal, animal fat (preserved with mixed tocopherols and citric acid), brewers rice, chicken liver flavor, soybean oil, flaxseed, potassium chloride, iodized salt, calcium carbonate, choline chloride, vitamin E supplement, vitamins (L-ascorbyl-2-polyphosphate [source of vitamin C], vitamin E supplement, niacin, thiamine mononitrate, vitamin A supplement, calcium pantothenate, biotin, vitamin B ₁₂ supplement, pyridoxine hydrochloride, riboflavin, folic acid, vitamin D ₃ supplement), minerals (ferrous sulfate, zinc oxide, copper sulfate, manganous oxide, calcium iodate, sodium selenite), DL-methionine, preserved with mixed tocopherols and citric acid, β -carotene, and rosemary extract
Products marketed as containing “no soy”		
3	OTC salmon and brown rice ^f	Salmon meal, brown rice, oatmeal, barley, canola oil, pumpkin seeds, ground flaxseeds, montmorillonite clay, natural chicken flavor, potassium chloride, vitamins (choline chloride, vitamin E supplement, ascorbic acid, biotin, niacin supplement, vitamin A acetate, D-calcium pantothenate, riboflavin supplement, pyridoxine hydrochloride, thiamine mononitrate, vitamin B ₁₂ supplement, carotene, vitamin D ₃ supplement, folic acid), sea salt, minerals (zinc proteinate, iron proteinate, manganese proteinate, copper proteinate, sodium selenite, ethylenediamine dihydriodide), direct-fed microorganisms (<i>Saccharomyces cerevisiae</i> yeast culture, dried <i>Enterococcus faecium</i> fermentation product, dried <i>Lactobacillus acidophilus</i> fermentation product, dried <i>Aspergillus niger</i> fermentation extract, dried <i>Trichoderma longibrachiatum</i> fermentation extract, dried <i>Bacillus subtilis</i> fermentation extract), inulin, flaxseed oil, apples, carrots, peas, chicken eggs, cottage cheese, mixed tocopherols with citric acid (a natural preservative), rosemary extract, freeze dried chicken, freeze dried turkey, freeze dried turkey liver, freeze dried turkey heart, ground chicken bone, butternut squash, broccoli, lettuce, spinach, dried kelp, salmon oil, apple cider vinegar, parsley, honey, blueberries, alfalfa sprouts, persimmons, olive oil, duck eggs, pheasant eggs, quail eggs, rosemary, sage, and clove
4	OTC salmon and potato ^g	Deboned salmon, peas, whole potatoes, whole ground brown rice, salmon meal (natural source of Ω 3 fatty acid), flaxseed (natural source of Ω 3 and 6 fatty acids), canola oil (naturally preserved with mixed tocopherols, A natural source of vitamin E), tomato pomace (natural source of lycopene), natural fish flavor, oatmeal, whole carrots, blueberries, cranberries, barley grass, dried parsley, alfalfa meal, dried kelp, <i>Yucca schidigera</i> extract, L-carnitine, L-lysine, turmeric, oil of rosemary, dried chicory root, calcium carbonate, β carotene, vitamin A supplement, thiamine mononitrate (vitamin B ₁), riboflavin (vitamin B ₂), niacin (vitamin B ₃), D-calcium pantothenate (vitamin B ₅), pyridoxine hydrochloride (vitamin B ₆), biotin (vitamin B ₇), folic acid (vitamin B ₉), vitamin B ₁₂ supplement, calcium ascorbate (source of vitamin C), vitamin D ₃ supplement, vitamin E supplement, iron amino acid chelate, zinc amino acid chelate, manganese amino acid chelate, copper amino acid chelate, choline chloride, sodium selenite, calcium iodate, salt, caramel, potassium chloride, <i>Saccharomyces cerevisiae</i> , <i>Lactobacillus acidophilus</i> , <i>Bacillus subtilis</i> , <i>Enterococcus faecium</i>
5	OTC bison and potato ^h	Bison, lamb meal, chicken meal, egg product, sweet potatoes, peas, potatoes, canola oil, roasted bison, roasted venison, natural flavor, tomato pomace, ocean fish meal, salt, choline chloride, dried chicory root, tomatoes, blueberries, raspberries, <i>Yucca schidigera</i> extract, dried fermentation products of <i>Enterococcus faecium</i> , <i>Lactobacillus acidophilus</i> , <i>Lactobacillus casei</i> , and <i>Lactobacillus plantarum</i> , dried <i>Trichoderma longibrachiatum</i> fermentation extract, vitamin E supplement, iron proteinate, zinc proteinate, copper proteinate, ferrous sulfate, zinc sulfate, copper sulfate, potassium iodide, thiamine mononitrate (vitamin B ₁), manganese proteinate, manganous oxide, ascorbic acid, vitamin A supplement, biotin, niacin, calcium pantothenate, manganese sulfate, sodium selenite, pyridoxine hydrochloride (vitamin B ₆), vitamin B ₁₂ supplement, riboflavin (vitamin B ₂), vitamin D supplement, and folic acid
6	OTC salmon and potato ⁱ	Salmon meal, potatoes, potato protein, ocean fish meal, canola oil (preserved with mixed tocopherols), peas, tomato pomace, natural flavor, choline chloride, uncured alfalfa meal, inulin (from chicory root), lecithin, sage extract, raspberries, β -carotene, rosemary extract, sunflower oil, <i>Yucca schidigera</i> extract, dried <i>Enterococcus faecium</i> fermentation product, dried <i>Lactobacillus acidophilus</i> fermentation product, dried <i>Lactobacillus casei</i> fermentation product, dried <i>Lactobacillus plantarum</i> fermentation product, dried <i>Trichoderma longibrachiatum</i> fermentation extract, vitamin E supplement, iron proteinate, zinc proteinate, copper proteinate, ferrous sulfate, zinc sulfate, copper sulfate, potassium iodide, thiamine mononitrate, manganese proteinate, manganous oxide, ascorbic acid, vitamin A supplement, biotin, calcium pantothenate, manganese sulfate, sodium selenite, pyridoxine hydrochloride (vitamin B ₆), vitamin B ₁₂ supplement, riboflavin, vitamin D ₃ supplement, folic acid, cobalt proteinate, papaya, and pineapple

(Table continues)

TABLE 1 (Continued)

Diet	Purpose	Ingredient List as Appearing on the Product ^c
Veterinary products marketed for food elimination trials		
7	Novel protein ^l	Brewers rice, salmon meal, trout, canola meal, animal fat preserved with mixed tocopherols (form of vitamin E), brewers dried yeast, canola oil preserved with tbhq, potassium chloride, fish oil, corn oil preserved with tbhq, salt, choline chloride, zinc sulfate, taurine, ferrous sulfate, vitamin E supplement, manganese sulfate, ascorbic acid, niacin, calcium carbonate, β -carotene, vitamin A supplement, calcium pantothenate, thiamine mononitrate, copper sulfate, riboflavin supplement, vitamin B ₁₂ supplement, pyridoxine hydrochloride, folic acid, vitamin D ₃ supplement, calcium iodate, biotin, menadione sodium bisulfite complex (source of vitamin K activity), and sodium selenite
8	Novel protein ^k	Potato, potato starch, venison, potato protein, pork fat (preserved with mixed tocopherols and citric acid), soybean oil (preserved with BHA, propyl gallate and citric acid), natural flavor, dicalcium phosphate, potassium chloride, powdered cellulose, venison meal, fish oil, iodized salt, calcium carbonate, choline chloride, vitamins (L-ascorbyl-2-polyphosphate [source of vitamin C], vitamin E supplement, niacin, thiamine mononitrate, vitamin A supplement, calcium pantothenate, biotin, vitamin B ₁₂ supplement, pyridoxine hydrochloride, riboflavin, folic acid, vitamin D ₃ supplement), DL-methionine, vitamin E supplement, taurine, minerals (ferrous sulfate, zinc oxide, copper sulfate, manganous oxide, calcium iodate, sodium selenite), L-tryptophan, preserved with mixed tocopherols and citric acid, β -carotene, and rosemary extract
9	Novel protein ^l	Potato, herring meal (source of fish oil), catfish, animal fat (preserved with mixed tocopherols, a source of vitamin E), dried beet pulp, fish digest, calcium carbonate, dicalcium phosphate, sodium hexametaphosphate, vitamins (vitamin E supplement, ascorbic acid, vitamin A acetate, calcium pantothenate, biotin, thiamine mononitrate [source of vitamin B ₁], vitamin B ₁₂ supplement, niacin, riboflavin supplement [source of vitamin B ₂], inositol, pyridoxine hydrochloride [source of vitamin B ₆], vitamin D ₃ supplement, folic acid), choline chloride, potassium chloride, DL-methionine, minerals (ferrous sulfate, zinc oxide, manganous sulfate, copper sulfate, manganous oxide, potassium iodide, cobalt carbonate), and ethoxyquin (a preservative)
10	Novel protein ^m	Oat flour, kangaroo, canola meal, animal fat (preserved with mixed tocopherols, a source of vitamin E), dried beet pulp, fish oil (preserved with mixed tocopherols, a source of vitamin E), calcium carbonate, salt, dicalcium phosphate, sodium hexametaphosphate, choline chloride, DL-methionine, potassium chloride, minerals (ferrous sulfate, zinc oxide, manganese sulfate, copper sulfate, manganous oxide, potassium iodide, cobalt carbonate), vitamins (vitamin E supplement, ascorbic acid, vitamin A acetate, calcium pantothenate, biotin, and thiamine mononitrate [source of vitamin B ₁], vitamin B ₁₂ supplement, niacin, riboflavin supplement [source of vitamin B ₂], inositol, and pyridoxine hydrochloride [source of vitamin B ₆], vitamin D ₃ supplement, and folic acid)
11	Hydrolyzed protein with an unstated average particle size ⁿ	Rice, soy protein isolate hydrolysate, chicken fat, dried beet pulp (sugar removed), natural flavors, monocalcium phosphate, sodium silico aluminate, soya oil, calcium carbonate, anchovy oil (source of EPA/DHA), fructo-oligosaccharides, potassium chloride, salt, taurine, vitamins (DL- α tocopherol acetate [source of vitamin E], inositol, niacin supplement, L-ascorbyl-2-polyphosphate [source of vitamin C], D-calcium pantothenate, biotin, pyridoxine hydrochloride [vitamin B ₆], riboflavin supplement [vitamin B ₂], thiamine mononitrate [vitamin B ₁], vitamin A acetate, folic acid, vitamin B ₁₂ supplement, vitamin D ₃ supplement), choline chloride, L-tyrosine, marigold extract (<i>Tagetes erecta</i> L.), trace minerals (zinc proteinate, zinc oxide, ferrous sulfate, manganese proteinate, copper proteinate, copper sulfate, manganous oxide, calcium iodate, sodium selenite), rosemary extract, preserved with natural mixed tocopherols (source of vitamin E), and citric acid
12	Hydrolyzed protein with an average particle size <12,200 daltons ^o	Starch, hydrolyzed soy protein isolate, vegetable oil, calcium phosphate, partially hydrogenated canola oil preserved with tbhq, powdered cellulose, corn oil, potassium chloride, vegetable gums (gum arabic, guar gum), choline chloride, DL-methionine, salt, magnesium oxide, lecithin, taurine, zinc sulfate, ferrous sulfate, vitamin E supplement, manganese sulfate, niacin, calcium carbonate, vitamin A supplement, copper sulfate, calcium pantothenate, thiamine mononitrate, riboflavin supplement, garlic oil, vitamin B ₁₂ supplement, pyridoxine hydrochloride, folic acid, vitamin D ₃ supplement, calcium iodate, biotin, menadione sodium bisulfite complex (source of vitamin K activity), and sodium selenite
13	Hydrolyzed protein with an average particle size <3,000 daltons ^p	Starch, hydrolyzed chicken liver, soybean oil (preserved with BHA, propyl gallate and citric acid), hydrolyzed chicken, powdered cellulose, dicalcium phosphate, calcium carbonate, glyceryl monostearate, potassium chloride, iodized salt, choline chloride, vitamins (vitamin E supplement, L-ascorbyl-2-polyphosphate [source of vitamin C], niacin, thiamine mononitrate, vitamin A supplement, calcium pantothenate, biotin, vitamin B ₁₂ supplement, pyridoxine hydrochloride, riboflavin, vitamin D ₃ supplement, folic acid), DL-methionine, minerals (ferrous sulfate, zinc oxide, copper sulfate, manganous oxide, calcium iodate, sodium selenite), preserved with mixed tocopherols and citric acid, taurine, β -carotene, and rosemary extract

OTC, over-the-counter

inhibitor and other soy protein antibodies to detect soy antigen in cooked food products. Samples positive for soy protein were quantitated by comparison with a known soy flour protein concentration gradient between 2.5 and 25 ppm. The lower limit of detection for that assay was the value of the lowest positive

control, which was 2.5 ppm soy flour protein. A sample with <2.5 ppm soy was not interpreted as having no soy but simply that the soy was below the level of detection. Soy proteins that had been significantly treated or altered with high temperatures, pressures, fermentation, or hydrolysis may not have been detected. The

methodology of the test was based on a technique using internal positive and negative controls conducted simultaneously with the test samples to validate the kit.^{13,16,17} A valid test was confirmed with controls corresponding to the kit performance criteria as set out by the Certificate of Analysis for each specific lot number.

Results

The positive and negative control diets were reaffirmed to contain >25 ppm and <2.5 ppm soy protein, respectively, which was consistent with the previous study.² Three of the four OTC diets with a “no soy” claim (diets 4–6) contained soy protein concentrations >2.5 ppm, while diet 3 contained <2.5 ppm (**Table 2**). Of the four novel protein veterinary therapeutic diets, diet 8 contained soy protein concentrations >25 ppm, diet 7 contained 4.6 ppm, and diets 9 and 10 contained <2.5 ppm. Of the three hydrolyzed veterinary therapeutic products, diets 11 and 12 tested for soy concentrations at 3.7 and 7.7 ppm, respectively, while diet 13 contained <2.5 ppm (**Table 2**).

Discussion

ELISA testing of foods is common practice to detect soy contamination because in people, soy and wheat are thought to be the most common plant allergens.¹⁸ Soy protein concentrations as low as 10 ppm have been documented to invoke a reaction in a soy-allergic person.¹⁹ Unfortunately, the lower limit of detection in dogs is unknown at this time. The immunoassay kits that have been developed to detect the presence of soy protein in various

foods are very specific and do not detect trypsin inhibitors from other legumes, such as lima beans or chickpeas.²⁰

Three “no soy” OTC diets evaluated in this study did contain soy protein making them unsuitable for an elimination trial if soy is a potential allergen for the patient undergoing a food elimination trial. All OTC diets evaluated advertised the product as containing no soy either directly on the product label or on the website. None of the OTC diets testing positive for soy protein had any identifiable source of soy listed on the label in this assessment. If the OTC diets claiming to not contain a specific ingredient selected in this study are representative of similar products in the market place, then OTC dry dog foods, regardless of claims, should not be used during a food elimination trial as a diagnostic tool in cases where a food allergy to soy is suspected.

The finding of soy in dry OTC dog foods is most likely due to cross contamination of ingredients during transportation and manufacturing. Cross contamination can occur when transportation vehicles and/or equipment used for different ingredients at different times are not thoroughly cleaned between the different ingredient types. Bulk ingredients are typically blown into mixing hoppers using forced air and conveyor belts, and airborne particles can spread from from nearby ingredients. Cross contamination between different ingredients is allowable within reason; however, “practices should minimize the potential for contamination” and contamination may occur “as unavoidable under good processing practices” or “except in such amounts as might occur unavoidably in good processing practices” according to AAFCO.²¹ Therefore,

TABLE 2
Results of Soy Protein Testing of Selected Dry Dog Foods

Diet	Diet	ELISA Soy (ppm)
Control diets		
1	Positive ^e	>25
2	Negative ^d	<2.5
Products marketed as containing “no soy”		
3	OTC dry adult dog food made with salmon & brown rice ^f	<2.5
4	OTC dry adult dog food made with salmon & potato ^g	7.9
5	OTC dry adult dog food made with bison and potato ^h	>25
6	OTC dry adult dog food made with salmon & potato ⁱ	>25
Veterinary products marketed for food elimination trials		
7	Prescription dry adult dog food made with salmon & rice ^j	4.6
8	Prescription dry adult dog food made with venison & potato ^k	>25
9	Prescription dry adult dog food made with fish & potato ^l	<2.5
10	Prescription dry adult dog food made with kangaroo & oats ^m	<2.5
11	Prescription dry adult dog food made with hydrolyzed soy protein & rice ⁿ	3.7
12	Prescription dry adult dog food made with hydrolyzed soy protein & starch ^o	7.7
13	Prescription dry adult dog food made with hydrolyzed chicken & starch ^p	<2.5

ELISA, enzyme-linked immunoassay; OTC, over-the-counter.

the presence of soy protein in OTC products should not be seen as a violation of regulations but as reasonably allowed contaminants that make interpreting the results of the dietary food trial more difficult. The issue of having a specific “no soy” claim on the product front panel, website, and associated marketing materials; however, will no doubt mislead pet owners and veterinarians considering such a product in a diet elimination trial.

The therapeutic veterinary diets evaluated in this study do not advertise the products as not having soy ingredients. This is apparent in reading the ingredient list where soybean oil and hydrolyzed soy protein isolate are clearly stated. Diets 7, 9, and 10 did not have soy-containing sources in the ingredient list however, diet 7 did test positive for soy protein at 4.6 ppm. In reviewing the January 2010 manufacturer’s therapeutic product guide for diet 7, it is noteworthy that other veterinary diets made by this manufacturer do contain soy ingredients. Therefore, if soy ingredients are present at the same plant, it is plausible that cross contamination of soy protein may have occurred in this novel protein diet. ELISA test results for soy protein for diets 9 and 10 (from the same company) was <2.5 ppm. Likewise, in reviewing the 2007 manufacturer’s product guide for diet 9 and 10, there is one therapeutic diet containing soy protein isolate, but soy is not a common ingredient used by this manufacturer. The limited use of soy ingredients is consistent with the decreased possibility of cross contamination.

Diets 11, 12, and 13 are marketed as hydrolyzed protein diets. Diets 11 and 12 contain hydrolyzed soy protein isolates, and diet 13 contains soybean oil. The process of developing the isolate hydrolysate from soy flour does result in smaller particle sizes of soy protein, but was not below the limit of detection in the ELISA test used. This may be due to difference in hydrolyzation methods that can affect the degree of hydrolyzation and variability in hydrolyzate fragment size. Diets 11 and 13 contained soybean oil but ELISA tested 3.7 and <2.5 ppm, respectively, probably because most of the proteins in soybean oil are as trypsin inhibitors and not typical soy protein flour allergens. This is consistent with the finding that refined soybean oil has not caused reactions in soy allergic people.^{22,23} In humans, hydrolyzed soy peptides tend to >20 kilodaltons to trigger an adverse allergic reaction. To date, no information is available for dogs, and the size of the hydrolyzed soy protein that can potentially trigger an allergic response is extrapolated from human studies but is thought to be between 18 and 36 kilodaltons.⁵ A limitation in the current study is that the size of hydrolyzed soy protein isolates was not determined. The degree of hydrolyzation was not evaluated and that could potentially offer another explanation for the levels of soy antigen detected in the hydrolyzed diets; however,

contamination cannot be ruled out. It is plausible that lower percentages of hydrolyzation would cause the resultant soy antigen detected.

The manufacturer was contacted to determine the origin of the inconsistent results between diets 8 and 13. Diet 8 (novel venison) contained >25 ppm soy protein antigen, whereas diet 13 (hydrolyzed chicken liver) contained <2.5 ppm. The difference could not be explained by the soybean oil ingredient because of the following: 1) soybean oil should be devoid of soy proteins, 2) the supplier is the same; however, there may be some variability in the refinement of the oil between shipments, and 3) both products are manufactured at the same processing plant with a standardized protocol for cleaning equipment between product runs^c (Dr. Dru Forrester, oral communication with the manufacturer, 2011).

Conclusion

If the diets evaluated in this study are representative of similar OTC “no soy” claiming products, those products should not be used during an elimination trial in a suspected soy allergic patient. Veterinary therapeutic diets need to be selected carefully based on the patient and the ingredient(s) of concern. A thorough diet history is needed to make the best recommendations for each patient. Based on these results, a soy allergy cannot be ruled out if a patient fails an elimination trial using a hydrolyzed soy diet. Compliance failure on the part of the owner during the elimination trial should not be assumed. ■

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FOOTNOTES

- ^a International Organization for Standardization. Available at: www.iso.org/iso/catalogue_detail.htm?csnumber=39883. Accessed August 13, 2014.
- ^b Elisa Technologies; Gainesville, FL. Available at: www.elisa-tek.com. Certificate Number: 2793.01 Accessed August 13, 2014.
- ^c Approximately 1 year later it was learned that a soy component was present in the natural flavoring used in diet 8 that was not present in diet 13 (per Dr. Dru Forrester).
- ^d Royal Canin Veterinary Diet Hypoallergenic Selected Protein Adult PD Canine; Royal Canin USA Inc., St. Charles, MO
- ^e Science Diet Adult Advanced Fitness Original; Hill’s Pet Products, Inc., Topeka, KS
- ^f Nature’s Variety Prairie Salmon Meal & Brown Rice Medley; Nature’s Variety, Lincoln, NE

- ^g Blue Buffalo Basics Salmon and Potato Formula; The Blue Buffalo Co., Wilton, CT
- ^h Taste of the Wild High Prairie Canine Formula with Roasted Bison and Roasted Venison; Taste of the Wild Pet Food, Meta, MO
- ⁱ CANIDAE Grain Free pureSEA with salmon meal; Canidae Corporation, San Luis Obispo, CA
- ^j Purina Veterinary Diets DRM Dermatologic Management Canine Formula; Nestlé S.A., Vevey, Switzerland
- ^k Hill's Prescription Diet d/d Canine Skin Support Potato & Venison Formula; Hill's Pet Products Inc., Topeka, KS
- ^l IAMS Veterinary Formula Skin & Coat Response FP; P&G Pet Care, Dayton, OH
- ^m IAMS Veterinary Formula Skin & Coat Response KO; P&G Pet Care, Dayton, OH
- ⁿ Royal Canin Veterinary Diet Hypoallergenic Hydrolyzed Protein Adult HP Canine; Royal Canin USA Inc., St. Charles, MO
- ^o Purina Veterinary Diet HA Hypoallergenic Canine Formula, Nestlé S.A., Vevey, Switzerland
- ^p Hill's Prescription Diet z/d Canine ULTRA Allergen-Free; Hill's Pet Products Inc., Topeka, KS
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