PATHOPHYSIOLOGY AND EVALUATION OF FOOD INTOLERANCE TO SOY USING AN ATOPIC DOG MODEL

A Thesis

by

ROBERT ALLEN KENNIS

Submitted to the Office of Graduate Studies
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

May 2003

Major Subject: Veterinary Microbiology

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Approved as to style and content by:

Ian Tizard (Chair of Committee)	Deb Zoran (Member)
Roger Smith III (Member)	Ann Kier (Head of Department)

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ABSTRACT

Pathophysiology and Evaluation of Food Intolerance to Soy Using an Atopic Dog Model. (May 2003)

Robert Allen Kennis, B.S., Michigan State University;

D.V.M., Michigan State University

Chair of Advisory Committee: Dr. Ian Tizard

The purpose of the study was to test the hypothesis that dogs sensitized to soy antigen would produce significantly greater amounts of antigen specific IgE antibody compared to a control population before and after challenges with soy, hydrolyzed soy, and non-soy diets. Further, we sought to evaluate important allergenic components of soy using Western blot analysis. Lastly, absorption and mucosal function testing using inert sugars were evaluated for our sensitized and non-sensitized controls.

Eight dogs (6 female, 2 male) were sensitized to whole soy using an established protocol. Seven dogs (3 female, 4 male) roughly age matched were used as controls. The dogs were randomly split into three groups. All dogs were fed an elimination diet of egg and Brewer's rice for six weeks. Samples were collected and each group was fed a diet of soy and rice flour, hydrolyzed soy and rice flour, or corn and rice flour for three weeks. Samples were collected and each group was fed the elimination diet followed by challenge with each of the diets. Serum was collected and stored for allergen specific IgE semi-quantitation and Western blot analysis using whole soy fractionated into globulin and whey components. A solution of monosaccharide and disaccharide sugars

was orally administered in a volume determined by weight. Six hours after administration the dogs were catheterized and the entire urine volume was collected for measurement of sugar recovery by high pressure liquid chromatography, followed by pulsed amphometric detection.

There was a statistically significant difference in serum IgE between sensitized and control dogs after the elimination diet, and also for each of the challenge diets. There were differences detected by Western blot analysis for allergens within the soy globulin and whey fractions for sensitized dogs compared to control dogs. There were no significant differences between sensitized and control dogs for sugar recovery for any of the diets. We conclude that although there were significant differences in measurable IgE between sensitized and control dogs, we were unable to differentiate these groups using gastrointestinal mucosal permeability and function testing.

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CHAPTER I

INTRODUCTION

Food allergy is a well recognized entity in veterinary and human medicine[1-3]. The suspectect incidence of affected dogs with clinical dermatologic symptoms may reach 8% of the general population and is the third most common allergy in dogs[4]. Due to its importance, an animal model to study food allergy has been sought. Recent publications have indicated that the atopic dog model may be suitable for studying Type I hypersensitivity reactions to foods[5,6]. Because these dogs are sensitized by subcutaneous injections rather than by naturally occurring oral sensitization, the validity of this model has been questioned. The purpose of the study was to evaluate the usefulness of this canine model for investigating spontaneously occurring dietary hypersensitivity in dogs.

The terminology used to describe adverse reactions to food is confusing. Proposed definitions based upon mechanistic classification have been employed in the human literature but have not been fully accepted in the veterinary community. Adverse food reactions may be the result of toxic or non toxic mechanisms[2]. An example of a toxic reaction is the ingestion of bacteria laden food items. Non toxic reactions depend upon individual susceptibilities and may be the result of immune mechanisms due to allergic hypersensitivity reactions. Other non toxic reactions may relate to intolerance due to pharmacologic properties of that food item. Strawberries and shellfish may be high in histamine and ingestion can lead to urticaria, edema, and anaphylaxis. An

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example of a non-toxic metabolic reaction is the ingestion of molds on grains that mimic estrogen hormones. Lastly, idiosyncratic responses may also occur. Any and all of example of a non-toxic metabolic reaction is the ingestion of molds on grains that mimic these reactions may occur in dogs. Therefore, it is probably more correct to use the term adverse food reactions in dogs until further clarification is established.

It has been suggested that food allergy may be a Type I, Type III, or a Type IV hypersensitivity reaction[4,7]. Affected dogs may present with a myriad of clinical signs involving the skin and/or the gastrointestinal tract. Clinical signs of skin involvement are associated with non-seasonal pruritus. The distribution pattern of pruritus may involve any location on the body. The most commonly affected locations are the ears, face, feet, and inguinal regions[8]. Secondary bacterial skin infections are common. Occasionally, dogs may present with a history of recurrent otitis externa. Additional clinical signs may include urticarial eruption, fever, malaise, and sometimes seizures[8]. Gastrointestinal signs may include vomiting, diarrhea, and weight loss[9]. Some dogs may present with both dermatologic signs and gastrointestinal disorders[10]. Interestingly, dogs with food allergy may not be responsive to anti-inflammatory dosages of corticosteroid medications[8]. Because of the clinical variability, it is hypothesized that there may be more than one type of reaction and it may be related to a Type I , IgE- mediated reaction.

In people, food intolerance probably accounts for the majority of adverse food reactions. Specific IgE mediated immediate hypersensitivity reactions have been clearly documented in adults and children[2]. These reactions are more prevalent in young children and those individuals with atopic disease[11]. Clinical symptoms from affected

skin, respiratory tract, and gastrointestinal tract may be seen minutes to hours after the offending allergen is ingested. Another clinical entity that is similar to the immediate hypersensitivity reaction is the oral allergy syndrome[2]. This type of contact reaction affects the oropharynx, from a localized release of histamine. Patients with oral allergy syndrome complain of pruritus or a tingling sensation of the mouth, tongue and lips. Angioedema or tinnitus may also be present[2]. Such symptoms may also be associated with pollen allergic individuals reacting to ingested fresh fruits or vegetables due to cross reactivity with antigenically similar pollen antigens. Birch pollen and fresh apples share similar antigens[12]. Cooking the offending food usually prevents oral allergy syndrome.

One of the common presenting signs of dogs with adverse reactions to food is pruritus of the face; especially the muzzle and the chin regions. Owners may observe that these clinical signs occur immediately or within hours after eating. These findings are suggestive of a reaction similar to the oral allergy syndrome. However, most dogs eat canned foods or dry kibble that has been heat processed that will alter the allergens. Once again, the diversity of clinical findings associated with adverse food reactions may suggest multiple etiologies, even in the same affected dog.

It is interesting to note that adverse reactions to foods in both dogs and humans may be associated with dermatologic symptoms. In humans, it has been shown that immunologically active food proteins can enter the circulation and travel to distant tissues such as the skin[13]. These proteins may stimulate a localized immunologic response. It is hypothesized that altered gastrointestinal permeability may lead to an

increase in food origin peptides in circulation. In dogs it has been demonstrated that repair of intestinal permeability and mucosal function has led to the resolution of clinical signs in dogs with suspected food allergy[14]. Although it has not been shown that food peptides are present in circulation in the dog, it is reasonable to assume that they are present. This assumption may explain why some dogs with adverse food reactions chew, bite, or lick at their feet.

Several methods are used for diagnosis of adverse reactions to food in humans. The best established and most reliable diagnostic test is the double blind, placebo-controlled, oral food challenge (DBPCFC)[14-16]. Patients are given gelatin capsules with measured amounts of allergen in increasing dosage until early clinical symptoms are noted. Neither the doctor nor the patient is aware of the ingredients until after the test is completed[15]. The test is accurate for diagnosis of an adverse reaction to a food but, it is not specific for IgE- mediated hypersensitivity reactions. Skin prick testing is frequently used as a screening tools for IgE mediated reactions[16]. A small amount of allergen is placed onto the skin surface and a superficial scratch or prick is made to allow the allergen to have contact with the dermis. If specific IgE antibody is present on mast cells in the dermis, the allergen will cross-link the antibiodies which will signal the mast cell to degranulate which leads to the release of histamine and other pro-inflammatory mediators and results in a wheal or urticaria formation. Glycerinated histamine and glycerine diluent are used as positive and negative controls, with a positive reaction defined by the wheal of the allergen becoming at least 3 mm greater than the negative control[11,16]. Skin prick testing in humans is associated with low sensitivity and high

specificity (few false positive reactions) and is used to rule out IgE- mediated food hypersensitivity. This test is frequently used for infants and young children that may not be amenable to oral food challenges. Skin prick testing is sometimes used in conjunction with patch testing to evaluate delayed reactions[11]. The net result is that skin prick testing has good negative predictability, especially in young children where IgE mediated food hypersensitivity is suspected.

Serum testing using radioallergen serum testing (RAST) or enzyme linked immunosorbant assay (ELISA) methodology is occasionally used as a screening test for humans[16,17]. In vitro evaluations of allergen specific IgE antibodies and occasionally IgG4 antibodies are semi-quantitatively compared to pooled normal sera[18-21]. There are several disadvantages to this diagnostic procedure. First of all, it has been shown that skin prick test negative, serum ELISA test negative patients demonstrated positive reactions to D.B.P.C.F.C.[22]. Further, these patients had gastrointestinal biopsy samples that were positive for a T-helper 2 cytokine pattern when treated by immunohistochemical staining. These results demonstrated that the IgE- mediated reaction can be localized. Others have hypothesized about the importance of localized IgE reactions, including the oral allergy syndrome[23,24]. Another study demonstrated that patients with allergies to pollen, may have high circulating IgE levels specific for food antigens without clinical evidence of food allergy[25]. Together these results make serum testing for IgE mediated food allergy less desirable than other diagnostic methodologies.

The only known diagnostic test to accurately assess IgE- mediated dietary hypersensitivity in the dog is the elimination test diet[8]. A diet of protein and

carbohydrate is prepared from home made novel food ingredients based upon previous dietary history. Ideally, the dog has not eaten either food item for greater than two months of its life. This recommendation is empiric and there is no scientific validity for the two month time frame. The home- cooked diet is to be fed for a minimum of six weeks[4]. Some contend that a six- week trial may not be long enough as some dogs did not show clinical improvement for twelve weeks or longer[8]. A home- cooked diet is preferred over commercially available diet products as there have been reports of some dogs having a relapse in clinical symptoms when commercial diets containing similar ingredients were fed[4]. There are some concerns about feeding an unbalanced diet for this duration of time. However, development of a nutritional deficiency in adults in this period of time would be uncommon[26]. Home cooked elimination diets supplemented with vitamins and minerals are available[27]. Another concern about commercially produced dry diets is the processing of ingredients through an extruder. Various animal and vegetable fats and proteins may be used in this processing, without a requirement of package listing[7]. It has been speculated that a diagnosis of dietary hypersensitivity may be missed in about 25% of the cases when a dry dog food is fed due to the addition of ingredients in processing[7]. Although most clinical dermatologists agree that six weeks to eight weeks of a food elimination diet are adequate for a diagnosis, there is not a standard protocol available that is internationally agreed upon[3]. Regardless of the time frame, once a dog has had significant improvement in clinical symptoms, the original diet is introduced to see if symptoms return. The adverse reactions may appear within several hours, suggesting an immediate hypersensitivity. In other cases it may be up to

14 days before adverse symptoms develop[7]. Once a cause and effect is noted, it is desirable to pursue individual food item challenges. This is accomplished by adding a small amount of a freshly prepared ingredient to the elimination diet and assessing the clinical response. The majority of dogs with adverse food reactions react to only one food antigen[28]. The most common ingredients found in a recent review were beef, corn, chicken, and wheat[29].

The elimination test diet is not specific for IgE- mediated hypersensitivity. Dogs suffering from a variety of non-immunologic gastrointestinal diseases and skin diseases may show clinical improvement. Gastrointestinal biopsy is sometimes used to aid in the diagnosis of dietary hypersensitivity. The reaction pattern is generally a mixed inflammatory pattern with variable severity and is non-specific[9].

In an attempt to further define the reaction pattern of dogs with dietary adverse reactions, the use of the gastroscope to inject allergens into the gastric mucosa has been proposed[30]. For this procedure, the dog is anesthetized and allergens are dripped onto the gastric mucosa or directly injected into the stomach submucosa. Hyperemia quickly develops in dogs suspected of having IgE- mediated hypersensitivity. Biopsy samples of injected regions are similar to biopsy samples of urticaria induced by intradermal skin test injections. Biopsy samples collected at the injection site 48 hours later revealed a reaction pattern suggestive of a late-phase IgE- mediated reaction[5]. These findings are suggestive that a true IgE mediated hypersensitivity maybe taking place.

Intradermal skin testing (IDST) with whole food proteins has been attempted as a diagnostic tool for IgE- mediated dietary hypersensitivity. Allergens are directly

injected into the dermis, along with histamine as a positive control and saline as a negative control. The IDST is similar similar to the skin- prick test used in humans in that it has low sensitivity and high specificity[29]. Although this test may have usefulness as a screening tool, it also has not become widely accepted for the diagnosis of dietary hypersensitivity. This may be because in the dog, clinical symptoms for all adverse dietary reactions may have overlapping clinical signs, yet only a small subset are truly IgE- mediated hypersensitivity. Intradermal skin testing is only valid for detecting dogs with IgE- mediated reactions.

Serum testing for allergen specific IgE for foods is commercially available for dogs. The serum assays are either RAST or ELISA and only measures IgE. Thus, they only aid in the diagnosis of those dogs with IgE mediated hypersensitivity. Although these tests are available, one company (Greer Laboratories) has a disclaimer indicating that there may not be any validity to the diagnostic usefulness of this procedure. Further, there have been no published reports documenting circulating IgE specific for food allergens in the normal canine population or in dogs with concurrent allergy. It is impossible to speculate on the validity of this diagnostic procedure until adequate baseline values are established.

Another novel approach for diagnosis of dietary hypersensitivity is gastrointestinal sugar absorption analysis. Disaccharide sugars exit the intestinal lumen through the tight junctions between cells while monosaccharide sugars exit via a transcellular route[30]. Sucrose, a disaccharide, is preferentially absorbed through the stomach. Lactulose and L-rhamnose absorption and recovery are a reflection of small

intestinal permeability[30]. The ratio of recovery of lactulose to rhamnose increases with mucosal damage. Xylose and methylglucose are absorbed through carrier transport mechanisms, thus a reduced absorption and urinary recovery would be related to reduced gastrointestinal function[14]. The percentage recovery ratio of xylose to methylglucose decreases with intestinal disease. Methylglucose absorption is relatively resistant to intestinal damage. Thus, the ratio goes down because xylose absorption is decreased[31]. Together, these five sugars can assess gastrointestinal permeability and mucosal function[31].

It has been demonstrated that the use of inert sugar testing can be used to differentiate dogs with adverse food reactions into those that have food hypersensitivity (not specifically IgE- mediated) and those with other gastrointestinal disease. Dogs with improvement in gastrointestinal permeability and mucosal function values after being fed an elimination diet were presumed to have dietary hypersensitivity while those without improvement had other gastrointestinal disease[14]. Although these findings do not define the immunologic process of the disease, they demonstrate that sugar absorption tests may be useful in ruling out non- immune causes of adverse food reactions in dogs.

The atopic dog model has been used for early investigations of human asthma and food allergy[5,32,33]. Atopy in dogs has been defined by those exhibiting clinical evidence of pruritus when exposed to aeroallergens. Because it is currently believed that these dogs exhibit a Type I, IgE- mediated hypersensitivity, intradermal injections of offending allergens will cause a wheal and flare reaction. Serum testing for IgE with

ELISA or RAST are inconsistently diagnostic leading to the suspicion that there may be a localized reaction confined to the skin[34].

A colony of highly inbred, mixed breed dogs has been formed because of their high levels of circulating IgE. It has been demonstrated that breeding dogs with high IgE levels with other similar dogs yields a population of high IgE producing puppies[33]. This may partially explain why certain purebred breeds have an increased prevalence of allergy compared to normal canine populations. A protocol has been developed to sensitize these predisposed allergic dogs to selected allergens including food items[6]. Thus this model may be useful to investigate IgE- mediated aspects of canine food allergy.

Because of the predisposition to develop IgE to various allergens, there is no way to prevent sensitization to naturally occurring exposure of inhaled allergens or to dietary food items. Therefore, it is inappropriate to attribute changes in pruritus or the gastrointestinal system to only those antigens that the dog was sensitized. Another criticism of the model is that the dogs are selected from a highly inbred group that may not represent the diversity of the general canine population.

The long range goal of the study is to advance the understanding of the pathophysiology of canine dietary hypersensitivity. The central hypothesis is that a Type I hypersensitivity reaction is the primary response in canine dietary hypersensitivity. A Type I hypersensitivity is an IgE- mediated process leading to the degranulation of mast cells. Mast cells release histamine and serotonin as the primary pro-inflammatory mediators. When this reaction takes place in the skin, wheal and flare

occur. The gastrointestinal symptoms that occur are not well defined. However, hyperemia may be visible with the aid of a flexible endoscopic examination of the gastric mucosa and late phase IgE reactions have been observed on histopathology[5]. To test the central hypothesis, several additional hypotheses have been developed using the atopic dog model. Clinically healthy, non-sensitized dogs without clinical evidence of dietary hypersensitivity will be used as controls.

Hypotheses:

1. Hydrolyzed soy protein fed orally, will not induce clinical evidence of dietary hypersensitivity in dogs sensitized to whole soy allergen.
2. Only sensitized dogs will exhibit wheal and flare reactions with intradermal injections of aqueous soy allergens.
3. Only sensitized dogs will have increases in serum IgE to soy when orally challenged with whole soy diets.
4. Only sensitized dogs will exhibit gastrointestinal permeability and mucosal function changes when orally challenged with whole soy diets.

The rationale for the overall study is to develop baseline knowledge about canine dietary hypsesensitivity; specifically, the IgE- mediated aspects of this response. A colony of highly inbred, mixed breed dogs with a genetic predisposition toward developing high IgE levels is available. One litter of eight has been sensitized to six different food allergens: soy, beef, corn, chicken, wheat, and cow's milk. Preliminary

data based upon intradermally injected whole food items indicated that all dogs reacted to at least one of the six allergens at a dilution of 1:100. These dogs are kept in a controlled environment, away from other research dogs. They are maintained on a commercial diet of egg and Brewer's rice because none of the dogs has been sensitized to these items. All of the dogs had clinical evidence of pruritus including interdigital erythema, erythematous pinnae, and periorbital alopecia that was self induced. None of the dogs had severe enough pruritus to warrant symptomatic medication. It is important to note that although these dogs were pruritic while being fed an elimination diet, the cause of the pruritus may have been due to inhaled allergy. These dogs are cared for by Texas A&M University Laboratory Animal Research Resources (L.A.R.R.) and meet the University Laboratory Animal Care Committee standards. An animal use protocol was approved prior to the study, for their humane care.

It is important to understand the pathogenesis of adverse food reactions so that innovative diagnostic and treatment options may be developed. Additionally, a better understanding of dietary hypersensitivity may ultimately lead to the development of diets that may prevent development of this problem. Finally, this animal model was developed to aid in the understanding of human food allergy. Thus, further understanding of this model may show that it is a model of naturally occurring dietary hypersensitivity that has implications for humans with similar disease.

The proposed study will evaluate canine adverse food reactions from a pathophysiologic view point. It will explore different soy protein allergens to determine which are most antigenic. Intradermal skin testing and serum ELISA analysis will be

evaluated as diagnostic tools for immediate hypersensitivity reactions. Gastrointestinal mucosal permeation and function will be evaluated using orally administered mono and disaccharide sugars before and after challenging our study dogs with various diets. These characterizations of the model, compared to clinically normal control dogs, will also allow for the systematic evaluation of hydrolyzed soy protein as a potentially non-allergenic canine protein source.

Specific Aim #1

Hydrolyzed soy protein fed orally will not induce clinical evidence of dietary hypersensitivity in dog sensitized to whole soy antigen.

The objective of this aspect of the study is to challenge dogs that were sensitized to whole soy allergen with diets containing soy or containing hydrolyzed soy protein and demonstrate the extent to which clinical symptoms are elicited. The working hypothesis is that hydrolysis of soy protein will yield a protein of a low molecular weight that will not be allergenic in our sensitized dogs. Further, it is hypothesized that feeding a hydrolyzed soy protein diet will not induce adverse clinical symptoms in those dogs not sensitized to soy. Assessment will be made with clinical observation of pruritus or vomiting and or diarrhea. The rationale for this aim is that amelioration of clinical symptoms is the ultimate objective of dietary therapy for those dogs exhibiting adverse reactions to food, regardless of the underlying mechanisms. It is expected that only those dogs with dietary hypersensitivity will experience clinical symptoms when fed a diet with corn or whole soy but not with the hydrolyzed soy diet.

Specific Aim #2

Only sensitized dogs will exhibit wheal and flare reaction with intradermal injections of aqueous soy protein.

It is believed that only those dogs with clinical dietary hypersensitivity will exhibit an IgE- mediated, Type I hypersensitivity reaction. The objective is to skin test the dogs with serial dilutions of antigen, before and after challenging them with the test diets. The hypothesis is that only those dogs sensitized with soy will exhibit a wheal and flare reaction to intradermally injected aqueous soy allergen. The rationale for this procedure is to generate statistical information validating this methodology as a diagnositic tool. It is expected that only those dogs sensitized to whole soy will react to intradermal injections and those reactions will diminish when a non-soy or hydrolyzed soy diet is fed.

Specific Aim #3

Only dogs sensitized with soy will have increased levels of serum IgE when challenged with a whole soy diet. Further, soy allergenic fractions will be evaluated using Western Blot immunoassay.

The objective of this part of the study is to demonstrate that dogs with clinical adverse reactions to food develop IgE antibodies specific for soy. Further, it is expected that these IgE antibodies will bind to specific fractions of soy proteins. The hypothesis is that only soy proteins over 20 kDa are antigenic. The strategy for evaluation will

incorporate Western Blot analysis for serum IgE before and after challenge with each test diet. Serum will be evaluated by an outside laboratory for soy specific IgE expressed as percent relative value (%R). The rationale of the study is to demonstrate the extent to which IgE antibodies are produced to the individual proteins of soy. It is expected that only those dogs sensitized with soy would develop greater than normal baseline amounts of soy specific IgE. It is also expected that these values would increase when a whole soy diet is fed, and decrease when a non-soy or hydrolyzed soy diet is fed. Humans with known soy allergy produce IgE to documented soy proteins and it is expected that similar results will be identified for dogs.

Specific Aim #4

Only sensitized dogs will exhibit gastrointestinal mucosal permeability and function changes when orally challenged with a whole soy diet. These changes can be evaluated via administration and recovery of mono and disaccharide sugar.

The objective of this section of the study is to administer a solution of five mono and disaccharide sugars orally and measure the extent to which each is absorbed by their excretion in urine. The hypothesis is that dogs with clinical evidence of dietary hypersensitivity will demonstrate altered gastrointestinal mucosal permeability and function when challenged with a whole soy diet. Specifically, the lactulose to rhamnose ratio would increase, demonstrating an increase in gastric mucosal permeability, and the xylose to methylglucose ratio would decrease, demonstrating a decrease in mucosal

absorption. Sucrose will be evaluated to assess absorption through the stomach, but is expected to be zero throughout the study. To test the hypothesis, all dogs will be evaluated at the end of the wash-out acclimation diet, and after each of the three challenge diets. The purpose is to demonstrate that only those dogs with dietary hypersensitivity will have altered gastrointestinal mucosal permeability and function, ultimately leading to an increase in absorption of glycoproteins which favors increased production of IgE. It is expected that only those dogs with clinical dietary intolerance will have abnormal gastrointestinal permeability and mucosal function. Further, it is expected that those dogs with abnormal gastrointestinal permeability and mucosal function values will become normal when a hydrolyzed soy diet is fed.

CHAPTER II

MATERIALS AND METHODS

Study Design

The study was conducted as a twenty seven week cross-over feeding trial. All dogs were fed a diet consisting of pelleted Brewer's rice and egg for 6 weeks. By definition, the diet was an elimination diet meaning that it contained none of the ingredients the study dogs were sensitized to via injection. This diet was complete and balanced and was fed at ½ kg. of dry food per dog per day. Each dog was weighed weekly and the amount of food was modified to prevent either weight gain or loss during the study. Water was provided ad libidim. At the end of the six week elimination diet, samples were collected, intradermal testing and the sugar study was done. The dogs were randomly split into three groups of five, containing both sensitized and non-sensitized control dogs. Each group was fed one of the three challenge diets for three weeks. The challenge diets were: 1) hydrolyzed soy and corn starch, 2) whole soy and corn starch, and 3) corn and corn starch. Each of the diets was pelleted and formulated to be complete and balanced. Complete nutritional information is provided in the appendix. The investigators were blinded with respect to which diet was being fed. Provisions were arranged for the care of a dog that may have an adverse reaction to any of the diets. Because the study dogs would be consuming food items that they had been sensitized to, there was a possibility that vomiting, diarrhea, or anaphylaxis could occur. After sample collection and testing, all dogs were again fed the elimination diet for six weeks. The groups were then fed one of the challenge diets for three weeks. Samples

again were collected and testing was performed after the challenge. This process continued until each group consumed all three of the challenge diets. Samples were collected after the first six week elimination diet period and then after each challenge, for a total of four collections.

Study Subjects

Eight sexually intact dogs (2 male, 6 female) from a single litter were sensitized to six food allergens including: corn, beef, chicken, wheat, soy, and cow's milk. These dogs were from a colony of highly inbred mixed breed dogs selected for their ability to produce high levels of serum IgE. Seven sexually intact, mixed breed, adult dogs (3 male, 4 female) with no history of adverse food reactions or clinical evidence of illness were selected to serve as control dogs. At the time of the study, all dogs were between 1.5-2 years of age.

Dog Sensitization Protocol

Dogs were injected on newborn day 1 subcutaneously (SQ) in the left axilla with: 0.1ml beef 1:10,000 (beef G 1:20, diluted with sterile saline), and 0.05ml alum; 0.1ml chicken 1:10,000 (chicken meat G 1:20, diluted with sterile saline) and 0.05ml alum; 0.1ml corn 1:10,000 (corn food G 1:40, diluted with sterile saline) and 0.05ml alum; 0.1ml cow's milk 1:10,000 (cow's milk G 1:40, diluted with sterile saline) and 0.05 ml alum. The dogs were also injected newborn day 1 subcutaneously (SQ) in the right axilla with: 0.1ml house dust mite mix (GS mite mix 1:100 diluted with sterile saline) and 0.05ml alum, 0.1ml soybean 1:10,000 (soybean food G 1:40, diluted with sterile saline) and 0.05ml alum, 0.1ml Western ragweed mix 1:10,000 (Western ragweed mix

1:40, diluted with sterile saline) and 0.05ml alum, and 0.1ml wheat 1:10,000 (whole wheat 1:20, diluted with sterile saline) and 0.05ml alum. All allergens were purchased from a single source [a] as stock solutions and diluted for sensitization. At ages 3,7, and 11 weeks of age, the puppies were vaccinated SQ in the dorsal scapular region with 1.0ml of a modified live virus canine distemper-adenovirus type 2- parainfluenza vaccine[b]. The vaccine was given not only for preventative health issues, but to serve as an immune stimulant to favor a t-helper 2, antibody response that favors the production of IgE. One and seven days after each vaccination, the dogs received the same food, mite, and pollen allergen extracts SQ (same dosages and site) that they received as newborns. Thereafter, the dogs were given the same allergen extracts SQ (same dosages and site) every eight weeks. Vaccinations were given annually. During the study, the dogs were given the allergens (SQ) immediately prior to feeding the elimination diet of each phase of the study (every 9 weeks) for consistency.

Intradermal skin testing

Whole freeze- dried allergens were purchased for each of the tested foods; corn, chicken, beef, wheat, soybean, and cow's milk from Greer Laboratories[a]. Each was diluted to a concentration of 1mg/ml with sterile buffered saline. Phenol was used in the diluent as a preservative. Serial dilutions were made of each food allergen for testing purposes. The dilutions were: 1:10, 1:100, and 1:1000. Briefly, 0.5ml of stock diluted allergen was added to 4.5ml sterile buffered saline. The process was continued until the testing dilutions were prepared. Each vial was labeled with a number corresponding to each of the food items and the dilution concentration. Once all testing vials were

prepared, they were placed into a bag and randomly placed in order in a testing rack. The first sample was always the negative control (sterile buffered saline with phenol) and the positive control was histamine phosphate diluted to 1:10,000 in sterile buffered saline. The allergens and positive control were prepared fresh before each testing procedure to ensure consistency in potency. Immediately prior to intradermal testing, 0.05 ml of allergen was drawn into a syringe with a 26 gauge intradermal bevel needle, and placed in numeric order corresponding to the allergen vial. The negative and positive controls were always the first and second injections accordingly; however, the allergen and dilution were blinded to the investigator. The code was not broken until the completion of the study.

Each dog was fasted overnight prior to sedation. Glycopyrrolate (2.5µg/kg, SQ) was given approximately 20 minutes prior to sedation to prevent bradycardia during anesthesia. An indwelling 20gauge 1.5 inch intravenous catheter was placed into either the right or left cephalic vein using aseptic technique. Thiopental 5% (20mg/kg) was then administered to achieve sedation enough to allow for intubation. The dog was then intubated with a sterile trachea tube and maintained on isoflurane anesthetic (2%) with oxygen using a non-rebreathable system for the procedure. The lateral chest wall was clipped using a #40 clipper blade in an area approximately 8 X 8 cm. Intradermal injections (0.05ml) were made for each allergen and dilution along with the negative and positive controls. After 15 minutes, the size of the wheal was measured along two axis and the mean wheal diameter size was recorded. Each wheal was also evaluated subjectively. A score of 0 was given for the negative control, while a score of 4 was

given to the positive control. The wheal was scored based upon size, color (redness), and turgidity, compared to the positive control. A score of 2 or higher indicated a significant positive reaction. For measured wheals, a mean diameter size greater than equal to ½ of the histamine positive control is considered a positive reaction. The subjective score is accepted as a standard for evaluation when the evaluator has expertise interpreting these test results. Descriptive statistics were used to evaluate the data.

IgE Semi-quantitation

At each sample collection time, 12cc of whole blood was collected for soy IgE antibodies. The sample was collected via jugular venipuncture using a 12cc syringe and 20 gauge needle using aseptic technique. The blood was transferred to a sterile red-top vacutainer and refrigerated until transferred to the laboratory. The whole blood was spun down in a centrifuge and the serum was separated from the blood cells. Each sample was labeled and frozen at -20° C until analyzed together at the end of the study. The samples were labeled and shipped cold, on dry ice to Greer Laboratories[a] for analysis. All samples were analyzed blind. An ELISA was performed and the values were expressed as a percentage of relative value (%R) compared to a pooled sample of clinically normal dogs. Reference serum of soy allergic dogs is not available. A Students T-test was used for statistical analysis with a p value of 0.05 considered significant.

Protein Separation and Western Blot Analysis

Whole organically grown soybeans (*Glycine max* spp.*soja*) were purchased from a local store. The beans were processed in a modified protocol described by

Awazuhara[18]. The beans were ground in a commercial food mill under a hood to prevent inhalation. 5 grams of ground soy was added to 20ml of N-Hexane and agitated for 30 minutes. The sample was poured through a filtering apparatus with a 48 mesh size. The solvent was discarded and the soy powder was re-suspended in 20ml of N-Hexane, agitated for 1 hour, and again filtered as above. The soy powder was then suspended in 50ml of ultrapure H_2O, and gently rocked for 1 hour. At that time, the sample was placed in a Beckman Microfuge at 10,000 x *g* for 10 minutes. The sample was again filtered through a 48 mesh with suction. This time the supernatant was saved and the soy powder was discarded. The supernatant was then centrifuged at 10,000 x *g* for 30 minutes at 20°C. The solution was again filtered, this time with a 2μ pore filter with suction. The sample was transferred to a beaker and adjusted to a pH of 4.6 using 0.5M HCl. The sample was again centrifuged at 10,000 x *g* for 30 minutes at 20°C. Whole soy was now fractionated into its two protein classes. The supernatant was the whey fraction and the precipitate was the globulin fraction.

Each sample fraction was dialyzed in 4L of ultra pure H_2O, refrigerated, with gentle stirring, for 3 days. The dialysis membranes contained 10kD pores. Samples of each fraction (globulin, whey) were transferred to Eppendorff tubes and frozen at -80°C. The protein concentrations were determined by using a bicinchoninic acid protein assay (Pierce, Rockford IL) and a Dynatech microplate reader (MRX). The globulin concentration was 0.8mg/ml, and the whey was 1mg/ml.

Proprietary hydrolyzed soy powder was obtained from Ralston Purina Inc. The powder was finely ground and required no further processing. The same steps were

performed for separation into globulin and whey fractions. Although the hydrolyzed soy apparently had the whey removed, a sample of whey protein was present.

All four samples (globulin, hydrolyzed globulin, whey, and hydrolyzed whey) were again measured for protein content using optical density and computer statistical analysis. The final concentrations were: globulin 1.82mg/ml, whey 1.79mg/ml, hydrolyzed globulin 7.44mg/ml, and hydrolyzed whey 13.34mg/ml.

Protein Electrophoresis using SDS-PAGE

One dimensional SDS-PAGE was performed using a Bio Rad Laboratories mini-gel apparatus. A 15% sodium dodecyl sulfate polyacrylamide gel and stacking gel was prepared fresh, as needed for the experiments. The volume prepared was enough for 2 gels. A 10% solution of ammonium persulfate was prepared with anhydrous ammonium persulfate granules diluted in sterile water. In a sterile beaker, 3.75ml of sterile water was added to 7.5ml acrylamide/ Bis (30%/0.8%). 3.75ml of 4xTris HCl (1.5M), containing 0.4% SDS, at pH 8.8 was added along with 0.05ml of the prepared 10% ammonium persulfate solution. The mixture was set aside while the glass plate sandwich was prepared. 1.0mm spacers were used in preparing the mini gels. Immediately before pouring the gels, 0.015 ml of Temed was added. 4.5 ml was quickly transferred to the glass plate sandwich. Finally, 3ml of sterile water was placed on the solution to remove any bubbles that may have formed. The gels were allowed to polymerize for 30 minutes. Meanwhile, the 4% stacking gel solution was prepared. In a vial, 3.05ml of sterile water was added to 0.6 ml of acrylamide/Bis (30%/0.8%). 1.25 ml of 4x Tris HCl (0.5M), containing 0.4% SDS, at pH 6.8 was added along with 0.05 ml of

the prepared 10% ammonium persulfate solution. Temed 0.015 ml was added immediately before pouring. Once the gel was set, the water was poured off. The stacking gel solution was added to the top of the gel. A 15 well comb was inserted.

Proteins were prepared for electrophoresis. Globulin and whey fractions were diluted 1:2 in sample buffer. The hydrolyzed globulin was diluted 1:4, and the hydrolyzed whey was diluted 1:8 in sample buffer. The samples were placed in a boiling water bath for 3 minutes for denaturing. 15µl was placed into each well for electrophoresis. Prestained marker was prepared using 5µl diluted in 100 µl of sample buffer. The marker was added to every 5^{th} lane.

The glass plate gel sandwiches were placed into the electrophoresis apparatus and the chamber was filled with 1x SDS electrophoresis buffer. The chamber was then attached to a power supply at constant 60 mili-amp current for 35 minutes or until the blue dye of the pre-stained marker reached the bottom of the gel. For evaluation of the protein separation, the gels were placed in a container and flooded with 0.1% Coomassie blue in 50% methanol and 10% acetic acid (Sigma Chemical Co, St. Louis MO) for one hour, with gentle shaking. The solution was removed and a destaining solution containing 10% ethanol, 7% acetic acid, and distilled water was added. Destaining with shaking was performed for 12 hours. The gel was dried on filter paper. Staining and destaining was not performed when the proteins were to be transferred for immunoblotting.

Protein electrophoresis was completed as above. An immunoblot sandwich was prepared. A Scotch-Brite pad (3M Co., St Paul MN) followed by a sheet of filter paper

pre-moistened with transfer buffer (0.02 M Tris, 0.15 M NaCl at a pH of 7.4 (Sigma Chemical Co. St. Louis MO) with 0.04% NaN_3 (Mallinckrodt Inc. Paris KY) as a preservative) was placed on the bottom ½ of the cassette. The gel was gently removed from the electrophoresis apparatus and placed on the filter paper. Transfer buffer was used to keep the gel moist. A pipette was rolled over the gel to remove any air bubbles. The transfer membrane was cut slightly larger than the size of the gel to enhance complete transfer. It was placed into 100% methanol for 30 seconds and rinsed three times with ultra-pure water. The membrane was then soaked in transfer buffer until being applied on top of the gel. A pipette was rolled over the membrane and gel to remove air bubbles and enhance contact. A moistened filter paper and sponge were stacked on top and the cassette was closed under moderate pressure. The membrane was placed to face the anode (positive side) when placed into the transfer tank. Transfer buffer was added to the tank to fill above the level of the cassette. The tank was attached to the power source and run at constant voltage (10 volts) overnight.

The membrane remained in the transfer buffer until ready for the immunoblot procedure to avoid drying. To confirm that the proteins were transferred, a sample of the membrane was placed in Coomassie blue stain solution for 30 seconds followed by rinsing in 10% methanol to decolorize.

A blocking solution was prepared using 1.5 grams powdered non-fat milk into 50 ml of calcium and magnesium free phosphate buffered saline (PBS, GibcoBRL Grand Island NY) and 25µl Tween 20 (0.05%). The solution was gently mixed until homogenous. A solution was prepared for washing using 10% PBS 100mls and 50µl

Tween 20. The transfer membrane was cut to include 5 lanes: pre-stained marker, globulin, hydrolyzed globulin, whey, and hydrolyzed whey. Each piece was placed into a shallow tray and 10 ml of the blocking mixture was added with gentle shaking for 2 hours. The sample was then washed three times, for 3 minutes with shaking, using the washing solution. The antibody solution was prepared for each dog using a 1:100 concentration (50µl of serum in 5ml blocking solution) and added to the membrane. Samples were shaken for 2 hours. Again, washing was performed as above. Goat anti-canine IgE (Bethel Laboratories) conjugated with alkaline phosphatase was diluted to 1:2500 (3.2µl in 8ml blocking solution), and 4 ml were added to each sample. The samples were shaken for 1 hour and then again washed. SigmaFast 5-bromo-4chloro-3indoyl phosphate / nitro blue tetrazolium (BCIP/NBT) (Sigma Chemical Co., St. Louis MO) tablets were diluted (1 tab in 10ml distilled H_2O) and 4 ml were added to each sample for 1 hour with shaking. The samples were then washed with distilled H_2O to stop the reaction and allowed to dry in a dark environment. Descriptive statistics were used to evaluate the data.

Sugar Absorption Study

All dogs were fasted overnight, but were allowed free access to water. The solution containing monosaccharide (methylglucose, rhamnose, xylose) and disaccharide (sucrose, lactulose) sugars were prepared freshly each day using tap water. Each 100 ml solution contained 1.0 g methylglucose, 2.5 g L-rhamnose, 2.5 g D-xylose, 10 g sucrose and 2.5 g lactulose. It was iso-osmotic at about 293mOsm. The volume given to the dog was based upon body weight: dogs less than 20kg received 200ml while dogs

greater than 20kg received 400ml of the solution. The dog was physically restrained while a urinary catheter was placed. All the urine was removed from the bladder. A 10ml sample of urine was saved for analysis and 10 µl of 0.1g/L NaN_3 (sodium azide) was added to each urine sample as a preservative. The dog was restrained as an orogastric tube was placed and the sugar solution was administered. The dog was placed in a metabolic cage for six hours with water withheld. Following the incubation period the dog was re-catheterized and the entire volume of urine was collected. If the dog had urinated in the cage, the urine was collected and measured. A 10 ml sample was saved with 10µl of sodium azide added as a preservative. After each sample collection period, each dog received a single injection of amoxicillin SQ (5mg/kg) to reduce the risk of urinary tract infection.

Urine samples were processed by the Gastrointestinal Laboratory at Texas A&M University. High pressure liquid chromatography followed by pulsed amperometric detection was used for sugar recovery[31]. Urinary sugar recoveries were expressed as percentage recovery for each sugar. The lactulose:rhamnose (assessment of gastrointestinal mucosal permeability)and xylose:methylglucose (assessment of gastrointestinal mucosal absorption) recovery ratios were calculated. Mean urinary recoveries for each sugar and mean urinary recovery ratios were compared between sensitized and control dogs using two-tailed T tests and ANOVA. A p- value of 0.05 was considered significant.

CHAPTER III

RESULTS

The first objective was to evaluate the effect of diet on sensitized and non-sensitized dogs. The original diet prepared as the "wash-out" diet (elimination diet) consisted of pelleted egg and rice flour. Although this diet was palatable and did not cause any dogs to vomit or to lose weight, all the dogs in the study developed diarrhea. All dogs, both sensitized and non-sensitized controls, developed watery feces consistent with large bowel diarrhea. At the end of the 6 week period, samples were collected and intradermal skin testing was performed. A new diet was formulated using Brewer's rice instead of rice flour and the wash-out feeding trial was re-started. The data from those samples were reported (see appendix). It also provided a chance to determine if further modification was required.

During the study, after switching to the egg and Brewer's rice diet for washout, none of the control dogs developed diarrhea. All the sensitized dogs developed diarrhea during some phase of the study. No particular diet was associated with the development of diarrhea. In fact, some sensitized dogs developed intermittent diarrhea while on the elimination diet. This was consistent with the prior observations that the sensitized dogs had intermittent diarrhea before the study was started. Thus, no conclusions could be made about the effects of the diet on the development of diarrhea.

Intradermal Skin Testing

The specific aim of this study was to test the hypothesis that only those dogs sensitized to whole soy allergen would develop wheal and flare reaction consistent with

a Type I hypersensitivity reaction. None of the control dogs developed a clinically significant positive reaction to intradermally injected soy antigen at 1:10, 1:100, or 1:1000 W/V during any phase of the study. The data were pooled over the entire study to evaluate the best testing concentration. (Table 1)

It was hypothesized that diet would have an effect on detecting clinically significant positive skin test results to soy. The total number of clinically significant reactions throughout the study at any testing concentration was 50. (Table 2) None of the control dogs had positive reactions to soy antigen. Only one sensitized dog (108) did not exhibit any clinically significant reactions to intradermally injected soy throughout the study. Of the 50 positive reactions, 8 occurred after feeding the washout diet (16%), 14 occurred after the hydrolyzed soy diet was fed (28%), 14 occurred after feeding the whole soy diet (28%), and 14 occurred after the corn based diet was fed (28%).

It was hypothesized that serum IgE specific to soy would increase after a whole soy diet was fed, and decrease when a hydrolyzed soy diet, a corn based diet, or an elimination diet was fed. In other words, consuming whole soy would increase IgE to soy in sensitized dogs. Serum levels of IgE to soy were reported as percentage of relative value (%R), compared to normal pooled canine serum. (Figure 1) There was a statistically significant difference ($p=0.0003$) between the control dogs and sensitized dogs for each diet including the washout diet. There were no statistically significant differences in IgE levels to soy when the washout diet and three challenge diets were compared among the sensitized dogs. The same was true for the non-sensitized control dogs.

Table 1 Number of clinically significant positive reactions to intradermal injections of soy protein at 1:10, 1:100, and 1:1000 W/V dilutions for all diets including the elimination diet, hydrolyzed soy, whole soy and corn based diet

	1:10		1:100		1:1000	
	Posititve	Negative	Positive	Negative	Postitive	Negative
Sensitized	25	7	20	12	5	27
Control	0	28	0	28	0	28
Total	25	35	20	40	5	55

Table 2 "X" indicates a significant positive reaction to intradermally injected soy antigen at each dilution for each diet. Dogs 101-108 were sensitized dogs. Non-sensitized control dogs did not have any significant positive reactions at any time during the study

Dogs	Elimination Diet			Soy			Hydrolyzed Soy			Corn			Total
	1:10	1:100	1:1000	1:10	1:100	1:1000	1:10	1:100	1:1000	1:10	1:100	1:1000	
101	X			X			X	X		X	X		
102	X	X		X	X		X	X		X	X		
103				X	X		X	X		X	X	X	
104	X			X	X	X	X	X		X	X		
105	X			X	X		X	X	X	X	X		
106	X	X	X	X	X		X	X		X	X	X	
107				X	X		X						
108													
Subtotal	5	2	1	7	6	1	7	6	1	6	6	2	
Total		8			14			14			14		50

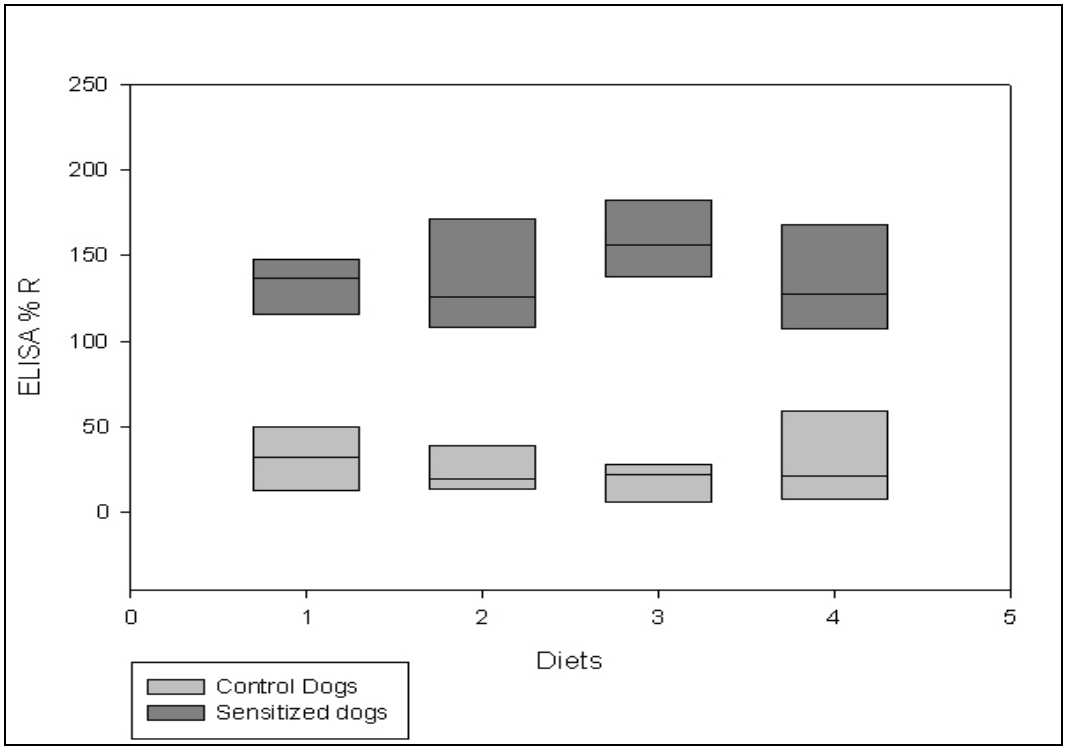

Figure 1 Box plot comparing ELISA values to soy, represented by relative percentage (%R) compared to pooled canine serum, in sensitized versus non-sensitized control dogs. Diet 1= elimination diet, Diet 2= hydrolyzed soy, Diet 3= soy, Diet 4= corn. The range and median are shown.

Western Blot Analysis

Western blots were performed for each patient after the washout diet and after each challenge. IgE binding was assessed compared to known major IgE binding proteins to soy in humans. Coomassie Blue stain was used on the transfer membrane to ensure protein transfer (Fig.2). Pre-stained molecular markers were used to estimate protein bands for the globulin and whey fractions of soy.

The major protein bands identified in the globulin fraction were 34 kD, 28 kD, 30 kD, 43-45 kD, and minor bands between 51-90kD. Because the hydrolyzed fraction of the globulin protein produced a smear of protein less than 28 kD, no discrete bands were identified. Whey binding was limited to the LMW and HMW components, and inconsistent binding between 37-90 kd. No discernable binding was present within the hydrolyzed whey fraction. An exceptional example of IgE binding was represented by dog 106 after being fed a whole soy diet (Fig 3). The molecular weight markers are in lane 1, the globulin fraction in lane 2, the hydrolyzed globulin fraction in lane 3, the whey fraction in lane 4 and the hydrolyzed whey fraction in lane 5.

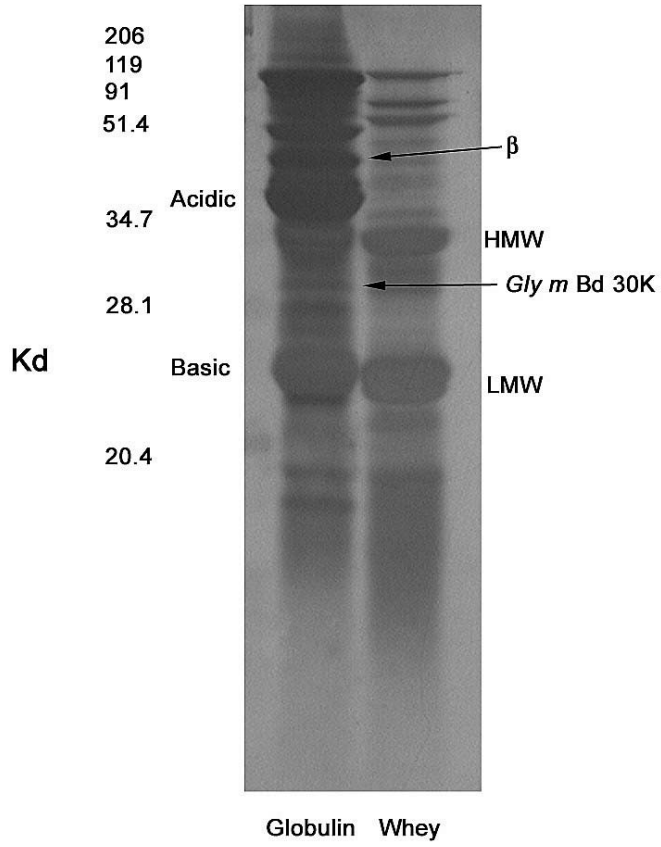

Figure 2 Coomassie stained membrane of globulin and whey fractions of whole soy. Molecular weigh markers kD are identified on the left lane. Important allergenic peptides in the globulin fraction are the acidic, basic, β, and Gly m Bd 30K. Important whey components are the high molecular weight peptide (HMW) and low molecular weight peptide (LMW)

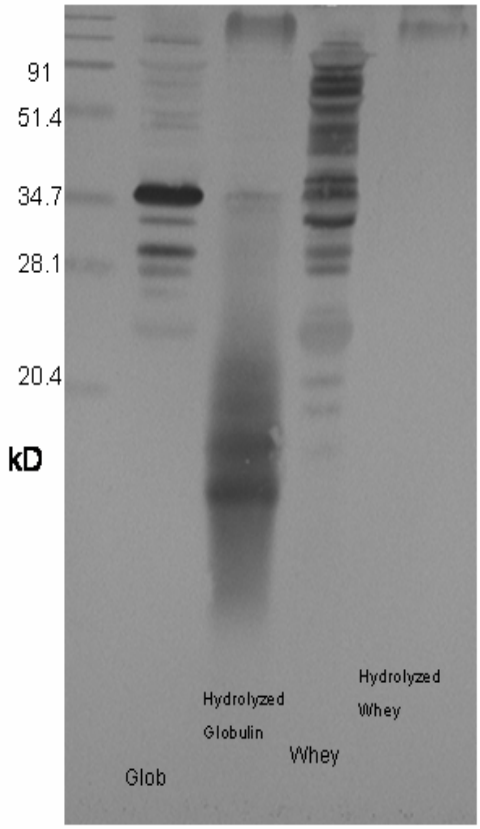

Figure 3 Western blot of serum IgE of sensitized Dog #106, to various fractions of soy. Lane 1= pre-stained markers, Lane 2=globulin fraction of whole soy, Lane 3=hydrolyzed globulin fraction of soy, Lane 4=whey fraction of whole soy, Lane6=hydroloyzed whey fraction of soy. Anti-canine IgE was labeled with alkaline phosphatase.

Sugar Absorption Study

The results of the sugar study were expressed in percentages of recovery from the total urine collected after 6 hours. All preliminary samples were zero and served as a negative control. These data are not shown. Table 3,4,5,and 6 represent the data for each dog and each diet over the course of the study. They represent the washout diet and each subsequent challenge diet respectively. The #101-108 represent sensitized dogs while #201-207 represent non-sensitized control dogs.

The effect of diet was compared for all dogs (sensitized and non-sensitized control dogs) for each sugar using unpaired t-test (Table 7). There were no statistically significant differences for any sugar nor for the lactulose:rhamnose (L:R) or xylose: methylglucose (X:M). The following p values were calculated for each sugar.

It was hypothesized that diet would cause alterations in gastrointestinal mucosal permeability and function in sensitized, but not non-sensitized control dogs. Unpaired t-tests were used to compare sensitized versus non-sensitized control dogs for each sugar and each diet (Table 8).

Table 3 Sugar absorption values for each dog after being fed the elimination diet. Dogs 101-108 represent sensitized dogs while dogs 201-207 represent controls. Mr= % methylglucose recovery, Rr= % rhamnose recovery, Xr= % xylose recovery, Sr= % sucrose recovery, Lr= % lactulose recovery, L/R= calculated ratio of lactulose to rhamnose recovery, X/M= calculated ratio of xylose to methylglucose.

Dog	Elimination Diet						
	Mr. %	Rr. %	Xr. %	Sr. %	Lr. %	L/R %	X/M %
106	43.83	14.06	27.18	0.00	1.78	0.13	0.62
104	69.12	24.84	36.18	0.00	1.35	0.05	0.52
107	59.47	23.75	30.69	0.00	1.24	0.05	0.52
105	67.51	27.36	40.08	0.00	1.65	0.06	0.59
101	49.04	13.02	32.24	0.00	1.05	0.08	0.66
102	14.46	4.10	10.47	0.00	0.18	0.04	0.72
103	63.24	24.66	38.46	0.00	0.84	0.03	0.61
108	55.66	14.72	36.80	0.00	0.92	0.06	0.66
201	71.76	31.98	38.71	0.00	2.44	0.08	0.54
202	5.60	5.45	4.40	0.00	0.73	0.13	0.79
203	77.63	35.16	39.88	0.00	3.21	0.09	0.51
204	32.41	24.43	29.43	0.00	19.66	0.80	0.91
205	67.68	27.00	36.84	0.00	1.68	0.06	0.54
206	49.89	18.28	31.44	0.00	1.22	0.07	0.63
207	66.19	23.95	42.70	0.00	1.47	0.06	0.65
Normal Range	32.8 - 81.0	17.3 - 42.6	16.0 - 43.8	0.0 - 0.6	1.5 - 5.8	0.05 - 0.15	0.4 - 0.59

Table 4 Sugar absorption values for each dog after being fed the hydrolyzed soy diet. Dogs 101-108 represent sensitized dogs while dogs 201-207 represent controls. Mr= % methylglucose recovery, Rr= % rhamnose recovery, Xr= % xylose recovery, Sr= % sucrose recovery, Lr= % lactulose recovery, L/R= calculated ratio of lactulose to rhamnose recovery, X/M= calculated ratio of xylose to methylglucose.

Dog	Hydrolyzed Soy						
	Mr. %	Rr. %	Xr. %	Sr. %	Lr. %	L/R %	X/M %
106	96.12	48.65	45.54	0.00	3.74	0.08	0.47
104	58.93	23.33	34.13	0.00	6.95	0.30	0.58
107	13.71	9.36	8.39	0.00	0.63	0.07	0.61
105	141.91	60.84	62.56	0.00	4.37	0.07	0.44
101	39.40	10.02	23.18	0.00	0.34	0.03	0.59
102	25.08	7.68	18.75	0.00	1.05	0.14	0.75
103	28.64	12.12	17.64	0.00	2.98	0.25	0.62
108	53.50	14.94	29.77	0.13	0.83	0.06	0.56
201	57.99	22.36	32.94	0.00	1.19	0.05	0.57
202	0.00	0.00	0.00	0.00	0.00	0.00	0.00
203	31.63	14.56	31.19	0.00	2.06	0.14	0.99
204	27.74	9.68	13.48	0.00	1.05	0.11	0.49
205	54.56	17.28	29.61	0.00	1.63	0.09	0.54
206	23.90	9.90	19.50	0.00	0.70	0.07	0.82
207	38.42	14.63	24.62	0.00	1.66	0.11	0.64
Normal Range	32.8 - 81.0	17.3 - 42.6	16.0 - 43.8	0.0 - 0.6	1.5 - 5.8	0.05 - 0.15	0.4 - 0.59

Table 5 Sugar absorption values for each dog after being fed the whole soy diet. Dogs 101-108 represent sensitized dogs while dogs 201-207 represent controls. Mr= % methylglucose recovery, Rr= % rhamnose recovery, Xr= % xylose recovery, Sr= % sucrose recovery, Lr= % lactulose recovery, L/R= calculated ratio of lactulose to rhamnose recovery, X/M= calculated ratio of xylose to methylglucose.

Dog	Whole Soy						
	Mr. %	Rr. %	Xr. %	Sr. %	Lr. %	L/R %	X/M %
106	32.75	9.41	20.59	0.00	0.78	0.08	0.63
104	49.66	25.29	37.31	0.00	1.50	0.06	0.75
107	40.09	16.91	18.57	0.00	1.90	0.11	0.46
105	58.14	18.70	35.28	0.00	2.38	0.13	0.61
101	34.96	11.59	20.79	0.00	1.35	0.12	0.59
102	54.60	13.28	35.70	0.00	0.68	0.05	0.65
103	69.09	32.95	21.27	0.00	6.05	0.18	0.31
108	92.29	17.29	60.69	0.00	4.35	0.25	0.66
201	58.24	21.94	30.23	0.00	2.06	0.09	0.52
202	124.25	45.67	66.39	0.00	5.80	0.13	0.53
203	110.50	54.40	51.98	0.00	14.25	0.28	0.47
204	0.00	0.00	0.00	0.00	0.00	0.00	0.00
205	52.52	23.50	36.14	0.00	1.95	0.08	0.69
206	46.36	15.71	29.59	0.00	0.92	0.06	0.64
207	40.73	19.80	36.90	0.00	1.86	0.09	0.91
Normal Range	32.8 - 81.0	17.3 - 42.6	16.0 - 43.8	0.0 - 0.6	1.5 - 5.8	0.05 - 0.15	0.4 - 0.59

Table 6 Sugar absorption values for each dog after being fed the corn based diet. Dogs 101-108 represent sensitized dogs while dogs 201-207 represent controls. Mr= % methylglucose recovery, Rr= % rhamnose recovery, Xr= % xylose recovery, Sr= % sucrose recovery, Lr= % lactulose recovery, L/R= calculated ratio of lactulose to rhamnose recovery, X/M= calculated ratio of xylose to methylglucose.

Dog	Corn diet						
	Mr. %	Rr. %	Xr. %	Sr. %	Lr. %	L/R %	X/M %
106	40.31	14.28	22.77	0.00	2.07	0.14	0.56
104	60.65	26.72	34.52	0.00	1.76	0.07	0.57
107	15.51	9.78	6.53	0.00	1.27	0.13	0.42
105	29.96	19.74	32.76	0.00	1.96	0.10	1.09
101	113.04	31.24	67.60	0.00	1.64	0.05	0.60
102	27.18	10.68	6.44	0.00	0.90	0.08	0.24
103	46.06	16.17	28.00	0.00	0.63	0.04	0.61
108	8.13	7.13	5.06	0.00	0.81	0.11	0.62
201	68.46	35.82	33.21	0.00	2.43	0.07	0.49
202	0.00	0.00	0.00	0.00	0.00	0.00	0.00
203	50.75	16.50	32.33	0.00	1.59	0.10	0.64
204	31.58	12.70	14.69	0.00	0.99	0.08	0.47
205	59.17	22.11	31.72	0.00	2.82	0.13	0.54
206	35.48	12.86	22.73	0.00	0.98	0.08	0.64
207	59.46	23.91	36.78	0.00	1.64	0.07	0.62
Normal Range	32.8 - 81.0	17.3 - 42.6	16.0 - 43.8	0.0 - 0.6	1.5 - 5.8	0.05 - 0.15	0.4 - 0.59

Table 7 Pooled data for all dogs for percentage recovery of each sugar and each diet. Values represent the mean value for each sugar. P values were calculated for each sugar using Student's T tests. There were no statistically significant differences. Mr= % methylglucose recovery, Rr= % rhamnose recovery, Xr= % xylose recovery, Sr= % sucrose recovery, Lr= % lactulose recovery, L/R= calculated ratio of lactulose to rhamnose recovery, X/M= calculated ratio of xylose to methylglucose.

	All Dogs Mean Vaules						
	Mr %	Rr %	Xr %	Sr %	Lr %	L/R %	X/M %
Elimination	52.90	20.90	31.70	0.00	2.63	0.12	0.63
Hydrolyzed Soy	46.10	18.40	26.10	0.00	1.95	0.10	0.58
Soy	57.60	21.60	33.40	0.00	3.06	0.11	0.56
Corn	43.10	17.30	25.00	0.00	1.43	0.08	0.54
P Value	0.51	0.72	0.30	0.00	0.54	0.84	0.63

Table 8 P values were calculated using unpaired Student's T test. Each value represents the comparison of sensitized to control dogs for each sugar and each diet throughout the study. There were no statistically significant differences (p=0.05). Mr= % methylglucose recovery, Rr= % rhamnose recovery, Xr= % xylose recovery, Sr= % sucrose recovery, Lr= % lactulose recovery, L/R= calculated ratio of lactulose to rhamnose recovery, X/M= calculated ratio of xylose to methylglucose.

		P Values						
		Mr. %	Rr. %	Xr. %	Sr. %	Lr. %	L/R %	X/M %
Elimination	Sensitized Control	0.98	0.26	0.95	0.00	0.20	0.23	0.50
Hydrolyzed Soy	Sensitized Control	0.34	0.33	0.54	0.00	0.23	0.49	0.23
Soy	Sensitized Control	0.30	0.08	0.20	0.00	0.30	0.98	0.59
Corn	Sensitized Control	0.58	0.44	0.74	0.00	0.32	0.92	0.83

CHAPTER IV

DISCUSSION AND CONCLUSIONS

The first specific aim of the study was to evaluate the clinical effects of feeding a hydrolyzed soy protein diet to dogs sensitized to whole soy. It was not possible to determine that diet had an effect on clinical symptoms. This was because all of the sensitized dogs had diarrhea of varying degrees on all of the diets including the elimination washout diet. None of the non-sensitized control dogs developed diarrhea or skin signs during any phase of the study. There were no observable changes in skin signs including pruritus throughout the study. All of the sensitized dogs had mild pruritus and none developed secondary bacterial infections or otitis. Dogs with clinical evidence of food hypersensitivity usually have moderate to severe pruritus with secondary complications. From the observable changes in the atopic dog model, it can be concluded that they did not represent spontaneously occurring dietary hypersensitivity associated with skin signs in the canine population.

Dogs with clinical evidence of dietary hypersensitivity usually improve when fed an elimination diet when diarrhea is the primary sign. This model did not demonstrate clinical improvement in diarrhea when fed an elimination diet or hydrolyzed soy diet. This may be because the elimination diet was fed for only six weeks and may have not been long enough. Another possibility is that the sensitized dogs had other gastrointestinal illness not relating to dietary hypersensitivity. From the clinical observations it was concluded that the atopic dog model did not share characteristics of dogs with clinical dietary hypersensitivity.

The results of the intradermal skin testing using soy antigen indicated that there was high sensitivity and also high specificity for the dogs in this study. Only those dogs that were sensitized to soy antigen exhibited clinically significant wheal and flare when injected with whole soy antigen. The results also demonstrated that at the 1:10 concentration there was 84% sensitivity. Sensitivity decreased with increased dilution factors. Specificity at all concentrations was 100%. It is intuitively obvious that higher concentrations would yield more clinically significant positive reactions. It is also reasonable to expect that there might be some false positive reactions at higher concentrations but this was not the case in the seven non-sensitized control dogs. These data are directly in conflict with those in the literature indicating that intradermal testing in both humans and dogs is highly specific but not very sensitive[2,34]. The sample size in this study was very small (15 dogs) and may not represent the true population. Also, our model is developed from dogs sensitized to soy by subcutaneous injections compared to the humans and dogs in the aforementioned studies that were sensitized by the oral route. There may be a clinically significant difference between the induction of IgE production by injection compared to oral route. This can be explored further using the same colony of dogs used for the study and comparing the changes in specific IgE to food items administered orally or injected as in our current model. Nonetheless, from the current data it could be concluded that the best sample concentration for intradermal skin testing is 1:10 because it represented the concentration with both the highest sensitivity and specificity. But because this test only detects the IgE mediated aspect of dietary hypersensitivity, it has little value in a clinical setting.

None of the non-sensitized control dogs had any clinically significant reactions to intradermal injections of soy antigent at any dilution during the study. False positive reactions were not a concern at these testing dilution concentrations. None of the sensitized dogs had reactions at weaker dilutions (1:1000 W/V) without concurrently having a positive reaction to the stronger concentrations. Because all the samples were blinded, these data demonstrate that greater sensitivity is seen at higher concentrations of injected soy antigen. When comparing the number of clinically significant positive reactions to soy, diet did not appear to have an impact. This may be due to the route of administration of the sensitizing antigen. Although our sensitized dogs produced significantly higher levels of soy specific IgE compared to the control population, oral challenges made no significant difference. This may be because the oral administration did not induce the production of circulating soy- specific IgE. Recent studies in humans have shown that there can be significant production of specific IgE in the gut without concurrent increases in circulating antibodies[22]. An alternative explanation is that injected antigen is more likely to produce circulating antigen specific IgE than when given by the oral route. It is this that makes the strongest argument that the atopic dog model is not representative of the at-large population of dogs with dietary hypersensitivity.

One of the sensitized dogs (#108) did not exhibit any wheal and flare to any injection of soy antigen during the study. The likely reason for this is twofold. First, it is expected that there is heterogeneity within any sample population, even within the same litter. Dog #108 may be an outlier with respect to her ability to produce IgE specific to

soy compared to her littermates. A simple evaluation of her ELISA values (mean 133, sensitized group mean 120) did not demonstrate that her IgE %R to soy were lower than her littermates. The alternative explanation for the lack of positive reactions to intradermal skin testing is more likely due to her "stress" associated with the study and testing procedures. Endogenous cortisol can be released during anesthesia that can suppress the ability of mast cells to undergo degranulation. Overall, she had weaker and fewer clinically significant positive reactions to any of the tested antigens than her littermates. Her positive controls were adequate for testing evaluation. However, the intradermal injection of histamine is not the same as the ability of mast cells to degranulate and release histamine leading to wheal and flare reaction. There may have been differences in the amount of mast cells present within her skin or differences in those cells to regenerate histamine after degranulation takes place. These changes are independent of the observation that she had circulating levels of soy specific IgE based upon ELISA testing. Intradermal skin testing alone may be inadequate to detect elevations in IgE.

ELISA values were compared for each diet over the course of the study (Figure 1). Although there may have been a trend toward an increase in %R after feeding the soy based diet, there was no statistically significant differences. These data correlate with the findings of our intradermal skin testing results. There were significant differences between the sensitized and non-sensitized control dogs but there was no effect of diet. Because the ELISA results and the intradermal skin tests yielded similar results, it is reasonable to surmise that either test would be acceptable to differentiate

sensitized from non-sensitized control dogs. There is no data to support that these results can be extrapolated to the whole canine population. Serum ELISA values are ineffective as a tool for detecting dietary hypersensitivities in dogs[34]. This may be because those dogs with clinical dietary hypersensitivity do not have high levels of circulating IgE. Comparing the ELISA values of this study with clinically affected dogs may clarify if any differences exist. Additionally, the small number of sensitized dogs in this study precluded extrapolation to the canine population at large.

Serial dilutions of soy globulin, whey, hydrolyzed soy, and hydrolyzed whey were run on 15% SDS-PAGE to optimize protein separation (data not shown). Samples of a known high IgE producing, sensitized dog were used to titrate the appropriate amount of serum to optimize visualizing the binding patterns. Because a known reactor was used and samples were titrated downward, the specificity was increased but sensitivity may have been compromised. The final serum dilution used for the study was 1:100 in blocking solution. It was interesting to note that some of the human studies incubated the Western blots with 3ml of whole serum[18]. This made overall comparisons with our study very difficult.

Western blot analysis results were compared to known major IgE binding proteins in soy in soy allergic humans. The allergenicity of soybeans is known to reside in the protein fractions and at least 16 soybean proteins ranging in size from 14 kD to 70 kD are recognized as major allergens in humans[35]. These proteins can be divided into the globulin fraction and whey fraction. The globulin fractions are divided into four components (2S, 7S, 11S, and 15S) based upon ultracentrifugation[36]. The whey

component makes up about 10% of the total soy protein. It also contains several biologically active proteins and enzymes, such as hemagglutinin, urease, and part of the 2S fraction, including trypsin inhibitors[37,38]. These proteins and enzymes are not likely to induce clinical allergy.

The two major globulin protein components are the 7S and 11S fractions. β-conglycinin is the major 7S globulin protein and accounts for about 50% of the 7S total fraction and about 20% of the soluble protein fraction[36]. It is a trimer composed of three major subunits with molecular weights of 76 kD, 72 kD, and 53kD. Many of the sensitized and non-sensitized control dogs exhibited binding to one or more of these proteins. The 11S globulin fractions account for about 40-50% of the total storage proteins in soybeans. They consist of the acidic and basic subunits of glycinin and have corresponding molecular weights of about 37 kD and 22 kD [36]. Studies on soy allergic humans have shown these proteins to be weakly allergenic [38] and the results of our data concur.

The strongest binding patterns were at the 33-35 kD site which corresponds to the 7S globulin fraction. Another band at about 30 kD represents a unique 7S globulin fraction protein called *Gly m Bd* 30K. The last 7S globulin fraction that was important in this study was at about 28 kD. In one study Ogawa identified humans with IgE binding to proteins in soybeans and the frequency among the patients with atopic dermatitis. The frequency of binding was 15.9% (33-35 kD), 65.2% (30 kD), and 23.2% (28 kD) accordingly[35]. Five of the sensitized dogs had binding to these proteins. In another study of soy allergic humans, 50% of the patients had binding to a protein at

about 31 kD [18]. This protein may have represented *Gly m* Bd 30K as it was discovered after the aforementioned study. Together these data support the importance of the 7S globulin fractions as important allergens in both humans and sensitized dogs.

There were very few proteins identified in the globulin fraction of soy that were less than 20 kD. Proteins smaller than this size are believed to be less allergenic. When hydrolyzed soy was processed in a similar manner as whole soy, and SDS-PAGE was performed, a streak of proteins less than 35 kD was present. The majority of the protein was seen less than 20 kD all the way to the bottom of the gel. No distinct banding was seen. When Western blot analysis was performed on the hydrolyzed soy fraction, IgE binding did occur. This was most likely due to these smaller protein fragments retaining antigenic epitopes of the larger 7S proteins. It could not be concluded that hydrolyzed globulin proteins were less allergenic.

The whey fraction of soy was less allergenic based upon the intensity of bands of both sensitized and non-sensitized control dogs. There was some binding at the LMW and HMW bands but because of the large size of these protein bands, there may have been some background reaction with the conjugate. It was interesting to note that some protein bands were present on SDS-PAGE above 31 kD. Some studies do not recognize proteins of this size as part of the whey fraction[18,35] while others discuss the importance of whey proteins at 41 kD, 58 kD, 75 kD, and 91 kD as allergenic [18]. The findings of our study support the findings of the latter. This may be due to the use of similar methodology used for the separation of the globulin and whey fractions. IgE specific for these proteins was only demonstrated in the sensitized dogs. Overall, the whey fraction

was not as allergenic as the globulin fraction in sensitized dogs, and there were few positive bands for the non-sensitized dogs for either soy component.

The hydrolyzed soy protein was processed into globulin and whey fractions in a similar manner as whole soybeans. This proprietary sample apparently had the whey component removed by the hydrolyzation process. Protein could be detected by the protein assay but could not be demonstrated on SDS-PAGE. Most likely, the proteins present were small peptides that migrated off of the 15% gel. The proteins can be run at higher percentage gels to better define the molecular weight of the hydrolyzed whey fraction. No binding to serum IgE was present at any phase of the study.

It was hypothesized that dogs sensitized to soy antigen would exhibit gastrointestinal changes associated with gastric permeability and mucosal function. All dogs (sensitized and non-sensitized controls) were pooled to evaluate the effect of diet on sugar recovery for the five sugars and L:R and X:M ratios. There was no statistically significant difference ($p<0.05$) for any diet, for each sugar (Table 7). The next evaluation was to compare sensitized dogs to non-sensitized control dogs for each sugar and each diet (Table 8). There were no statistically significant differences ($p<0.05$).

Gastrointestinal mucosal permeability and function testing using sugar solutions did not reveal statistically significant differences between the two groups of dogs. Thus, the explanation for the differences in IgE to soy between the groups is likely not because of increased damage and increased absorption of soy antigen. Further, soy allergic dogs did not exhibit mucosal function changes after being fed soy diet. The rational explanation for the differences in IgE to soy must be because of the route of sensitization

(injection). It was demonstrated in previous studies that dogs with dietary hypersensitivity developed normal values after being fed an elimination diet[14]. The current study did not demonstrate differences among sensitized versus control dogs, or differences based on diet. The conclusion is that the atopic dog model is different than clinically affected dogs with dietary hypersensitivity. This atopic model demonstrates differences in IgE to soy between sensitized and non-sensitized control dogs independent of diet, but not due to gastrointestinal mucosal permeability and function testing.

Methylglucose is actively transported in the small intestine and is relatively resistant to intestinal damage. This sugar recovery percentage is an assessment of absorptive capacity. By itself methylglucose is not an assessment of intestinal mucosal function. Xylose is also actively transported and absorption decreases with disease. When methylglucose absorption is compared to xylose absorption in the ratio of X:M, the ratio provides a more useful evaluation of mucosal function. This ratio tends to decrease with small intestinal disease because xylose absorption is decreased. This demonstrates reduced absorption and decrease in mucosal function. An increase in the X:M ratio is of no known significance other than the possibility that it may represent recovery or improvement in dogs that previously had abnormal values. Three values were zero (Fig 7) because the dogs vomited the sugar solution and could not be assessed during that sample collection period. Only two dogs had a slightly decreased ratio but the overall changes were not significant for any diet, or for sensitized versus control dogs. Because there was not a statistically significant difference in mucosal function or IgE levels measured by ELISA, it is reasonable to consider that the differences between

sensitized dogs and non-sensitized control dogs was attributed to the method of sensitization.

The lactulose to rhamnose ratio (L:R) of percentage of sugar recovery in the urine is an assessment of small intestinal permeability. This ratio tends to increase with disease and is an indicator of mucosal damage. It was hypothesized that dogs sensitized to soy antigen might undergo mucosal damage when fed a whole soy diet due to inflammation associated with dietary hypersensitivity. Further, it was hypothesized that mucosal damage might allow increased permeability of antigen leading to increases in IgE measured by serum ELISA. One non-sensitized dog (#204) had an extremely high lactulose value after eating the washout diet. This was a true outlier and the abnormal value cannot be explained as her other samples were within normal limits. Two dogs had an increase in the L:R ratio after eating whole soy (one sensitized, one control) and two dogs had an increase in L:R after eating hydrolyzed soy (both sensitized). The remainder of the dogs was within normal limits for the duration of the study. When the sensitized dogs were compared to the non-sensitized control dogs using ANOVA, there was no significant difference ($p=0.84$). Critical review of the study would lead to the conclusion that the sensitized dogs were not different than the non-sensitized control dogs with respect to intestinal permeability as measured by L:R. Previous studies have demonstrated that dogs with clinical dietary hypersensitivity had improvement in intestinal permeability after being fed an elimination diet[14]. The current study did not demonstrate similar findings. Although the sample size was small, this study suggests that the atopic dog model may not be suitable for further studies associated with

measurement of intestinal permeability if the route of sensitization is subcutaneous injection.

It might be interesting to use the genetic offspring of the atopic dog model and attempt to sensitize the dogs by oral rather than subcutaneous route. By repeating the sugar and IgE evaluation tests, it might be possible to demonstrate that this model may more closely resemble the population of dogs that have dietary hypersensitivity.

Conclusions

This study was designed to evaluate the atopic dog model, the effects of diet on IgE production and gastrointestinal permeability and mucosal function, the methods of evaluation of IgE detection by ELISA and intradermal testing, and evaluation of soy protein antigens in sensitized versus non- sensitized dogs. Overall, diet did not have a statistically significant effect on IgE detected by ELISA or assessed by intradermal skin testing. There were no statistically significant differences between sensitized and non-sensitized control dogs with respect to gastrointestinal permeability or mucosal function evaluation, for any diet. Soy specific IgE was significantly different between sensitized and non-sensitized control dogs as measured by ELISA, but there was no statistically significant effect of diet. Intradermal skin testing yielded a high sensitivity (84%) and high specificity at a testing concentration of 1:10 W/V. These data were different than those reported for dogs with naturally occurring dietary hypersensitivity. Lastly, immunoblot analysis demonstrated important soy antigens, mostly subunits of the 7S globulin fraction. There were differences between sensitized dogs and non-sensitized control dogs that correlated with ELISA values. Diet did not influence IgE ELISA

values and similar results were seen with Western blot analysis. Because diet had no influence on any parameter of the study, it can be concluded that the atopic dog model does not represent dietary hypersensitivity. IgE antibodies induced by subcutaneous sensitization are not synonymous with food allergy.

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APPENDIX

IGE ANTIBODIES TO SOY ANTIGENS, IN SENSITIZED AND CONTROL DOGS, DETECTED BY WESTERN BLOT ANALYSIS AND ELISA.

Kennis RA[1], Tizard I[2], Hannah S[3], Ermel R[2], Bauer J[1]

1. Texas Veterinary Medical Center, Department of Small Animal Medicine and Surgery, Texas A&M University, College Station, TX 77843-4474
2. Texas Veterinary Medical Center, Department of Pathobiology, Texas A&M University, College Station, TX 77843-4467
3. Ralston Purina Company, Pet Products Group, Checkerboard Square, St. Louis, MO 63164

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The objective of the study was to test the hypothesis that dogs sensitized with whole soy antigen would produce higher levels of IgE antibodies to soy, compared to non-sensitized control dogs. Further, we investigated the IgE binding to whey and globulin fractions of soy antigens by immunoblotting with the sera of sensitized and control dogs.

Methods: 8 intact (6 female, 2 male) dogs from a single litter were sensitized to whole soy antigen beginning shortly after birth, in a manner previously described. 7 intact (4 female, 3 male) mixed breed dogs, roughly age matched (1.5-2 years), served as controls. All dogs were fed a pelleted soy free diet (rice flour and egg) for 6 weeks. Serum samples were collected and frozen (–20c) until analysis. Blinded serum samples were sent to an outside laboratory (Greer Laboratory®) for ELISA analysis. Soy proteins were separated into whey and globulin components and analysed by 15% SDS-PAGE. Immunoblottting for IgE was performed from the sera of each dog.

Results: ELISA test results were presented in terms of relative values (%R) with respect to pooled canine sera. A Student's T-test showed that there was a statistically significant difference ($p < 0.003$) between the sensitized and control dogs. Western blot analysis showed that IgE binding to whey and globulin protein fractions of soy was variable among all dogs.

Conclusions: These data validate the model and methodologies necessary for the further investigation of canine IgE to soy antigens.

CHANGES IN IGE ANTIBODIES TO SOY IN SENSITIZED AND CONTROL DOGS DETECTED BY ELISA AFTER CHALLENGE WITH THREE DIFFERENT DIETS, IN A CROSS-OVER STUDY.

Kennis RA[1], Tizard I[2], Hannah S[3], Ermel R[2], Dziezyc J[1], Bauer JE[1]

1. Texas Veterinary Medical Center, Department of Small Animal Medicine and Surgery, Texas A&M University, College Station, TX 77843-4474
2. Texas Veterinary Medical Center, Department of Pathobiology, Texas A&M University, College Station, TX 77843-4467
3. Ralston Purina Company, Pet Products Group, Checkerboard Square, St. Louis, MO 63164

Funding: *Sponsored by a research grant through Ralston Purina Company*

The objective of the study was to test the hypothesis that sensitized and control dogs would produce higher levels of IgE antibodies to soy after being challenged with a whole soy diet, compared with an egg based diet (elimination diet), and challenges by a hydrolyzed soy diet, and corn based (non-soy) diet.

Methods: 8 dogs were sensitized with 6 food allergens by subcutaneous injections. Seven dogs (roughly age matched) were used as non-sensitized control dogs. All dogs were fed an elimination diet containing egg and Brewer's rice (to neither of which any dog had been sensitized) for 6 weeks. Serum samples were collected and frozen (-20C) until submission to Greer Laboratories ® for ELISA analysis. The dogs were split into 3 groups and were fed a pelleted diet consisting of either hydrolyzed soy and corn starch (Purina HA®), intact soy and corn starch, or intact corn (non-soy) diet. After a 3 week challenge, serum samples were collected and submitted. All dogs were placed on the elimination diet for 6 weeks prior to group cross-over and challenge.

Results: ELISA test results were presented in terms of relative values (%R) with respect to pooled canine sera. There was a statistically significant difference between sensitized and control dogs ($p < 0.0003$) for all diets including the elimination diet. There was not a statistically significant difference in serum IgE levels to soy for any of the diets.

Conclusions: We were unable to reject the null hypothesis that sensitized and control dogs would produce higher levels of IgE to soy when challenged with a whole soy diet, compared with challenges by a hydrolyzed soy diet (Purina HA®) and corn based (non-soy) challenges.

Nutritional Information

	Diet 1 No soy, No corn	Diet 2 Modified soy, corn starch	Diet 3 Soy based diet	Diet 4 Corn based diet
Brewer's Rice	X		X	
Corn Starch		X		X
Corn				X
Egg Albumin	X			
Modified Soy Protein		X		
Soy Protein			X	
Moisture (%)	7.8	8.02	8.73	8.23
Protein (%)	24.4	22.2	21.7	22.8
Fat (%)	10.1	10.9	9.58	11.5
Fiber (%)	2.99		3	

All diets contained the same amounts of additives including:
canola oil, coconut oil, cellulose, calcium carbonate, dicalcium phosphate, potassium chloride, nutroloid fiber plus, trace mineral mix, choline chloride, magnesium oxide, salt, vitamin mix, selenium, flavor mix, and vitamin E

VITA

Robert Allen Kennis
716 Aster
College Station, TX 77845

EDUCATIONAL BACKGROUND

B.S. Biology	Michigan State University	1983
D.V.M.	Michigan State University	1989
Residency (Dermatology)	Michigan State University	1993
M.S. Veterinary Microbiology	Texas A&M University	2003

ACADEMIC / EMPLOYMENT EXPERIENCE

July 1989 to March 1991	Associate Veterinarian, Lake Orion, MI
March 1991 to July 1991	Associate Veterinarian, Birmingham, MI
July 1991 to July 1993	Dermatology Resident, Michigan State University
August 1993 to January 1996	Staff Dermatologist, Kaneohe, HI
February 1996 to Sept. 1998	Clinical Assistant Prof., Texas A&M University
September 1998 to Present	Assistant Professor, Texas A&M University

BOARD CERTIFICATION

Diplomate American College of Veterinary Dermatology (ACVD) 1996

SELECTED HONORS AND AWARDS

2000-2001 Montague Center for Teaching Excellence Scholar, TAMU
April 2001 Texas VMA Faculty Achievement Award for outstanding accomplishment in teaching
April 2002 Carl J. Norden Distinguished Teacher Award

PUBLICATIONS

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