Healthy Holistic

BLUE

NATURAL

Veterinary Diet™

Enhanced with Vitamins, Minerals and Other Nutrients

VOLUME NO.7

BLUE BUFFALO CLINICAL REPORT

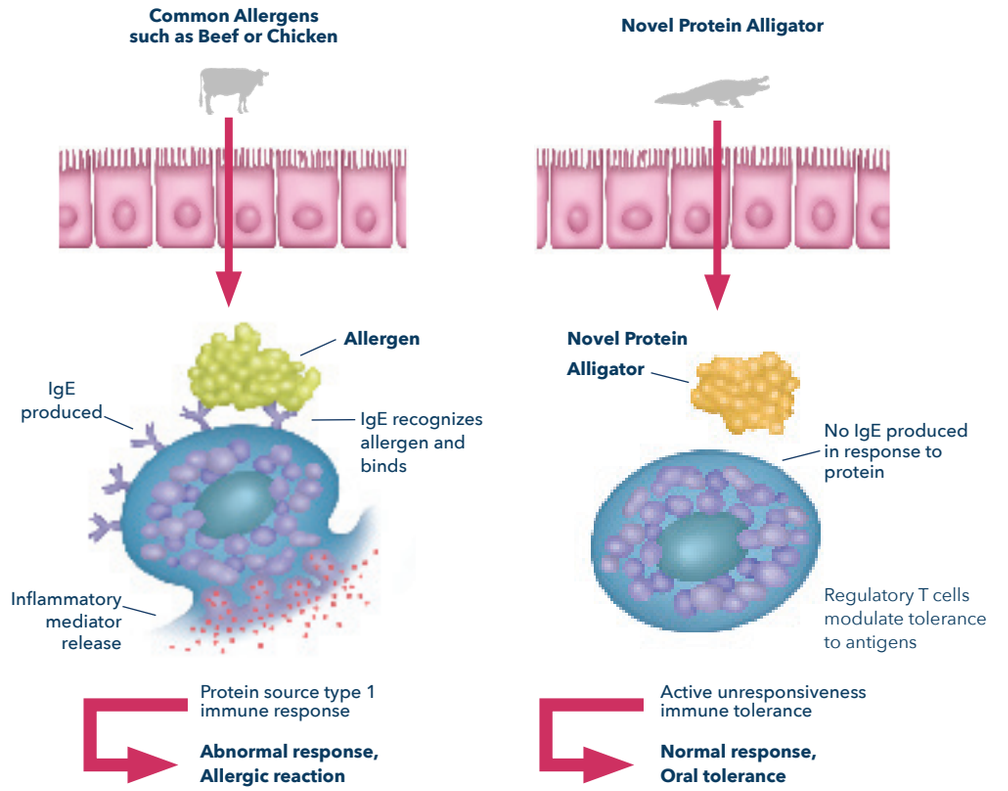
Clinical Evidence for: NP Novel Protein

KEY POINTS

1. BLUE Natural Veterinary Diet NP Novel Protein–Alligator features alligator, a novel protein source, to help reduce the risk of adverse reactions to food.

2. Multiple research study findings support that BLUE Natural Veterinary Diet NP Novel Protein–Alligator provides an ideal approach to nutritionally manage pets with adverse food reactions:
   - Novel protein, alligator
   - High digestibility
   - High palatability

Figure 1. Novel proteins like alligator help avoid immune reactions in allergic pets.



BLUE Natural Veterinary Diet NP Novel Protein–Alligator

Food hypersensitivity (food allergy) is the term used to describe the clinical disease induced by food ingestion in which there is an immunological response.[1] When a foreign antigen triggers an allergic reaction, the response is typically due to IgE-mediated type I hypersensitivity.[2] An allergic reaction to food suggests a defect in tolerance and may involve components of the gut-associated lymphoid tissue (GALT), the mucosal barrier and the systemic immune response. Food antigens, commonly proteins that survive cooking temperatures, stomach acid and digestive enzymes, are the components responsible for eliciting an allergic food reaction in hypersensitive pets. At initial exposure, the protein antigens are absorbed via specialized M cells or enterocytes. These antigens pass through the GALT to stimulate lymphocytes to produce antibodies that are specific for the allergen which migrate by way of the intestinal lymphatics to mesenteric lymph nodes, ultimately reaching the systemic circulation. These antibodies are released and attach to the surface of mast cells in different tissues throughout the body. Subsequent ingestion of the food antigen will bind to mast cell-bound IgE antibodies and directly provoke mast cell degranulation (Figure 1), releasing histamine and other chemical mediators.[2, 3] Dogs and cats can experience a variety of dermatologic and gastrointestinal responses to food allergens depending on the tissue or organ in which these chemical mediators are released.[4, 5]

A NOVEL PROTEIN APPROACH TO HELP MANAGE PETS WITH ADVERSE FOOD REACTIONS

1) NOVEL PROTEINS

Multiple clinical studies have demonstrated that feeding a novel intact protein source, one to which a patient has not been previously exposed, is a very effective approach for managing dietary hypersensitivities in both dogs and cats. In a 2001 study, 95% of dogs with confirmed dietary sensitivity were managed successfully with 1 of 3 commercially available selected protein-source diets.[1] A 2007 study showed that dogs with food-responsive IBD responded to an elimination diet of intact protein[6] and in a 2010 retrospective study of cats, 100% responded to dietary change using a different intact protein with or without additional pharmacologic therapy.[7]

Although any molecule in the diet has the ability to induce a hypersensitivity reaction, proteins are more likely to cause such reactions than are other nutrients. All proteins contained in food are foreign to the body and, therefore, potentially allergenic. When the ingestion of foreign antigens leads to a state of specific and active unresponsiveness this is referred to the phenomenon known as oral tolerance[8] (Figure 1). Patients with adverse food reactions must be carefully managed to minimize the potential for allergen exposure and triggering of immune responses. The most common food allergens are proteins with a molecular weight between 10 kDa and 70 kDa.[9, 10] Smaller proteins are normally too little to elicit an immune reaction, while larger proteins cannot normally access the body across the GI mucosa. The most commonly identified food allergens in dogs and cats are listed in Table 1.[9] Reactions to carbohydrate sources, such as corn, rice and potato, have been reported but appear to be much less common.[11]

TABLE 1. MOST COMMONLY IDENTIFIED FOOD ALLERGENS IN DOGS AND CATS[9]

BEEF	•	SOY
DAIRY	•	CORN
CHICKEN	•	EGG
WHEAT	•	PORK
LAMB	•	RICE

BEEF	•	CORN
FISH	•	DAIRY
CHICKEN	•	LAMB
WHEAT	•	PORK

By utilizing alligator as a protein source, BLUE Natural Veterinary Diet NP Novel Protein–Alligator provides an ideal novel protein approach for nutritionally managing pets with adverse reactions to food. In addition to being a novel protein source, alligator is also high in protein, low in fat and an excellent source of linoleic acid and omega-3 fatty acids. Table 2 shows how alligator compares with other protein sources on a variety of key nutritional factors.

TABLE 2. KEY NUTRITIONAL FACTORS FOR SELECTED PROTEIN SOURCES[12-14]

Key Nutritional Factor	Alligator (100 g)	Beef (100 g)	Duck (100 g)
Protein (g)	46	20	19
Total Fat (g)	4	12.7	28
Saturated Fat (g)	0	5.2	9.7
Cholesterol (mg)	0	75	84
	Meat, bone removed	Top Sirloin, raw	Duck breast with skin, bone

STUDY: FINISHED PRODUCT ELISA-TEK™ ANTIGEN AND PCR TESTING

PURPOSE

Ensure that BLUE Natural Veterinary Diet NP Novel Protein–Alligator finished product does not contain other common protein sources that might elicit an immune response.

STUDY DESIGN

Samples of BLUE Natural Veterinary Diet NP Novel Protein–Alligator finished product from every production run are subjected to protein contaminant testing by commercially available enzyme-linked immunosorbent assay, ELISA-TEK™.[15] This highly sensitive testing is designed to detect the presence of common food protein sources including beef, poultry, egg and soy. Test samples are also collected throughout the production run to verify each run prior to release and to validate the effectiveness of steps taken in the manufacturing process, such as equipment cleanout and burnout (a high-heat process to sterilize the equipment), before and after each manufacturing run.

PCR testing is performed in addition to ELISA to validate that NVD NP Alligator products are free of poultry contamination because ELISA testing for poultry on alligator-based formulas has not yielded accurate results. The very sensitive PCR test is able to detect and amplify even microscopic pieces of poultry DNA.

RESULTS[16]

Results confirm that BLUE Natural Veterinary Diet NP Novel Protein–Alligator meets our strict standards for evidence of contaminating proteins prior to release of the finished product and validates our cleanout procedures.

2) ENHANCED DIGESTIVE EFFICIENCY

The use of highly digestible, novel protein diets has long been recommended for managing food allergies. Studies show that BLUE Natural Veterinary Diet NP Novel Protein–Alligator is highly digestible as well as results in ideal stool quality.

STUDY: NUTRIENT ANALYSIS AND DIGESTIBILITY

PURPOSE

Prove that dry BLUE Natural Veterinary Diet NP Novel Protein–Alligator for Food Intolerance is a highly digestible pet food.

STUDY DESIGN

Two groups of adult dogs (n=6 each for Canine Digestibility Studies 1 and 2) and 2 groups of adult cats (n=7 each for Feline Digestibility Studies 1 and 2) from a commercial research facility were enrolled in the studies. All animals selected were clinically healthy. Animals were individually fed the species-appropriate BLUE Natural Veterinary Diet NP Novel Protein–Alligator dry diet once daily as their sole source of nutrition for 10 days. Animals were maintained individually in standard, species-appropriate housing and managed consistently during the study, including providing access to activity/exercise. Food consumption was monitored daily and body weights were recorded on days 1 through 6 and on day 10. On the last day of the study, a fecal sample from each animal as well as a sample of BLUE Natural Veterinary Diet NP Novel Protein–Alligator diet was sent to a commercial laboratory for nutrient analysis. The results of these analyses were used to calculate digestibility values, including dry matter digestibility. Digestibility analysis was performed according to the recommended protocol for use in the determination of metabolizable energy of pet food as defined by AAFCO.[17]

RESULTS[18]

Mean results from two studies in each species showed that BLUE Natural Veterinary Diet NP Novel Protein–Alligator dry is highly digestible.

TABLE 3. DIGESTIBILITY RESULTS[18]

Digestibility Values	Mean Protein	Mean Fat	Mean Caloric
DOG	90.1	94.9	91.6
CAT	87.4	92.2	89.8

DIGESTIVE HEALTH

Although fiber can impact digestibility, the dry BLUE Natural Veterinary Diet NP Novel Protein–Alligator formulation is rich in fermentable fiber ingredients such as dried chicory root, pumpkin, and pea fiber. Individually and combined, these natural ingredients provide important and beneficial effects on the digestive system. Chicory root is a source of inulin, a prebiotic that helps promote digestive health by stimulating the normal, beneficial bacteria in the digestive tract. In addition to being great sources of fiber, pumpkin and pea fiber are packed with vitamins, minerals, and antioxidants, that actively facilitate digestive health. Promoting digestive health along with the highly digestible formulation make BLUE Natural Veterinary Diet NP Novel Protein–Alligator a great choice for pets with dietary hypersensitivity.

STUDY: DETERMINING STOOL QUALITY

PURPOSE

Multiple studies were conducted to show that feeding BLUE Natural Veterinary Diet NP Novel Protein–Alligator can result in ideal stool quality (fecal consistency) in healthy dogs and cats.

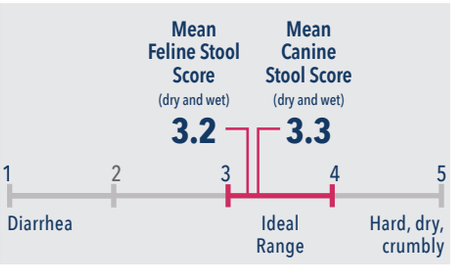
STUDY DESIGN

Two groups of adult dogs and 2 groups of adult cats (n=10 each for Canine Stool Quality Studies 1 and 2 and for Feline Stool Quality Studies 1 and 2) were enrolled in the studies. All animals selected were clinically healthy. Animals were individually fed the species-appropriate BLUE Natural Veterinary Diet NP Novel Protein–Alligator diet once daily as their sole source of nutrition for 7 days. For cats, the diet was made available over a 4-hour period. Animals were maintained individually in standard, species-appropriate housing and managed consistently during the study, including providing access to activity/exercise. Food consumption was monitored daily and body weights were recorded prior to study initiation and on study days 1, 3 and 5. Stool quality observations were made at least twice daily and scores were recorded. The scoring scale ranged from 1 for diarrhea to 5 for hard, dry crumbly feces and was aided by photographs of examples. A stool score between 3 and 4 is considered to represent ideal fecal consistency for dogs and cats.

RESULTS[19]

Overall, feeding BLUE Natural Veterinary Diet NP Novel Protein–Alligator dry and wet formulas in both dog and cat studies resulted primarily between moist, formed (score of 3) and well-formed, sticky (score of 3.5) stools, which are considered ideal fecal scores.

FIGURE 2. STOOL QUALITY SCORING[19]



3) HIGH PALATABILITY[20]

Because of its impact on compliance and acceptability, high palatability is an important component of the nutritional approach to adverse food reactions. Feeding studies in dogs and cats show that BLUE Natural Veterinary Diet NP Novel Protein–Alligator is highly palatable.

STUDY: URINE RELATIVE SUPERSATURATION (RSS) EVALUATION

PURPOSE

To show that feeding BLUE Natural Veterinary Diet NP Novel Protein Alligator food can result in clinically significant urine RSS values for struvite and calcium oxalate to limit the formation of struvite and calcium oxalate uroliths.

STUDY DESIGN

Two groups of adult dogs (n=10 each for Canine RSS Studies 1 and 2) and 2 groups of adult cats (n=10 each for Feline RSS Studies 1 and 2) were

REFERENCES

1. Leistra MHG, Markwell PJ, Wilemse T. Evaluation of selected-protein source diets for management of dogs with adverse reactions to foods. JAVMA, 2919(10):1411-1414, 2001.

2. Scott, D.W., Miller, W.H., Griffin, C.E. Skin immune system and allergic skin diseases. in: Muller and Kirk's small animal dermatology. 6th ed. WB Saunders, Philadelphia; 543–666, 2001.

3. Walker WA. Pathophysiology of intestinal uptake and absorption of antigens in food allergy. Annals of Allergy 1987; 59: 7-16.

4. Roudebush P, Guilford WG, Jackson HA. Adverse reactions to food. In Hand MS, Thatcher CD, Remillard RL, et al. (eds): Small Animal Clinical Nutrition, 5th ed, Topeka, Kan, 2010, Mark Morris Institute, p 609-635.

5. Kennis RA. Use of atopic dogs to investigate adverse reactions to food. JAVMA 221(5):638-640, 2002.

6. Allenspach, K. et al. Chronic enteropathies in dogs: Evaluation of risk factors for negative outcome. J Vet Intern Med 2007; 21: pp 700-208.

7. Jergens, AE, et al. A clinical index for disease activity in cats with chronic enteropathy. J Vet Intern Med., 2010 Sep-Oct;24(5):1027-33.

8. Janeway CA Jr, Travers P, Walport M, et al. Immunobiology: The Immune System in Health and Disease. 5th edition. New York: Garland Science; 2001. The mucosal immune system. Available from: https://www.ncbi.nlm.nih.gov/books/NBK27169/

9. Mueller RS et al. Critically appraised topic on adverse food reactions of companion animals (2): common food allergen sources in dogs and cats. BMC Vet Res (2016) 12:9.

10. Bryan J, Frank L. Food allergy in the cat: a diagnosis by elimination. J Fel Med Surg, 12:861-866, 2010.

11. Gaschen FP, Merchant SR. Adverse food reactions in dogs and cats. Vet Clin North Am Small Anim Pract 41:361-379, 2011.

12. Florida Department of Agriculture and Consumer Services. http://www.freshfromflorida.com/Consumer-Resources/Buy-Fresh-From-Florida/Florida-Alligator-Meat-Skins-and-Hides/About-Alligator accessed November 20, 2017.

13. United States Department of Agriculture. https://ndb.nal.usda.gov/ndb/foods/show/4081 accessed November 20, 2017.

14. United States Department of Agriculture. https://ndb.nal.usda.gov/ndb/foods/show/963 accessed November 20, 2017.

15. ELISA-TEK™ Sigma-Aldrich® Elisa Technologies, Inc. Gainesville, FL, 2017.

16. Blue Buffalo Co., Ltd., ELISA testing, data on file, 2017.

17. AAFCO (2015) Official Publication of the Association of American Feed Control Officials Inc., Champaign, IL.

18. Blue Buffalo Co., Ltd., Digestibility testing, data on file, 2017.

19. Blue Buffalo Co., Ltd., Stool Quality testing, data on file, 2017-2018.

20. Blue Buffalo Co., Ltd., Palatability testing, data on file, 2017.

21. University of Tennessee Pharmacology Laboratory, College of Veterinary Medicine

22. Blue Buffalo Co., Ltd., data on file, 2017-2020

enrolled in the studies. All animals selected were clinically healthy. Animals were maintained in standard, species-appropriate housing and managed consistently during the study, including providing access to activity/exercise. The study protocols were reviewed and approved by the research facility's institutional animal care and use committee. Animals were fed the species-appropriate BLUE Natural Veterinary Diet NP food for 23 days. An amount of food calculated to maintain body weight was offered once daily and available for 1 hour for dogs and for 20 hours for cats. On day 22, a 24-hour urine sample was collected from each animal, using a metabolism cage with a urine collection system for dogs and a specialized litter box for cats. From that sample, urine pH was measured via pH meter and 2 aliquots were frozen and shipped to The University of Tennessee for RSS analysis.[21] Those aliquots included a 1-ml sample that was diluted with 1.5 ml 1N HCl, and a 10- to 15-ml sample placed in a sterile container. For the RSS analysis, urine sodium, potassium, chloride, calcium, magnesium, phosphorus, citrate, oxalate, ammonia, pH, creatinine, and uric acid were measured.

RESULTS[22]

Feeding BLUE Natural Veterinary Diet NP dry dog food and dry and canned cat foods resulted in urine RSS values <1 for struvite and <10 for calcium oxalate. Feeding NP dog canned food resulted in urine RSS values <10 for calcium oxalate. These RSS values have been shown to dissolve and limit the formation of struvite and limit the formation of calcium oxalate uroliths.

STUDY: AAFCO FEEDING TRIALS[22]

nimal feeding trials using AAFCO procedures substantiate that BLUE Natural Veterinary Diet NP Novel Protein – Alligator for Dogs and Cats dry formulas provide complete and balanced nutrition for growth and maintenance, including growth of large sized dogs (70 lbs. or more as an adult).

CLINICAL IMPACT

These studies provide evidence supporting the digestibility and palatability, ideal stool scores, and ELISA and PCR testing results for BLUE Natural Veterinary Diet NP Novel Protein–Alligator. These findings support that BLUE Natural Veterinary Diet NP Novel Protein–Alligator provides an ideal novel protein approach to help nutritionally manage pets with adverse food reactions.

For more information about Blue Buffalo Quality Assurance Testing and Clinical Research please visit BLUEVetConnect.com.

Healthy Holistic

BLUE

NATURAL

Veterinary Diet™

Enhanced with Vitamins, Minerals and Other Nutrients

Learn more about BLUE Natural Veterinary Diet formulas
Call 888-323-BLUE or visit BLUEVetConnect.com