

Clinical Evidence for: **NP** Novel Protein

KEY POINTS

1

• BLUE Natural Veterinary Diet NP 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 Alligator provides an ideal approach to nutritionally manage pets with adverse food reactions:

- Novel protein, Alligator
- High digestibility
- High palatability

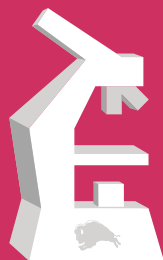
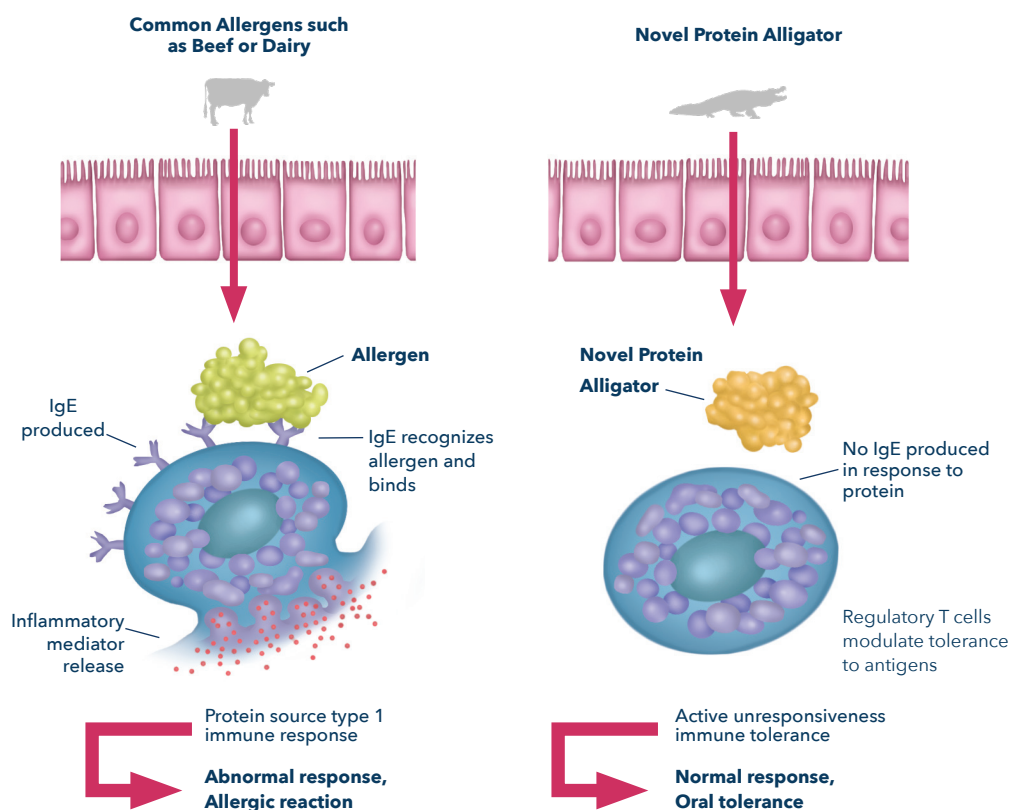


Figure 1. Novel proteins like alligator help avoid immune reactions in allergic pets.



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 protein⁶ 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 an 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 tolerance⁸ (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 CATS⁹



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



BEEF	•	CORN
FISH	•	DAIRY
CHICKEN	•	LAMB
WHEAT	•	

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 SOURCES¹²⁻¹⁴

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 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 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.

RESULTS¹⁶

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 proteins has long been recommended for managing food allergies. Studies show that BLUE Natural Veterinary Diet NP Alligator is highly digestible as well as results in ideal stool quality.



STUDY: NUTRIENT ANALYSIS AND DIGESTIBILITY

PURPOSE

Prove that BLUE Natural Veterinary Diet NP 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 Alligator 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 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

RESULTS¹⁸

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

DIGESTIVE HEALTH

Although fiber can impact digestibility, the BLUE Natural Veterinary Diet NP 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 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 Novel Protein Alligator can result in ideal stool quality (fecal consistency) in healthy dogs and cats.

STUDY DESIGN

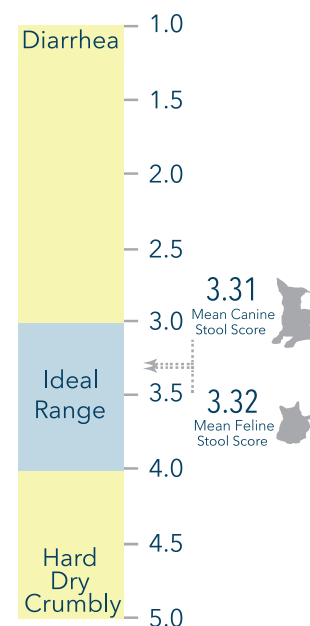
Two groups of adult dogs and 2 groups of adult cats (n=10 each for Canine Stool Quality Studies 1 and 2 and for Feline Stool Quality Studies 1 and 2) were enrolled in the studies. All animals selected were clinically healthy. Animals were individually fed the species-appropriate BLUE Natural Veterinary Diet NP 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.

RESULTS¹⁹

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

FIGURE 2. STOOL QUALITY SCORING



3) HIGH PALATABILITY

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 Alligator is highly palatable.

STUDY: PALATABILITY TESTING UTILIZING TWO-PAN STANDARD ASSAY TECHNIQUES

PURPOSE

Compare responses of dogs and cats to BLUE Natural Veterinary Diet NP Novel Protein Alligator palatability versus leading novel protein therapeutic pet foods.

STUDY DESIGN^{20, 21}

Thirty adult dogs and 30 adult cats were enrolled in the palatability studies. All animals selected were from calibrated palatability panels and were clinically healthy. Animals were maintained individually in standard, species-appropriate housing and managed consistently during the study, including providing access to activity/exercise. Per standard protocol, each animal was individually offered 2 stainless steel bowls, one containing BLUE Natural Veterinary Diet NP Alligator and the other containing a leading novel protein therapeutic pet food once daily for 2 days. Dogs were offered 400 g of each diet and cats were offered 100 g of each diet. Bowl placement was reversed daily and both bowls were presented for 30 minutes for dogs and for 4 hours for cats. If one diet was completely consumed prior to the end of the test period, both bowls were removed. Food consumption was recorded for each animal and each diet on each day.

RESULTS²²

All formulations, canine and feline, dry and canned, of NP Alligator performed well and were highly palatable. Examples of the intake ratio and the first choice palatability results are demonstrated in the cat dry results shown in Charts 1 and 2 below.

CHART 1. FELINE DRY PALATABILITY INTAKE RATIO

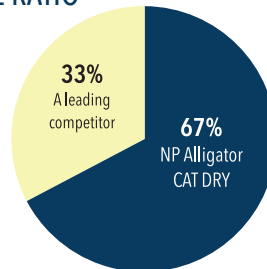
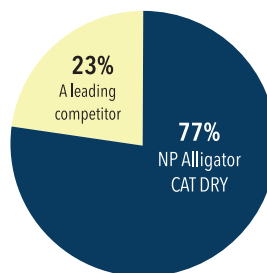


CHART 2. FELINE DRY FIRST CHOICE



CLINICAL IMPACT

These studies provide evidence supporting the digestibility and palatability results for BLUE Natural Veterinary Diet NP Novel Protein Alligator. These findings support that BLUE Natural Veterinary Diet NP 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 TrueBLUEVets.com.



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

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.
20. Smith J.C., Rashotte M.E., Austin T., Griffin R.W. Fine-grained measures of dogs' eating behavior in single-pan and two-pan tests. *Neurosci. Biobehav. Rev* (8)243-251, 1984.
21. Aldrich GC, Koppel K. Pet Food Palatability Evaluation: A Review of Standard Assay Techniques and Interpretation of Results with a Primary Focus on Limitations. *Animals*. 5(1):43-55, 2015.
22. Blue Buffalo Co., Ltd., Palatability testing, data on file, 2017.