

## Clinical Evidence for: **HF** Hydrolyzed for Food Intolerance

### KEY POINTS

1

BLUE Natural Veterinary Diet HF Hydrolyzed for Food Intolerance features salmon hydrolysate, a novel protein with a mean molecular weight of 2 kDa to help reduce the risk of adverse reactions to food.

2

Multiple research study findings support that BLUE Natural Veterinary Diet HF provides an ideal approach for nutritionally managing pets with adverse food reactions:

- Novel low molecular weight protein
- No animal protein antigen contamination
- High digestibility
- High palatability
- Ingredients preferred by client
- Clinically shown to be efficacious in managing clinical signs associated with adverse food reactions

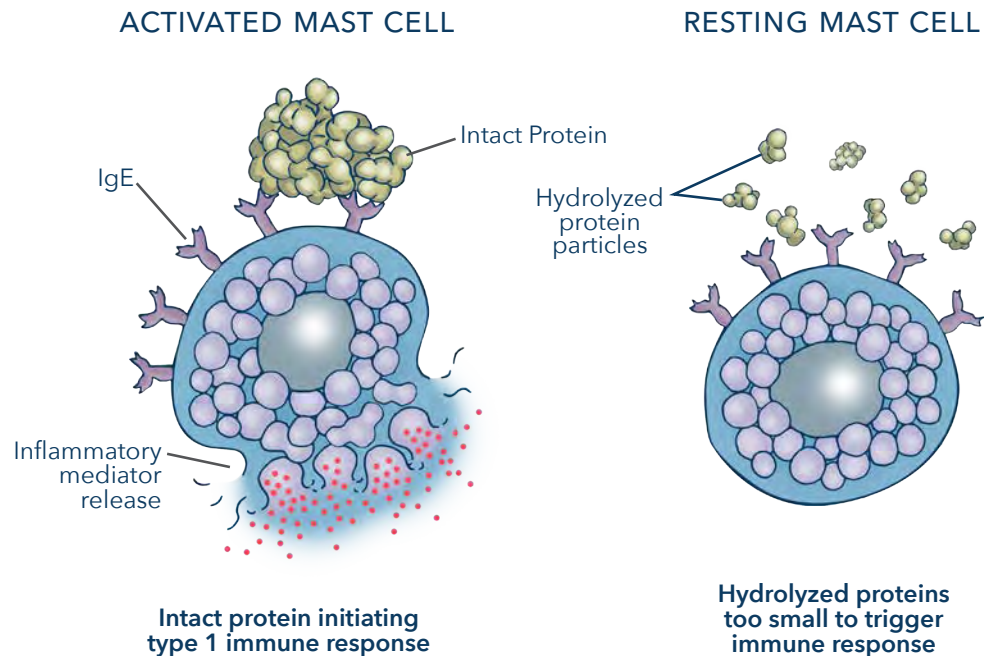


Figure 1. Hydrolyzed proteins help avoid immune reactions.

## BLUE Natural Veterinary Diet HF Hydrolyzed for Food Intolerance

Often times the ingredient exclusions in a diet are just as important as the inclusions. This is essential in cases of true food allergies as well as food intolerance. Food hypersensitivity (allergy) is the term used to describe the clinical disease induced by food ingestion in which there is an immunological reaction. This response is typically due to IgE-mediated type I hypersensitivity; however, types III and IV also are highly suspected.<sup>1</sup> The immunological reaction is usually attributed to dietary water-soluble glycoproteins that have molecular weights ranging from 10 to 70 kDa.<sup>2,3</sup>

Food intolerance (also known as food sensitivity) is the term used for an adverse

reaction to food due to a non-immunological abnormal physiological response. Digestive enzyme deficiencies, garbage ingestion, vasoactive amines, contaminants such as bacteria, metabolic, toxic, idiosyncratic or pharmacological effects of foods or food additives all can contribute to food intolerance.<sup>1,4,5</sup>

In a clinical setting, food allergy and food intolerance are rarely differentiated and frequently respond to a similar dietary approach. Because the precise immunologic processes of most adverse food reactions are usually not known,<sup>4,6,7</sup> on a practical level, the phrase adverse food reactions (AFR) is used to reference both conditions.

## NUTRITIONAL APPROACH TO HELP MANAGE PETS WITH ADVERSE FOOD REACTIONS

### 1) PROTEIN HYDROLYSATES

Once diagnosed, patients with adverse food reactions must be carefully managed to minimize the potential for allergen exposure and triggering of immune responses. One clinically proven option is the use of hydrolyzed protein diets, in which the protein source is hydrolyzed to small molecular weights that are not allergenic<sup>8</sup> (Figure 1). Studies have shown that 50% to 80% of dogs allergic to intact proteins have clinically improved when fed foods with protein hydrolysates.<sup>3, 9, 10</sup>

### STUDY: PROTEIN POLYPEPTIDE HYDROLYSATES MOLECULAR WEIGHT ANALYSIS

#### PURPOSE

To show that BLUE Natural Veterinary Diet HF Hydrolyzed for Food Intolerance is formulated to reduce the risk of immune responses, with highly hydrolyzed salmon protein as the sole animal protein source.

#### STUDY DESIGN

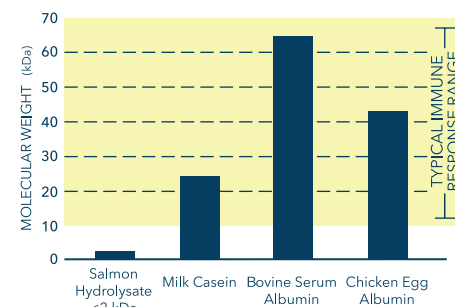
Salmon hydrolysate intended for use in BLUE Natural Veterinary Diet HF Hydrolyzed for Food Intolerance formulas is regularly subjected to molecular weight analysis by conventional testing methodology at AGROBIO Laboratories.<sup>11</sup>

#### RESULTS<sup>12</sup>

The majority of the protein hydrolysis products from the salmon hydrolysate in BLUE Natural Veterinary Diet HF Hydrolyzed for Food Intolerance have a mean molecular weight of 2 kDa or less.

- 78.2% of the water-soluble peptides in the salmon hydrolysate ingredient sample had a molecular weight of 2 kDa or less
- 97.3% of the water-soluble peptides in the salmon hydrolysate ingredient sample had a molecular weight of 10 kDa or less

CHART 1. HF MOLECULAR WEIGHT COMPARED WITH COMMON ALLERGENS



### 2) AVOIDING COMMON FOOD ALLERGENS

Since allergies are inappropriate or abnormal reactions of the immune system against a normal protein, allergies can form to any protein. The most common food allergens are proteins with a molecular weight between 10 kDa and 70 kDa.<sup>2, 3</sup> 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.<sup>6</sup> Reactions to carbohydrate sources, such as corn, rice and potato, have been reported but appear to be much less common.<sup>10</sup>

TABLE 1. MOST COMMONLY IDENTIFIED FOOD ALLERGENS IN DOGS AND CATS<sup>6</sup>

| Dogs        | Cats    |
|-------------|---------|
| Beef        | Beef    |
| Dairy       | Fish    |
| Chicken     | Chicken |
| Wheat       | Wheat   |
| Lamb/Mutton | Corn    |
| Soy         | Dairy   |
| Corn        | Lamb    |

Additionally, to ensure that the finished product does not contain other common protein sources that might elicit an immune response, tests were conducted for any evidence of common animal protein contaminants.

## STUDY: FINISHED PRODUCT ELISA-TEK™ ANTIGEN TESTING

#### PURPOSE

Ensure that BLUE Natural Veterinary Diet HF Hydrolyzed for Food Intolerance dry formula does not contain other common protein sources that might elicit an immune response.

#### STUDY DESIGN

Samples of BLUE Natural Veterinary Diet HF Hydrolyzed for Food Intolerance finished product from every production run are subjected to protein contaminant testing by commercially available enzyme-linked immunosorbent assay, ELISA-TEK™.<sup>13</sup> 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.

#### RESULTS<sup>12</sup>

This testing continues to show results that meet Blue Buffalo's strict standards for evidence of contaminating proteins prior to release of the finished product and validates the manufacturing cleanout procedures.

*In summary, by utilizing salmon as the protein source for hydrolysis, BLUE Natural Veterinary Diet HF uniquely addresses both nutritional approaches for AFR: protein hydrolysis AND avoidance of common food allergens. This combined benefit of a novel protein source and a low molecular weight helps reduce the risk of immune responses or other adverse reactions to food.*

### 3) ENHANCED DIGESTIVE EFFICIENCY

The use of highly digestible proteins has long been recommended for managing food allergies. Hydrolysis of a protein enhances digestive efficiency and studies show that BLUE Natural Veterinary Diet HF is highly digestible as well as results in ideal stool quality.<sup>12</sup>

## STUDY: NUTRIENT ANALYSIS AND DIGESTIBILITY

#### PURPOSE

Prove that BLUE Natural Veterinary Diet HF Hydrolyzed for Food Intolerance dry formula is a highly digestible pet food.

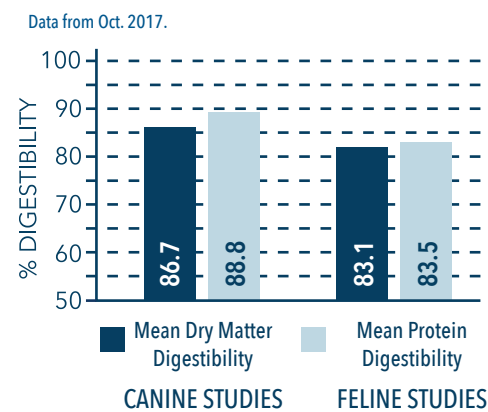
#### STUDY DESIGN

Three groups of adult dogs (n=6 each for Canine Digestibility Studies 1, 2 and 3) and 3 groups of adult cats (n=7 each for Feline Digestibility Studies 1, 2 and 3) 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 HF Hydrolyzed for Food Intolerance 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 HF Hydrolyzed for Food Intolerance 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.<sup>14</sup>

#### RESULTS<sup>12</sup>

Mean results from three studies in each species showed that BLUE Natural Veterinary Diet HF Hydrolyzed for Food Intolerance dry formulas are highly digestible.

CHART 2. HIGH DIGESTIBILITY RESULTS



### STUDY: DETERMINING STOOL QUALITY

#### PURPOSE

These 5 studies were conducted to show that feeding BLUE Natural Veterinary Diet HF Hydrolyzed for Food Intolerance dry formula would result in ideal stool quality (fecal consistency) in healthy dogs and cats.

#### STUDY DESIGN

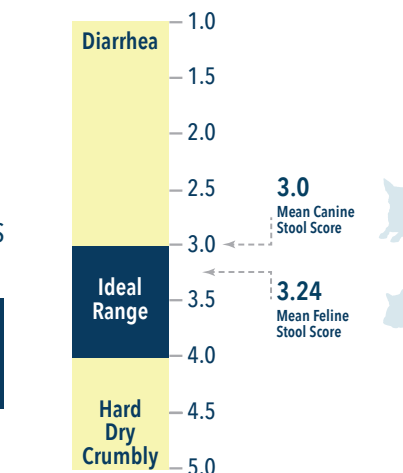
Three groups of adult dogs and 2 groups of adult cats (n=10 each for Canine Stool Quality Studies 1, 2 and 3 and for Feline Stool Quality Studies 1 and 2) were enrolled in the studies. All animals selected were clinically healthy. Animals were individually fed the species-appropriate BLUE Natural Veterinary Diet HF Hydrolyzed for Food Intolerance dry diet once daily as their sole source of nutrition for 7 days. For cats, 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. In this study, a stool score between 3 and 4 is considered to represent ideal fecal consistency for dogs and cats.

#### RESULTS<sup>12</sup>

Overall, feeding dry BLUE Natural Veterinary Diet HF Hydrolyzed for Food Intolerance dry formulas in both dog and

cat studies resulted in stools between moist, formed (score of 3) and well-formed, sticky (score of 3.5), which are considered ideal in dogs and cats.

FIGURE 2. STOOL QUALITY SCORING



### 4) HIGH PALATABILITY

Because of its impact on compliance and acceptability, high palatability is an important component of the nutritional approach to adverse food reactions. BLUE Natural Veterinary Diet HF Hydrolyzed for Food Intolerance is highly palatable.

### STUDY: URINE RELATIVE SUPERSATURATION (RSS) EVALUATION

#### PURPOSE

To show that feeding BLUE Natural Veterinary Diet HF dry food can result in clinically significant urine RSS values to help manage urolithiasis.

#### 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 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 dry BLUE Natural Veterinary Diet HF Hydrolyzed for Food Intolerance food for 23 days. An amount of food calculated to maintain body weight was





## REFERENCES

1. 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.
2. Biourge VC, Fontaine J, Vroom MW. Diagnosis of adverse reactions to food in dogs: efficacy of a soy-isolate hydrolyzate-based diet. *J Nutr* 134:2062-2064, 2004.
3. Puigdemont A, Brazis P, Montserrat S, et al. Immunological responses against hydrolyzed soy protein in dogs with experimentally induced soy hypersensitivity. *Am J Vet Res*, 67:484-488, 2006.
4. Kennis RA. Use of atopic dogs to investigate adverse reactions to food. *JAVMA* 221(5):638-640, 2002.
5. Leistra MHG, Markwell PJ, Willems T. Evaluation of selected-protein-source diets for management of dogs with adverse reactions to foods. *J Am Vet Med Assoc* 219:1411-1414, 2001.
6. 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.
7. Bryan J, Frank L. Food allergy in the cat: a diagnosis by elimination. *J Fel Med Surg*, 12:861-866, 2010.
8. 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.
9. Jackson HA. Food allergy in dogs—clinical signs and diagnosis. *Eur J Comp Anim Pract*. 19: 230-233, 2009.
10. Gaschen FP, Merchant SR. Adverse food reactions in dogs and cats. *Vet Clin North Am Small Anim Pract* 41:361-379, 2011.
11. GROUPE Qualtech, Agrobio Laboratories analysis report, January 2016.
12. Blue Buffalo Co., Ltd., data on file, 2015.
13. ELISA-TEK™ Sigma-Aldrich® Elisa Technologies, Inc. Gainesville, FL.
14. AAFCO (2015) Official Publication of the Association of American Feed Control Officials Inc., Champaign, IL.
15. GfK scanned data, 2015.
16. 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.
17. 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.
18. Blue Buffalo Co., Ltd., Consumer Study, July 2015.
19. Raditic DM et al. ELISA testing for common food antigens in four dog dry food used in dietary elimination trials. *J Anim Physiol Anim Nutr* 2011; 95: 90-97
20. Lewis T et al. Evaluation of a new hydrolyzed salmon-based diet in dogs with suspected cutaneous adverse food reaction: a multi-center randomized triple-blinded clinical trial. Presented at NAVDF April 10-13, 2019, Austin, TX.
21. Blue Buffalo Company, Ltd., data on file, 2016.

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.<sup>17</sup> 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<sup>18</sup>

Feeding BLUE Natural Veterinary Diet HF Hydrolyzed for Food Intolerance dry formula for dogs and canned formula for cats resulted in a struvite RSS of <1, indicating these diets are appropriate for both prevention and dissolution of struvite urolithiasis. BLUE Natural Veterinary Diet HF dog canned formula, and dry cat formula resulted in struvite RSS <1.2, indicating these formulas are appropriate for prevention of struvite urolithiasis. BLUE Natural Veterinary Diet HF dog dry and canned, as well as cat dry and canned formulas all resulted in calcium oxalate RSS values less than 7.25, indicating that these formulas are appropriate for the prevention of calcium oxalate urolithiasis.

## STUDY: EVALUATION OF DOGS WITH SUSPECTED CUTANEOUS ADVERSE FOOD REACTION

### PURPOSE

Therapeutic nutrition is commonly used to diagnose and treat dogs with cutaneous adverse food reactions (CAFR) as over-the-counter diets may contain undeclared ingredients or contaminants.<sup>19</sup> The purpose of this study was to compare the efficacy, palatability, and tolerability of BLUE Natural Veterinary Diet HF Hydrolyzed for Food Intolerance dry canine formula (BLUE HF) to that of the leading hydrolyzed poultry feather diet (HPFD) which historically has been considered the “gold standard” for diagnosing and managing CAFR.

### STUDY DESIGN<sup>20</sup>

Fifty-seven dogs from a multi-centric dermatology specialty hospital group were

enrolled in this triple-blinded, crossover clinical trial. Each dog was randomized to receive BLUE HF or HPFD for 8 weeks, then the alternative diet for an additional 8 weeks. Dogs were evaluated at 4-week increments using Canine Atopic Dermatitis Extent and Severity Index (CADESI)-4, owner-scored pruritus visual analog scale (VAS) and any adverse events were recorded.

## RESULTS

Of the 57 enrolled, 47 dogs completed the study. Only BLUE HF significantly decreased CADESI scores, BLUE HF and HPFD were effective at lowering the VAS scores. More adverse events were recorded in dogs fed HPFD (16) than BLUE HF (7). The findings of this study indicate that BLUE HF is as efficacious as the “gold standard” therapeutic nutrition for diagnosing and managing CAFR, while causing less adverse events.

## STUDY: AAFCO FEEDING TRIALS<sup>21</sup>

Animal feeding tests using AAFCO procedures substantiate BLUE Natural Veterinary Diet HF Hydrolyzed for Food Intolerance for Dogs and Cats Dry provides complete and balanced nutrition for maintenance of adult dogs and cats.

## CLINICAL IMPACT

The studies discussed in this Clinical Report provide evidence supporting the molecular weight analysis, antigen testing, digestibility, ideal stool scores, RSS for urinary health, and clinical study of efficacy in dogs with CAFR for BLUE Natural Veterinary Diet HF. These findings support that BLUE Natural Veterinary Diet HF Hydrolyzed for Food Intolerance provides an ideal approach to nutritionally manage pets with adverse food reactions while satisfying pet owner preferences for quality, natural ingredients.

For more information about Blue Buffalo Quality Assurance Testing and Clinical Research please visit [TrueBLUEVets.com](http://TrueBLUEVets.com) or call 1-888-323-BLUE.



**NATURAL**  
—Veterinary Diet—

Learn more about BLUE Natural Veterinary Diet formulas  
Call 888-323-BLUE or visit [TrueBLUEVets.com](http://TrueBLUEVets.com)