

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 protein plus low molecular weight
- No animal protein antigen contamination
- High digestibility
- Preferred palatability
- Ingredients preferred by clients

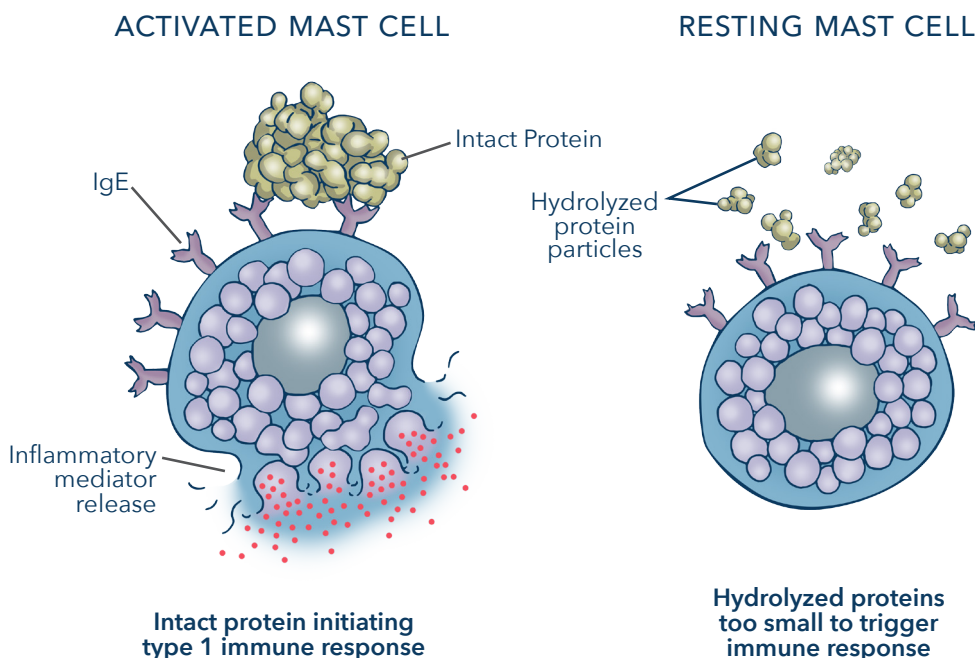
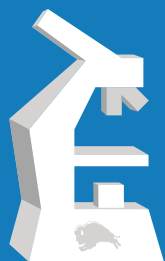


Figure 1. Hydrolyzed proteins help avoid immune reactions.

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.¹ The immunological reaction is usually attributed to dietary water-soluble glycoproteins that have molecular weights ranging from 10 to 70 kDa.^{2,3}

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.^{1,4,5}

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,^{4,6,7} 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 allergenic⁸ (Figure 1). Studies have shown that 50% to 80% of dogs allergic to intact proteins have clinically improved when fed foods with protein hydrolysates.^{3, 9, 10}

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

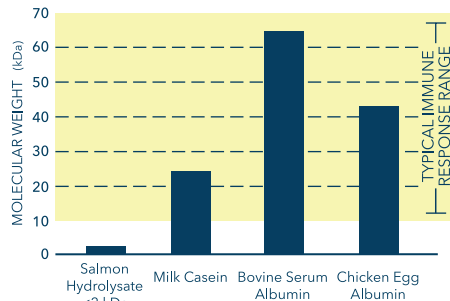
Salmon hydrolysate intended for use in BLUE Natural Veterinary Diet HF Hydrolyzed for Food Intolerance formulas is regularly subjected to molecular weight analysis by conventional testing methodology at AGROBIO Laboratories.¹¹

RESULTS¹²

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. 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 1.⁶ Reactions to carbohydrate sources, such as corn, rice and potato, have been reported but appear to be much less common.¹⁰

TABLE 1.
MOST COMMONLY IDENTIFIED FOOD ALLERGENS IN DOGS AND CATS⁶

Dogs (n=198)	Cats (n=89)
Beef (36%)	Beef (20%)
Dairy (28%)	Dairy (14.6%)
Wheat (15%)	Fish (13%) (e.g. sardines, tuna)
Egg (10%)	Lamb (6.7%)
Chicken (9.6%)	Poultry (4.5%)
Lamb/Mutton (6.6%)	Barley/Wheat (4.5%)
Soy (6%)	Diverse (11%)

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

STUDY: FINISHED PRODUCT ELISA-TEK™ ANTIGEN TESTING

PURPOSE

Ensure that BLUE Natural Veterinary Diet HF finished product 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™.¹³ 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.

RESULTS¹²

This testing continues to show results that meet our strict standards for evidence of contaminating proteins prior to release of the finished product and validates our 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.¹²



STUDY: NUTRIENT ANALYSIS AND DIGESTIBILITY

PURPOSE

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

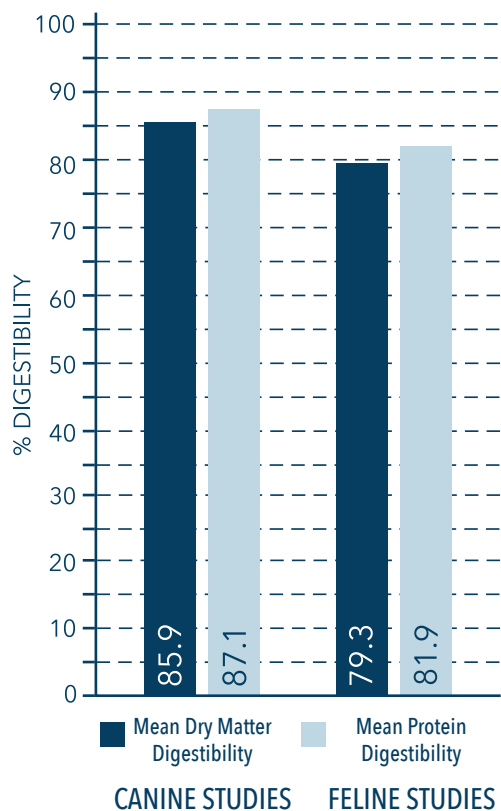
STUDY DESIGN

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 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.¹⁴

RESULTS¹²

Mean results from three studies in each species showed that BLUE Natural Veterinary Diet HF is 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 could result in ideal stool quality (fecal consistency) in healthy dogs and cats.

STUDY DESIGN

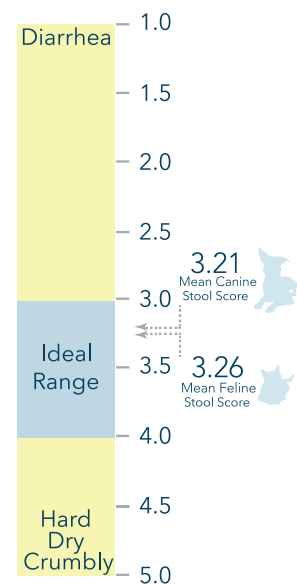
Three groups of adult dogs and 2 groups of adult cats (n=10 each for Canine Stool Quality Studies 1, 2 and 3 and for Feline Stool Quality Studies 1 and 2) were enrolled in the studies. All animals selected were clinically healthy. Animals were individually fed the species-appropriate BLUE Natural Veterinary Diet HF Hydrolyzed for Food Intolerance 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.

RESULTS¹²

Overall, feeding BLUE Natural Veterinary Diet HF Hydrolyzed for Food Intolerance in both dog and cat studies resulted primarily in moist, formed (score of 3) or well-formed, sticky (score of 3.5) stools.

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. Studies show dogs and cats prefer BLUE Natural Veterinary Diet HF Hydrolyzed for Food Intolerance over the leading hydrolyzed therapeutic pet food.¹⁵

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STUDY: PALATABILITY TESTING UTILIZING TWO-PAN STANDARD ASSAY TECHNIQUES

PURPOSE

Compare responses of dogs and cats to BLUE Natural Veterinary Diet HF Hydrolyzed for Food Intolerance palatability versus the leading hydrolyzed therapeutic pet food.¹⁵

STUDY DESIGN^{16, 17}

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 HF Hydrolyzed for Food Intolerance and the other the leading hydrolyzed therapeutic pet food (leading diet) 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.

RESULTS¹²

CHART 3. FELINE PALATABILITY INTAKE RATIO

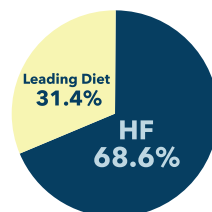
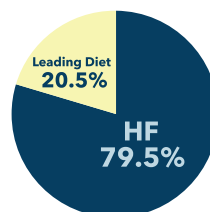


CHART 4. CANINE PALATABILITY INTAKE RATIO



Dogs and cats consumed significantly ($P < 0.001$) more HF Hydrolyzed for Food Intolerance than the leading diet.

5) CLIENT INGREDIENT PREFERENCE

PET OWNER INSIGHTS¹⁸

In a survey of 300 pet owners, owners report that they prefer the top ingredients in BLUE Natural Veterinary Diet HF 5 to 1 over the ingredients in the leading hydrolyzed protein therapeutic diet. Meeting client needs and preferences is key to encouraging increased client compliance.

CLINICAL IMPACT

The studies discussed in this Clinical Report provide evidence supporting the molecular weight analysis, antigen testing, digestibility, palatability and consumer insights for BLUE Natural Veterinary Diet HF. These findings support that BLUE Natural Veterinary Diet HF 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 or call 1-888-323-BLUE.

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