

GLYCEMIC RESEARCH LABORATORIES
GLYCEMIC SOLUTIONS

111 SECOND AVENUE N.E.
SUITE 512
ST. PETERSBURG, FL 33701

OFFICIAL CLINICAL TRIAL
LABORATORY

GLYCEMIC RESEARCH INSTITUTE®

BOARD CERTIFIED
HUMAN IN VIVO CLINICAL TRIALS

CLINICAL STUDY

STATEMENT OF INVESTIGATION

CLINICAL ASSESSMENT
of GLYCEMIC PERIMETERS

ANALYTICAL REPORT FOR:
NATURAL POLYMER
INTERNATIONAL CORPORATION
2009

www.GlycemicResearchLaboratories.com

GLYCEMIC RESEARCH INSTITUTE
Washington, D.C.

PET FOOD TESTING PROTOCOLS

GRIPetFoods.com

**Independent Clinical & Analytical
Rating on Pet Foods**

PET FOOD RATINGS
PET FOOD AWARDS
PET FOOD TREATS
PET FOOD OF THE YEAR

ABOUT THE GLYCEMIC RESEARCH INSTITUTE

The Medical Advisory Board, Senior Staff, and Associate Research Fellows of the Glycemic Research Institute® provide *Independent Pro Bono Certification* for products that pass the strict criteria for “Low Glycemic” foods, by authorization of the United States government, the Canadian government and the United Kingdom government.

The Federal Glycemic Research Institute® Certification Marks and Seals of Approval are based on ongoing Human In Vivo Clinical Trials that determine the glycemic index, glycemic load, diabetic response, Lipoprotein Lipase, and adipose tissue fat-storing properties of foods, beverages, Nutraceuticals, and Pharmaceuticals. Determining how humans and animals metabolize foods allows scientists to gain insights as to the varying effects different foods have on metabolism and longevity.

Federal authorization for the Glycemic Research Institute Certification Marks can be seen at the Official United States, Canada, and UK government websites.

25-Years of Research & Clinical Trial Expertise
Official Glycemic Research Institute Websites

Glycemic.com
GlycemicResearchLaboratories.com
GRIKidFriendly.com
GRIPetFoods.com

PET FOOD PROGRAM

The Glycemic Research Institute (GRI) conducts Independent Clinical and Analytical research on Pet Foods and Pet Treats.

GRI has examined hundreds of pet foods from 2004 to the present in order to determine the healthiest overall products, focusing on anti-aging, diabetes, arthritis, glycemic, blood glucose and insulin response, in the canine and feline.

As a Non-Profit independent research organization, GRI has no ties to the pet food industry, and does not accept monies from any company for the *Pet Food of the Year* Award or any other GRI Pet Food Award.

www.GRIPetFoods.com

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GLYCEMIC RESEARCH INSTITUTE

**601 Pennsylvania Avenue
Suite 900
Washington, D.C
20004**

DEFINING LOW GLYCEMIC & DIABETIC-FRIENDLY PET FOODS

As a species, dogs and cats are evolutionarily designed to utilize animal proteins and fat as their primary fuel, with Low Glycemic fruits and vegetables as a secondary fuel. It is important to include Low Glycemic species-friendly fruits and vegetables in canine and feline pet foods.

In the canine and feline, diets high in sugars and carbohydrates accelerate the aging process, reduce lifespan, and exacerbate cancer, diabetes, and other disease states.

One symptom of improper diet is skin diseases, which range from mild to severe, and are manifested by itching and scratching, rashes, and dull and dry coat.

HIGH-QUALITY INGREDIENTS

Glycemic Research Institute's pet food recommendations mandate high-quality proteins as the primary ingredient in any pet food.

According to *PetEducation.com*:

“High-quality ingredients are essential for a healthy pet. Some economy brands of dog food are made from inexpensive ingredients that are not easily digested, and therefore, do not provide the best nutrition. While they may technically meet the legal specifications for percentages of protein, fat, carbohydrates, etc., these foods have lower energy values and lower-grade proteins.

Because of this, many health-building nutrients may pass right through your dog's system without being absorbed.

It also means that you have to feed larger amounts of that lower quality food to provide your pet with the same nutrition as a smaller amount of premium food. When you compare the cost of these foods on a per-serving basis, and realize how quickly you go through a bag, economy foods may actually cost more in the long run.

When you are looking for a healthy food for your dog, reviewing the list of ingredients on the back of the bag is a good place to start. By law, pet food labels must list their ingredients by weight.

Look for meat, fish, egg, or some type of meat meal or fish meal as the first or second ingredient. Meat, fish, and eggs all have a high biological value, which means they have a high percentage of protein in the form of digestible, usable amino acids.”

CORN IS CONTRAINDICATED

Corn is typically found in dog foods, and is a prime example of inappropriate pet food ingredients. When is the last time you saw a dog grazing in a corn field? Corn is the worst possible product to feed a dog, even in small amounts, as their metabolism reacts to corn extremely negatively. Corn elevated blood glucose levels in the canine and feline, which causes skin problems, diabetes, and a plethora of other physical problems.

HIGH GLYCEMIC INGREDIENTS

Ingestion of corn and grains directly affect blood sugar levels in humans, as well as dogs and cats. The canine biochemistry is hard-wired to produce glucose from amino acids (proteins), which balances blood sugar levels.

Dogs can detect the smell of amino acids more distinctly than any other odor, as they have evolved into an efficient meat-detecting machine.

High Glycemic ingredients are contraindicated in canine and/or feline pet foods and treats.

High Glycemic ingredients over-elevate blood glucose levels, and increase risk of epilepsy, hypothyroidism, allergies, yeast infections, cancer and diabetes.

- Primary (high) levels of High Glycemic grains in canine and feline pet foods are not acceptable. These include wheat, corn, rice, and brown rice.
- Primary (high) levels of High Glycemic carbohydrates include potatoes, potato starch, beets, and cooked carrots.

Many pet food manufacturers have recently begun to add potato starch to dog and cat food. This practice is unfortunate, because most consumers are not aware that potato starch is High Glycemic and contraindicated in pet foods of any type.

The Glycemic Research Institute does not accept canine/feline foods or treats that contain high levels of potato starch. It is added to pet food because it is a very inexpensive raw material that adds bulk to the formula. This is also the case with corn.

Unlike regular potatoes and potato starch, sweet potatoes do not have the same negative effect on the canine, and are an acceptable ingredient in pet foods, as long as the ratios of proteins-to-carbs are in alignment.

CANINE ARTHRITIS

Pain management in the arthritic canine requires reducing inflammation, and reducing excess adipose tissue body fat. Sore and inflamed joints are a common side effect of arthritis in dogs. In the lean, muscular canine, arthritic-soreness is reduced as compared to the overweight dog.

Excess adipose tissue fat is exacerbated in the canine and feline via ingestion of grains and starches, which can be fattening.

TOXIC INGREDIENTS

Many foods and plants are safe for humans to consume, but are quite toxic to dogs and cats. Xylitol and chocolate can cause death in the canine.

The following foods and plants are contraindicated in dogs/cats.

Foods that can cause death, even in small doses, are tagged as *LETHAL*.

- XYLITOL (Lethal)

The *Animal Poison Control Center of the American Society for the Prevention of Cruelty to Animals* reports a substantially increased number of cases involving xylitol poisoning in dogs. Found in sugar-free chewing gum, candy, and baked goods, xylitol is a sweetener that can cause serious and sometimes life-threatening problems for pets.

- CHOCOLATE (Lethal)

Includes milk chocolate, dark chocolate, Cocoa powder, baking chocolate, dry cocoa powder, chocolate milk, chocolate candy, coca nut, chocolate ice cream, chocolate chip cookies, chocolate cake.

- THEOBROMINE (Lethal)
- CAFFEINE (Lethal)
- DRINKS THAT CONTAIN CAFFEINE & ENERGY DRINKS
- COFFEE & COFFEE GROUNDS (Lethal)
- GRAPES, RAISINS
- YEAST DOUGH, BREADS MADE WITH YEAST
- ONIONS & GARLIC
- SOY (endocrine disruptions)
- TEA
- AVOCADO
- ALCOHOL
- MACADAMIA NUTS
- VEGETABLES: Vegetables in the nightshade family, such as tomatoes, potatoes, eggplant and peppers should be avoided in canine/feline foods, as these foods can aggravate arthritic-inflammation and weight gain

TOXIC PLANTS

Many household and yard plants can sicken and/or kill your pet.

- Lilies, Lily of the Valley, oleander, azalea, yew, foxglove, rhododendron and kalanchoe (heart problems if ingested).

- Rhubarb leaves and shamrock contain substances that can produce kidney failure. Certain types of lilies (*Lilium* and *Heimerocallis* species) are highly toxic to cats, resulting in kidney failure, even if small amounts are ingested.
- Sago palms (*Cycad* species) can cause liver damage, especially if the nut portion of the plant is consumed.
- Fungi: certain varieties of mushrooms can cause liver damage and other medical problems.
- Philodendron, castor bean, corn plant, mother-in-law's tongue, Hibiscus and hydrangea.

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PROTOCOLS for ANALYZING & TESTING PET FOODS

2009

The following methodologies are utilized in determining the Glycemic and Diabetic properties of pet foods. Following HPLC/U-HPLC analysis, the data is cross-analyzed with *Glycemic Research Institute* (GRI) records of known glycemic indices of raw materials, and assigned a glycemic and diabetic index. Per GRI Certification Protocols, pet foods that meet the criteria are legally (FDA/FTC) allowed to display the GRI Certification Mark (s) on labels and attendant marketing material, which signify specific claims.

High Speed Liquid Chromatography HPLC and U-HPLC

- Conventional and ultra-high pressure modes
- Optimized performance for sub two micron particle columns
- Isothermal injection and separation enhances reproducibility
- Innovative LightPipe™ technology for increased sensitivity
- Rapid peptide identification and quantitation
- Fast efficient separations of metabolic profiles
- Ballistic gradient small molecule identification
- New columns geometries and particle sizes for improved speed, resolution and/or sensitivity

QUATERNARY PUMP/ISOTHERMAL INJECTION

A quaternary pump with only 65 microliters of delay volume, assuring rapid transfer of even the most complex gradients from the pump to the column. Isothermal injection and separation provide superior reproducibility by eliminating all external environmental influences to the chromatography.

HPLC ANALYTICAL ANALYSIS

Food compounds in pet foods analyzable by HPLC include amino acids, peptides, proteins, lipids, carbohydrates, sugars, sweeteners, alcohols, vitamins, organic acids, organic bases, mycotoxins, additives, preservatives, colorants, antimicrobial residues, pesticide residues, bittering substances, phenolic compounds, pigments, nitrosamines, growth promoters, anions, and cations.

ASSAY PRINCIPLES: PROTEINS

Protein interactions are analyzed using a label-free detection method. Sample in solution is injected over a sensor surface on which potential interacting partners are immobilized.

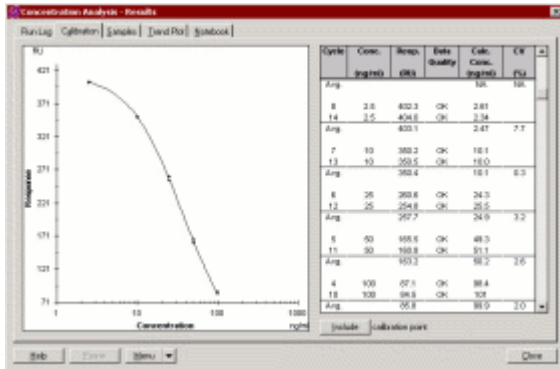
As the injected sample interacts with the immobilized partners, the refractive index at the interface between the sensor surface and the solution alters to a degree proportional to the change in mass at the surface.

Surface plasmon resonance (SPR) is exploited to detect these changes in real time and data are presented in a “sensorgram” (SPR response plotted against time).

Concentration test assays are designed as indirect (inhibition) assays. A known concentration of a relevant binding protein is mixed with the sample and injected over a sensor surface on which a corresponding derivative is immobilized.

Any target molecules present in the sample bind to the binding protein and so inhibit it from binding to the sensor surface. The higher the concentration of the target molecule in the sample, the higher the level of inhibition, and hence the lower the SPR (see below) response.

Concentrations are calculated by interpolation of the binding responses on a calibration curve.



CALIBRATION CURVE

DATA SYSTEMS

Method transfer calculators for isocratic method and gradient method transfer

Xcalibur MS data system

ChromQuest Chromatography Data System (CD)

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SUITE 512
ST. PETERSBURG, FL 33701

STATEMENT OF INVESTIGATION
PET FOOD ASSESSMENT

RESEARCH CONDUCTED FOR:

Natural Polymer International Corporation (NPIC)
1909 10th Street
Suite 100
Plano, Texas USA 75074

Telephone:	(972) 509-0449
Contact:	Shin Shi Chen
Analytical Trials:	Pet Treats/Canine
Report Date:	August/September 2009
Test Foods:	Get Naked Low Calorie Biscuits Twistix Oral Care
Report ID:	NPIC-1-2009

PROTOCOL

Assessment of the Glycemic Perimeters, including the Diabetic and Metabolic Responses were investigated on two (2) orally ingested pet products (herein the TEST FOODS) submitted by Natural Polymer International Corporation (NPIC). Standard protocols were followed per Glycemic Research Institute *Protocols for Analyzing & Testing Pet Foods 2009*.

The Test Foods were submitted in a ready-to-eat form.

IMPAIRED GLUCOSE METABOLISM IN THE CANINE

It is currently estimated that one out of every 100 dogs seen by a practicing veterinarian is diabetic. Obesity is estimated to occur in approximately 40% of the dog population.

In older dogs, (10% of the canine population in America are 11 + years old) diabetes and impaired glucose metabolism disorders are more common.

Impaired glucose metabolism in the canine is directly tied to hypoglycemia, diabetes (insulin-dependent and non-insulin dependent), obesity, advanced age (geriatrics), and can occur during canine gestation (pregnancy).

Disorders of glucose metabolism are also triggered by ingestion of high glycemic pet foods and treats, as well as ingestion of high glycemic human foods, such as bananas, rice, rice flour, brown rice flour, corn, and bread. These foods are to be avoided in the canine diet.

If high glycemic pet foods, treats, and pet diets are administered over a long period of time, the canine risk of diabetes and other glucose-metabolism disorders rises as age progresses. Diagnosis is often delayed, as the diagnosis of diabetes or impaired glucose metabolism in dogs and cats typically occurs after a hyperglycemic episode for the animal.

Borderline diabetes and other disorders involving *moderate* impairment of glucose metabolism, exhibit a significant risk of misdiagnosis, with undiagnosed progression of symptoms continuing over the lifespan of the canine, resulting in reduced lifespan.

CANINE RESPONSE TO PET FOODS & TREATS

Oral ingestion of any pet food, pet treat, or human food results in a specific postprandial glucose response. This response is characterized by an elevation in blood glucose levels, which range from normal to abnormal.

Following ingestion of a high glycemic meal, blood glucose and insulin levels are over-elevated.

The postprandial glucose and insulin response to a canine meal is extremely important in *avoiding* accelerated aging, obesity, and risk of impaired glucose metabolism disorders. Maintaining long-term health and homeostasis in the canine mandates *avoidance* of high glycemic foods and treats.

Ingestion of high glycemic foods in the canine exacerbates hyperinsulinemia, a metabolic disorder associated with obesity and diabetes.

Avoiding the negative effects of hyperinsulinemia requires avoidance of high glycemic ingredients fed to the canine. Lowering serum insulin levels controls the negative effects of hyperinsulinemia and reduces the risk and progression of canine obesity and diabetes.

Hyperinsulinemia also increases the risk of canine cancers, and reduces long-term survival in canine hormonal cancers.

DIETARY FIBER IN CANINE FOODS

Dietary fiber exhibits an effect on the glycemic and diabetic properties of all foods and beverages. This includes canine pet foods and treats.

Controlling the glycemic and diabetic effects of pet foods has a profound effect on risk of diabetes in companion animals. The utilization of certain soluble fibers, such as guar and pectin, has been found to yield a *reduced* postprandial rise in blood glucose levels.

However, not all soluble fibers provide benefits, and some result in undesirable side effects for the animal including diarrhea, flatulence, and abdominal cramping in both humans and companion animals.

The selection and inclusion of soluble fibers added to pet food and treats must be judicious, with specific focus on fibers that deliver glycemic benefits and are free from gastrointestinal side effects.

REVIEW SUMMARY

A Low Glycemic food plan, including Low Glycemic pet foods and treats, is the most effective means of preventing and controlling the postprandial glycemic and insulin response in canine companion animals, thereby preventing long-term impaired glucose/insulin metabolism disorders, and extending lifespan.

The postprandial regulation of glucose and insulin response in animals is required for longevity, reduced risk of obesity, and diabetes. This is particularly relevant in prevention of chronic over-elevation of glucose and insulin, which results in impaired glucose/insulin metabolism.

In the healthy canine, with no evidence of diabetes, prevention of the development of impaired glucose metabolism is recommended via administration of a Low Glycemic food plan.

This approach can prevent the onset of non-genetic chronic diabetes, and is particularly effective in managing the geriatric, obese, or diabetic companion animal.

TEST FOOD RESULTS

(2) TEST FOODS SUBMITTED:

Get Naked Low Calorie Biscuits

Twistix Oral Care

NPIC-1-2009

Test Food Number 1: Get Naked Low Calorie Biscuits

This product was duly submitted to Glycemic Research Laboratories for analysis per the *Glycemic Research Institute Protocols for Analyzing and Testing Pet Foods*. The results of said Protocols are as follows:

Get Naked Low Calorie Biscuits qualify as a Low Glycemic Pet Treat and additionally qualify to utilize the Glycemic Research Institute Seal of Approval for Low Glycemic Pet Treats (per the guidelines as outlined by the Glycemic Research Institute).

Test Food Number 2: Twistix Oral Care

This product was duly submitted to Glycemic Research Laboratories for analysis per the *Glycemic Research Institute Protocols for Analyzing and Testing Pet Foods*. The results of said Protocols are as follows:

*Twistix Oral Care does **not** qualify as a Low Glycemic Pet Treat and additionally does **not** qualify to utilize the Glycemic Research Institute Seal of Approval for Low Glycemic Pet Treats (per the guidelines as outlined by the Glycemic Research Institute).*

Note: The first ingredient in *Twistix Oral Care* is listed as “Brown Rice Flour.” This type of flour is quickly digested and absorbed, which elevates blood glucose and insulin levels in the canine.

Canine and human food formulas that contain sugar beet fiber do not elicit a significant reduction in blood glucose ($P < 0.05$) and/or serum insulin response ($P < 0.025$), and/or serum hydroxyproline response ($P < 0.025$).

In addition, barley flour has a high glycemic index.

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