Effect of a ketogenic food on canine hypertriglyceridemia

by

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Abstract

Canine hypertriglyceridemia (HTG) is a metabolic disorder that can present as a transient postprandial state, a primary idiopathic condition, or secondary to other endocrine diseases (Xenoulis, 2015). Traditional nutritional management of hyperlipidemia relies on strict dietary fat restriction to decrease circulating lipids (Ford & Ludlow, 2010b; Xenoulis, 2015); however, emerging evidence suggests that modulating other macronutrients, particularly carbohydrates, may offer a more comprehensive mechanism for improving systemic lipid metabolism (Clifton & Keogh, 2007). Chapter 1 explores the literature on canine dietary preferences and the physiological basis for an alternative nutritional strategy: replacing dietary carbohydrates with protein and fat to formulate a ketogenic, low-carbohydrate food that addresses metabolic dysfunction.

Chapters 2 and 3 detail the first study, to the best of the author's knowledge, to evaluate the clinical and metabolic impacts of this novel ketogenic food. Nineteen adult dogs with confirmed HTG were randomly assigned to receive either a traditional low-fat (Control) food or the experimental ketogenic (Test) food. Over the 10-week treatment period, a significant diet x day interaction was observed in caloric intake (Kcal/day, $P = 0.03$). Additionally, diet had an effect on average caloric intake ($P < 0.01$). Specifically, dogs fed the Test food maintained adequate caloric intake, while the Control group exhibited transient fluctuations in daily caloric intake, before stabilizing by week 10. Body weight demonstrated a significant effect dependent of day ($P < 0.01$), with no overall difference between the treatments. Although the Control group experienced a temporary within-group fluctuation in body weight ($P = 0.03$), both groups concluded the study with stable weights ($P > 0.05$). Analysis of serum triglyceride concentrations demonstrated a significant effect of day ($P = 0.03$). Importantly the Test food

performed similar to the nutritional standard of care Control food, with both foods achieving a mean reduction from baseline (Test:-161 mg/dL and Control: -112 mg/dL). The macronutrient shift in the Test food induced ketosis, evidenced by a significant diet x day ($P < 0.01$) interaction, as well as an effect of day ($P < 0.01$), and diet ($P = 0.01$) for β-hydroxybutyrate (B-HBA). Dogs in both groups started the study with similar B-HBA concentrations (Test: 0.93, and Control: 0.88, mg/dL; $P = 0.52$). However, by end of study dogs on the Test food continued to increase B-HBA concentrations while the Control group remained unchanged (Test: 1.4, and Control: 0.85, mg/dL; $P < 0.01$), when compared to baseline. Driven by the replacement of carbohydrates with fat and protein, the Test food increased proteolytic fecal short-chain fatty acids (SCFAs) including 2-methylbutyric acid ($P = 0.03$) and isobutyric acid ($P < 0.01$) and decreased saccharolytic SCFAs including propionic acid ($P < 0.01$). Conversely, the Control group demonstrated an increase in saccharolytic SCFAs including propionic acid ($P < 0.01$), and acetic acid ($P < 0.01$) when compared to baseline and demonstrated no changes in proteolytic SCFAs including 2-methylbutyric acid ($P= 0.67$), isobutyric acid ($P= 0.93$), and isovaleric acid ($P= 0.24$). Additionally, both foods modulated hormones involved in appetite regulation; circulating ghrelin exhibited a diet x day interaction ($P = 0.04$), with final concentrations remaining stable between dietary groups ($P = 0.04$). However, the Test food induced an increase in ghrelin at week 5 ($P = 0.04$) through study end when compared to baseline, whereas the control group exhibited no change ($P = 0.78$). Leptin and peptide YY (PYY) demonstrated no diet x day interactions ($P > 0.05$) and were influenced solely by time ($P < 0.05$). Finally circulating glucose-dependent insulinotropic polypeptide (GIP) and pancreatic polypeptide (PP) remained stable throughout the study, showing no significant variations ($P > 0.05$). Collectively, this research is the first study to evaluate the potential clinical efficacy of a ketogenic food in

managing canine HTG, by maintaining body weight and intake, and improving systemic lipid metabolism. More research is needed to determine the full potential a ketogenic food could represent as a therapeutic alternative to traditional low-fat dietary interventions for certain chronic endocrine disease management in dogs.

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Morgan A. Key

Chapter 1 - Literature Review

Pet food represents a growing industry, having experienced notable expansion over the recent decades. In 1986, pet food sales in the United States were $5.1 billion (Enterline, 1986). By 1995, these sales had almost doubled to $9.3 billion, and over a decade later reached $14.4 billion (Crane et al., 2010). Today, this expansion has transformed the U.S. pet food market into a major economic driver, generating $51.7 billion in annual sales and directly employing nearly 35,000 people (Pet Food Institute, 2024). Furthermore, the industry heavily supports agricultural and rural economies by purchasing over $13.2 billion in farm-based ingredients annually (Pet Food Institute, 2024). The continuous surge in industry revenue and agricultural demand, highlights a wider trend, wherein a majority of pet owners in the United States regularly feed their pets commercially prepared foods. The widespread reliance on commercial foods is not restricted to the United States; European and Asian countries have also embraced commercially prepared pet foods. Crane et al. (2010) notes that North America constitutes the largest market for pet food, followed by Western Europe and Asia.

The evolution of dog food since its creation in 1860 is significant. Initially developed to offer pet owners a convenient, economical, and easily accessible alternative that delivers "complete and balanced" nutrition. Commercial dog food represented a substantial shift from the traditional practice of feeding table scraps (Kazimierska et al., 2021). Today, commercially prepared pet foods have become a staple, providing more than 90% of the calories consumed by dogs and cats in many parts of the world (Zicker, 2008). Companies have continued to revolutionize the pet food space by finding new ways to appeal to pet parent’s perception of their pet’s nutritional needs (Crane et al., 2010). According to Crane et al. (2010), the pet food industry employs a variety of marketing strategies to appeal to consumers. These tactics can be

categorized by their nutritional claims, ingredient focus, and overall branding. Each approach operates under specific regulatory requirements to ensure product safety and accurate labeling.

The following marketing concepts are based on the nutritional content and purpose of the pet food (Crane et al., 2010). Specialty formulas are designed to provide targeted nutrition for a specific purpose, such as weight management or joint health. In contrast, all life stage foods are formulated to meet the nutritional requirements of a pet throughout its entire life. A more economical approach, the value brand, provides adequate nutrition at an affordable price point. The "more is better" strategy suggests that pets may require higher nutrient levels than what is officially deemed nutritionally adequate.

Ingredient focused marketing often highlights either the presence or absence of specific components (Crane et al., 2010). Some brands emphasize the presence of an ingredient to signal associated health benefits. Conversely, promoting the absence of an ingredient appeals to consumers who desire fewer fillers or specific components in their pet's food. The "people food" concept markets products that resemble human meals to suggest a higher quality or more palatable food.

Beyond ingredients, branding and product names are crucial. Brands may use specific product names to suggest a premium or specialized product. Additionally, commercial pet food labels can utilize terms such as "natural," "holistic," and "organic" to appeal to consumers seeking products with fewer synthetic additives, or those influenced by anthropomorphic feeding practices; however, the application of these terms is subject to strict regulatory definitions and standards (Crane et al., 2010). Finally, offering a wide flavor variety addresses the consumer assumption that pets want or need dietary diversity.

Given the variety of marketing concepts employed by the pet food industry, a robust framework of governance is essential to ensure product safety, quality, and accurate labeling (Roudebush et al., 2010; Zicker, 2008). Consequently, several agencies are responsible for establishing and enforcing these guidelines while others exist to support the industry (see Table 1.1).

Marketing claims used in the pet food industry are supported by a robust framework of scientific data and regulatory standards (Zicker, 2008). Most commercial pet foods are formulated to meet specific nutritional objectives for a pet's life stage, such as growth, adult maintenance, or reproduction, based on recommendations from the Association of American Feed Control Officials (AAFCO, 2025). These nutritional profiles are supported by scientific data and the NRC as a foundation, to determine the essential nutrient levels—including calories, protein, fat, carbohydrates, vitamins, and minerals—required for optimal health (Zicker, 2008).

To create more practical nutrient profiles, AAFCO established the Canine Nutrition Expert (CNE) and Feline Nutrition Expert (FNE) subcommittees in 1990 and 1991, respectively (AAFCO, 2025). This initiative led to the development of distinct AAFCO profiles for each species: one for growth and reproduction, and another for adult maintenance. By specifying minimum and, for certain nutrients, maximum inclusion levels, these profiles were carefully designed to prevent both nutritional deficiencies and toxicities. The AAFCO Dog and Cat Food Nutrient Profiles have since replaced the NRC recommendations as the primary basis for substantiating label claims in the United States (Roudebush et al., 2010).

While AAFCO does not possess direct regulatory authority, it plays a foundational role in the pet food regulatory framework. By collaborating with key officials, AAFCO develops the guidelines, regulations, and policies widely adopted by state authorities (Roudebush et al., 2010).

Established in 1959, the AAFCO Pet Food Committee works alongside the U.S. Food and Drug Administration (FDA), industry representatives, and other stakeholders to oversee and propose revisions to the AAFCO Official Publication (Roudebush et al., 2010). This committee also organizes educational initiatives to protect consumers and promote orderly business practices (AAFCO, 2025). Notably, AAFCO has established comprehensive guidelines for pet food labels that most states incorporate into their own legal frameworks, though individual state regulations may vary slightly. The AAFCO labeling guidelines dictate required label components, including a nutritional adequacy statement that indicates whether a product provides "complete and balanced" nutrition for a specific life stage of a dog or cat (AAFCO, 2025). Any assertion of nutritional adequacy must be validated either by formulating the diet to meet established AAFCO dog or cat food nutrient profiles or by successfully passing standardized AAFCO animal feeding trials (Roudebush et al., 2010).

At the federal level, the FDA enforces its own labeling requirements for any animal food products crossing state lines, which align with AAFCO standards (AAFCO, 2025). The FDA regulates pet food similarly to other animal feeds under the Federal Food, Drug, and Cosmetic Act (FD&C Act). This legislation mandates that all animal foods must be safe for consumption, produced under sanitary conditions, free of harmful substances, and truthfully labeled (Miller et al., 2010). Furthermore, canned pet foods require processing conformance with low-acid canned food regulations (21 CFR Part 113) to ensure the final product is free of viable microorganisms (FDA, 2024; Price et al., 1993; Van Houweling et al., 1977).

The FDA’s Center for Veterinary Medicine (CVM) is specifically responsible for regulating pet food labeling in cooperation with state authorities (Roudebush et al., 2010). Federal regulations require labels to include a proper product identifier, a net quantity statement,

the manufacturer's name and location, and a complete ingredient list arranged in descending order by weight (FDA, 2024).

The FD&C Act distinguishes between a "food" and a "drug." Under the Federal Food, Drug and Cosmetic Act (FFDCA) 201(f), a food is defined as an "article used for food or drink for man or other animals," which includes products used primarily for nutrition, taste, or aroma (AAFCO, 2025). In contrast, a drug is defined as an "article intended for use in the diagnosis, cure, mitigation, treatment, or prevention of disease" or one intended "to affect the structure or any function of the body (FDA, 2022)." Unlike animal drugs, pet foods are not subject to mandatory pre-market review by the FDA, except for new food additives (FDA, 2022). However, they must still comply with the FD&C Act's requirements for safety, sanitation, and truthful labeling.

Specific pet foods are formulated to nutritionally manage particular conditions, such as renal disease or diabetes. According to AAFCO (2025), therapeutic and veterinary medical foods must carry a statement of nutritional adequacy. However, due to their specialized clinical indications, the nutrient profiles of these formulations are sometimes modified to levels below the standard recommendations for healthy animals. In such instances, the product label must state that the food is intended for "intermittent or supplemental" feeding unless nutritional adequacy has been substantiated through a completed feeding trial to retain a “complete and balanced” (AAFCO, 2025). Depending on their intended use and specific health claims, the FDA may regulate these products as food, drugs, or both (FDA, 2022). As outlined in the agency's 2016 Compliance Policy Guide, the FDA mandates that these veterinary-exclusive foods be distributed solely through or under the supervision of a licensed veterinarian to ensure appropriate clinical application (FDA, 2022).

Health claims on pet food labels are subject to regulation by the CVM (Dzanis, 2008; Roudebush et al., 2010). The FDA specifically reviews claims related to urinary health and hairball control (FDA, 2024). Ingredients must be approved food additives (21 CFR 573), generally recognized as safe (GRAS) substances (21 CFR 582), or defined by AAFCO for their intended use (Roudebush et al., 2010). Pet food labels are prohibited from making false, misleading, or disease prevention claims. Products that are found to be adulterated or misbranded can be subject to regulatory actions, including seizure (Dzanis, 2008).

Commercial pet foods have traditionally been categorized by their moisture content and processing methods into three main types: dry, semi-moist, and canned (Zicker, 2008). While new commercial formats, such as raw and fresh foods, are increasingly entering the market, regulatory bodies like the AAFCO have not yet established formal definitions for these emerging categories (AAFCO, 2025). Despite this lack of standardized classification, the FFDCA mandates that all animal foods, be safe for consumption, produced under sanitary conditions, free of harmful substances, and truthfully labeled (FDA, 2022). Dry food, which contains less than 11% moisture, is the most common and cost effective option due to its convenience for storage and shelf life (Crane et al., 2010). The manufacturing of dry food relies on extrusion, a process that creates consistent kibble similar to human breakfast cereals and was first adopted for pet food in the mid 1950s (Aldrich, 2006). Extrusion refers to a process where a mixture of ingredients is steam conditioned, compressed, and forced through the die of an extruder (Tran et al., 2008).

Canned foods, which have a moisture content of 60% to 87%, tend to be highly palatable and often have an appearance that mimics meat chunks or ground meat (Crane et al., 2010; Zicker, 2008). Manufacturers use gums and gelling agents to maintain the structure of these high

moisture products, which often contain higher protein levels than dry foods (Zicker, 2008). Canned foods are available in various packaging formats, including steel cans, aluminum trays, pouches, and plastic containers (Crane et al., 2010). Semi-moist foods, with 25% to 35% moisture, represent the smallest market share (Zicker, 2008). They are manufactured similarly to extruded foods but require humectants and acidification to control moisture and prevent mold. Like canned foods, semi-moist products may contain higher protein and sugar content than dry pet food, to enhance palatability (Case et al., 2010; Crane et al., 2010; Zicker, 2008).

In recent years, the pet food market has seen a growing trend toward “human-grade”, "natural" and "homemade" foods, such as home-cooked or raw meat-based foods (Oba et al., 2019; Schlesinger & Joffe, 2011). This shift is largely driven by the humanization of pets. A phenomenon where owners treat dogs as family members, attribute human emotions and needs to them, and purchase products that reflect a human lifestyle (Michel, 2006; Stoltz et al., 2020). Pet parents who choose these unconventional foods often do so due to a distrust of conventional commercial diets, a desire to address specific health requirements, or a need to align feeding practices with personal beliefs and lifestyle choices (Dodd et al., 2018; Parr & Remillard, 2014; Vandendriessche et al., 2017). Recent findings suggest significant differences in the lifestyle and informational habits of owners who feed conventional foods versus those who choose unconventional options, such as "Biologically Appropriate Raw Food" (BARF) (Freeman et al., 2013; Hoummady et al., 2022). Proponents of these alternative diets claim a wide range of benefits, ranging from improved dental and skin health to chronic disease prevention (Davies et al., 2019; Freeman et al., 2013; Towell, 2008). However, despite these health claims, there is limited scientific evidence to support the benefits of feeding unconventional pet foods. Furthermore, the rationale for these foods often overlooks the genetic and physiological

adaptations of modern dogs, who have evolved a significantly greater capacity to digest dietary carbohydrates than their wild ancestors (Freeman et al., 2013; Schlesinger & Joffe, 2011).

Among these unconventional options, fresh and raw foods have gained significant traction in the commercial space. Fresh foods are typically heat-treated without excessive heat damage, utilizing techniques such as kettle cooking, steam cooking, or the separate cooking of individual ingredients followed by blending (Geary et al., 2024; Tanprasertsuk et al., 2021). Fresh foods are usually refrigerated or frozen and thawed prior to consumption. Conversely, raw meat-based foods are thermally unprocessed (Vecchiato et al., 2022). To mitigate the risk of microbial contamination without traditional cooking, most commercially available raw pet foods undergo alternative manufacturing treatments. These include high-pressure processing (HPP, a non-thermal technique using isostatic pressure with water), radio frequency (RF, which uses moisture to create volumetric heat) pasteurization, electric fields, plasma, freeze-drying (which reduces water activity to slow bacterial growth), and chemical treatments (Shukla, 2011; Song, 2020). Beyond processing challenges, research has shown that both homemade and commercial raw foods are highly susceptible to nutritional imbalances and deficiencies(Freeman et al., 2013; Vecchiato et al., 2022). This is often due to a lack of rigorous formulation, an absence of standardized feeding studies, and the tendency for owners to simplify homemade recipes, which ultimately compromises the food's nutritional adequacy (Davies et al., 2019; Freeman & Michel, 2001; Kölle & Schmidt, 2015; Mehlenbacher et al., 2012; Towell, 2008).

In addition to nutritional concerns, raw foods present a unique safety challenge because contaminating pathogenic bacteria and zoonotic viruses are not always rendered inert prior to feeding (Davies et al., 2019; Hellgren et al., 2019; van Bree et al., 2018; Weese et al., 2005). Unconventional pet foods pose a significant zoonotic risk due to frequent contamination with

bacterial pathogens such as Yersinia, Salmonella, Escherichia coli, Campylobacter, and Listeria monocytogenes (Freeman & Michel, 2001). These pathogens can be easily transmitted to owners and the household environment by pets (Finley et al., 2007; Fosse et al., 2008; Fredriksson-Ahomaa et al., 2009; Ionică et al., 2025; Joffe & Schlesinger, 2002; Morgan et al., 2024; Nemser et al., 2014; Pedrinelli et al., 2017; Sealey et al., 2024; Solís et al., 2022; van Bree et al., 2018; Wideman et al., 2016). For example, the reported prevalence of Salmonella contamination in commercial raw diets varies, with most studies indicating an approximate 20% contamination rate among tested foods (Vecchiato et al., 2022; Weese et al., 2005). Consequently, there remains a potential zoonotic risk for disease transmission, necessitating proper handling practices by pet owners (Vecchiato et al., 2022). The FDA maintains a strict zero-tolerance policy for pathogen contamination in all commercial pet foods. However, it is important to note that bacterial contamination is not exclusive to raw foods; extruded pet foods and products from other manufacturing processes can also experience contamination events (FDA, 2023).

Beyond primary foods, additional categories of canine and feline nutrition include treats and supplements. Unlike fresh, raw, or conventional commercial foods, these products are not typically intended to serve as a pet's sole source of nutrition (Crane et al., 2010; Zicker, 2008). Instead, treats and supplements are utilized to reward behavior, strengthen the human-animal bond, or nutritionally support specific health concerns identified by the pet owner (Crane et al., 2010).

The pet industry is dynamic, with its evolution driven by changes in pet ownership. Humanization of dogs has not only affected new pet food categories but also the economic landscape of the pet industry (Stoltz et al., 2020). According to the American Veterinary Medical Association’s (AVMA) Pet Ownership and Demographics Sourcebook, dogs have consistently

been the most popular pet in American households (AVMA, 2024). A majority of dog owners (88%) consistently report that their dog is considered a member of the family (AVMA, 2024). From 2011 to 2024, the total number of dogs in households increased by approximately 16.5 million. While the total number of dog owning households has risen since 2011, there was a decrease of about 3 million dogs per household between 2020 and 2024, a change attributed to the COVID-19 pandemic (AVMA, 2024).

Dog management and human dog relationships in the U.S. have evolved considerably over the last 40 years, with increased expenditures on dogs reflecting a growth in responsible ownership behaviors (Rowan & Kartal, 2018). Recent surveys confirm the strong family bond between owners and their pets. A 2023 poll of 5,073 U.S. adults found that 62% of Americans own a pet, and nearly all pet owners (97%) consider their pets part of the family (PRC, 2023). A separate poll found that 91% of owners are willing to make financial and personal sacrifices to keep their pets (Poll, 2024a).

The strength of the pet owner bond can correlate to the level of veterinary care provided. A study by Lue et al. (2008) characterized a strong bond by the owners' feelings, time spent, and shared activities with their pets, concluding that a stronger bond correlates with more frequent preventative veterinary care. An increased financial commitment is evident in spending habits. A 2024 Harris Poll identified a category of "all in pawrents" who agreed that their pet is like their child (Poll, 2024a). These individuals outspend other pet owners on yearly expenses, with an average annual expenditure of $5,049 compared to $4,366 for other owners (Poll, 2024b). The same poll found a generational divide in spending, with Generation Z spending over $6,000 annually, significantly more than Millennials ($5,150), Generation X ($3,878), and Baby Boomers ($2,454) (Poll, 2024b)

The bond between owner and pet has significantly influenced the evolution of feeding behavior in domesticated dogs, *Canis familiaris* (Bradshaw, 2006). Historically the diets of ancestral dogs were primarily meat based, and their co-evolution with humans shifted their dietary habits toward omnivory (Axelsson et al., 2013). As such, the dietary evolution parallels the descent of the modern dog from the wolf, Canis lupus (Bradshaw, 2006). Evidence suggests that the co-evolution of dogs and humans began in the Upper Paleolithic period (~35,000 BP), making dogs one of the first domesticated plant or animal species (Bradshaw, 2006; Galibert et al., 2011).

The earliest archaeological evidence differentiating dogs from wolves dates to approximately 14,000 years ago, though mitochondrial DNA indicates a much older common ancestor, over 100,000 years old (Bradshaw, 2006). By the Sumerian period (~7,000–6,000 BP), carvings from the Fertile Crescent depict dogs working alongside humans, with the Saluki identified as one of the oldest breeds (Galibert et al., 2011). Significant breed diversification occurred during the Medieval and Renaissance periods, driven by selective breeding for traits like coat color, size, and body shape (Galibert et al., 2011). Today, the remarkable diversity within the domestic dog species complicates broad generalizations about the entire cohort (Bradshaw, 2006).

The diversity within the species correlates with variations in feeding behaviors. Some breeds are known for consuming large meals rapidly, a trait inherited from their wolf ancestors (Lord et al., 2016). This ravenous behavior, associated with scavenging in early domesticated and feral dogs, means that ad libitum feeding is not recommended for such breeds (Bradshaw, 2006). The domestication of dogs may have reduced their innate hunting abilities as they became more reliant on humans for food, which allowed for preferential selection based on appearance,

odor, flavor, and texture (Bradshaw, 2006). This growing reliance on human food sources also drove profound metabolic changes. Specifically, domestication drove the selection of three genes with key roles in starch digestion: AMY2B, MGAM, and SGLT1 (Axelsson et al., 2013). These genetic adaptations provided dogs with the metabolic capacity to utilize starch-rich diets, distinguishing them from their carnivorous wolf ancestors and marking a pivotal milestone in canine domestication (Axelsson et al., 2013). Furthermore, this dietary shift illustrates a remarkable co-evolution, demonstrating how the agricultural revolution and its newly starch-rich diets drove similar adaptive genomic responses in both humans and dogs (Perry et al., 2007).

In canines, food preference is a complex behavior influenced by a variety of factors, including sensory evaluation and behavioral strategies like neophilia, neophobia, and food aversion (Bourgeois et al., 2006). When presented with a choice, dogs use their senses to evaluate available foods. Beyond this sensory assessment, they may also employ several behavioral strategies (Bourgeois et al., 2006). One such strategy is neophilia, a preference for new or recently unencountered foods. In the wild, this behavior would help dogs diversify their diet and ensure nutritional adequacy (Bourgeois et al., 2006). However, the duration of neophilia is dependent on the relative palatability of the new food. If a new food is less palatable than the dog's usual food, its novelty driven appeal will be short-lived (Ferrell, 1984).

Conversely, neophobia is the general avoidance of new foods and is often referred to as "fixation of food habits" (Bourgeois et al., 2006). Animals developed neophobia as a protective mechanism to avoid potentially harmful foods in the wild (Bourgeois et al., 2006). This behavior is most common when meals are served under unusual conditions or when the animal is stressed (Bradshaw & Thorne, 1992). This explains why a dog may refuse new food when stressed by

factors like pain, illness, separation from its owner, or a visit to a veterinary clinic (Bourgeois et al., 2006).

Several solutions have been proposed to combat neophobia and encourage sick pets to eat. One strategy is to provide a multi-day transition period, allowing the pet to experiment with the new food before fully removing the original food (Cheney & Miller, 1997). In a study of cats, neophobia to lamb flavored food disappeared after just three days of exposure. However, this neophobic response reappeared after three months of no exposure, highlighting the importance of regular introduction to new flavors (Bradshaw, 1986). Another approach leverages the fact that while many species exhibit neophobia toward new foods, they rarely show neophobia to drinking water. For instance, rats that were neophobic to mint flavored food readily drank mint flavored water, which eliminated their neophobia after five days (Cheney & Miller, 1997).

Food aversion is a form of negative conditioning in which an animal avoids a food that it associates with a negative experience, such as distress, digestive upset, or an unpleasant smell (Cheney & Miller, 1997). This behavior can develop very quickly, particularly in cats, and is often difficult to reverse, though it can also occur in dogs (Bradshaw et al., 1996). In addition to these behavioral strategies, an animal's dietary history and experiences can significantly influence an animal’s food preferences (Bourgeois et al., 2006). For example, a study comparing the dietary preferences of pet dogs living in owner's homes versus those in kennel environments found that the two groups had different preferences for dry and semi-moist foods. This difference was attributed to their distinct dietary histories and housing experiences (Griffin et al., 1984).

For familiar foods, a dog's choice can be influenced by their relative availability, a behavior known as apostatic or anti-apostatic selection (Bourgeois et al., 2006). Apostatic selection occurs when a predator chooses the most abundant food option, while anti-apostatic

selection involves selecting the least abundant option (Church et al., 1996). Both of these behaviors add another layer of complexity to understanding canine dietary preference and consumption.

The intricate relationship between taste, eating behavior, and metabolism is not fully defined, but specific conditions can make these correlations more explicit. Endocrine diseases, for instance, are characterized by complex interactions among carbohydrate and lipid metabolism, which are regulated by hormones essential for maintaining metabolic homeostasis in both health and disease (Zicker et al., 2010). Dogs with endocrinopathies may exhibit a range of signs, including altered appetite, such as polyphagia (excessive hunger) leading to obesity, or anorexia (a decrease or loss of appetite) (Batchelor & German, 2020; Delaney, 2006).

Hyperlipidemia is a common metabolic disorder characterized by the abnormal accumulation of lipids in the bloodstream, specifically presenting as elevated levels of circulating triglycerides, cholesterol, or both (Ford & Ludlow, 2010b; Zicker et al., 2010). It is estimated to affect up to 12.5% of the canine population (Barriga & Fontúrbel, 2011). Despite this relatively high prevalence, canine endocrinopathies and lipid disorders have historically been under researched (Xenoulis et al., 2011a). Clinically, hyperlipidemia is classified as either primary or secondary. Secondary hyperlipidemia is the most common presentation in dogs, typically resulting from underlying systemic diseases or the administration of certain medications (Nelson et al., 2004; Xenoulis & Steiner, 2010).

Accurate diagnosis relies on distinguishing between normal physiological responses and pathological states. While transient postprandial hyperlipidemia is an expected response to a meal that typically resolves within 12 hours, persistent fasting hyperlipidemia is clinically significant and indicates an underlying primary or secondary condition (Bauer, 2004; Xenoulis &

Steiner, 2010). If left unmanaged, this persistent elevation of circulating lipids can lead to a variety of nonspecific clinical signs, including vomiting, diarrhea, abdominal discomfort, seizures, and a decreased appetite (Ford, 1993, 1996). To fully understand the clinical scope of these conditions, it is crucial to differentiate the specific underlying causes and their unique metabolic signatures. A comprehensive summary of the main primary and secondary lipid disorders in dogs, along with their respective lipid and lipoprotein abnormalities, is provided in Table 2.1 (Xenoulis & Steiner, 2010).

Among the types of hyperlipidemias, hypertriglyceridemia (HTG) is particularly noteworthy. Although once considered a benign and relatively common condition, recent research suggests that HTG may have greater clinical significance than previously thought (Nelson et al., 2004; Xenoulis & Steiner, 2010). Similar to other hyperlipidemias, HTG can be post-prandial, primary (idiopathic), or secondary to a range of endocrine and other systemic diseases, including pancreatitis, diabetes mellitus (DM), Cushing's syndrome (HAC), hepatobiliary disease, and ocular disease (Bauer, 2004; Ford & Ludlow, 2010a; Xenoulis & Steiner, 2010). A diagnosis of idiopathic HTG is made after all secondary causes are ruled out and this diagnosis is particularly common in Miniature Schnauzers (Bauer, 2004; Ford, 1993; Rogers et al., 1975a, 1975b).

HTG is characterized by an excessive (>108 mg/dL) concentration of triglycerides in the blood, which can lead to a lipemic, milky white plasma that becomes opaque in severe cases (Ford & Ludlow, 2010b). The clinical presentation of HTG varies depending on its etiology. While dogs with secondary HTG frequently exhibit clinical signs related to their underlying primary disorder, those with primary HTG often remain entirely asymptomatic (Xenoulis & Steiner, 2010). When clinical signs do manifest, the most common presentations include

vomiting, diarrhea, abdominal discomfort, and, in rare instances, seizures (Bauer, 2004; Bodkin, 1992; Ford & Ludlow, 2010b; Ford, 1993, 1996; Rogers et al., 1975a).

A definitive diagnosis of HTG relies heavily on the laboratory analysis of serum triglycerides collected from a fasted patient (Ford & Ludlow, 2010b). Visually, the presence of lipemia or cloudy serum serves as a strong preliminary indicator of HTG, as this opacity is consistently associated with triglyceride concentrations exceeding 300 mg/dL (Xenoulis & Steiner, 2015). However, reference intervals can be highly variable between laboratories, with some studies citing upper limits ranging from 109 to 151 mg/dL (Ford & Ludlow, 2010b; Kim et al., 2019; Xenoulis & Steiner, 2015; Xenoulis et al., 2007, 2008). Notably, a recent study demonstrated that serum triglyceride levels can exhibit significant intra-individual variability over time, even when dogs are maintained on a consistent food (Xenoulis et al., 2020a). Although the exact mechanism driving this variability remains unknown, this finding suggests that at least two fasting samples should be collected at different time points during the diagnostic evaluation to ensure an accurate assessment of HTG severity (Xenoulis et al., 2020a). While a direct correlation between triglyceride concentrations and the severity of clinical signs has not been definitively established, dogs with elevated serum triglycerides are generally considered to be at an increased risk for developing clinical complications (Armstrong & Ford, 1989; Chapman, 1980; Rogers et al., 1975a).

The interconnectedness of HTG with other endocrine disorders is a focus of more recent research (Kim et al., 2019). The relationship between HTG and pancreatitis appears to be bidirectional, with HTG potentially causing pancreatitis and inflammation from pancreatitis leading to HTG (Xenoulis, 2015; Xenoulis et al., 2011b). This bidirectional link is supported by some evidence, but more research is needed in dogs with naturally occurring states of disease to

confirm the relationship definitively (Whitney, 1992; Williams & Steiner, 2005; Xenoulis et al., 2011a, 2011b; Xenoulis & Steiner, 2010).

Pancreatitis is an inflammatory disorder of the pancreas that can manifest in distinct clinical forms. The acute form is a rapid onset inflammatory process that, despite ranging from mild edema to severe necrosis, can be reversible (Mansfield, 2012). In contrast, chronic pancreatitis is a persistent inflammatory condition that results in irreversible fibrosis and functional deficits (Watson, 2007).

The true prevalence of canine pancreatitis is likely underestimated, due to the challenges of achieving a definitive antemortem diagnosis (Mix & Jones, 2006; Trivedi et al., 2011; Watson, 2007). The condition's nonspecific clinical presentation, which can range from mild signs like lethargy and anorexia to severe outcomes such as multi-organ failure, often complicates accurate clinical assessment (Cridge et al., 2022; Dröes & Tappin, 2017; Hess et al., 1999; Mitchell et al., 2024). Postmortem studies corroborate these diagnostic difficulties; one necropsy analysis of 200 dogs revealed a significantly higher prevalence of chronic pancreatitis (34%) compared to the acute form (2.6%) (Watson, 2007). This discrepancy is partially explained by the reversible nature of acute pancreatitis, which can resolve without leaving permanent histological evidence (De Cock et al., 2007; Watson, 2007). According to Cridge et al. (2021), histopathology remains the standard for diagnosing and distinguishing between chronic and acute pancreatitis. However, due to the invasive nature and requirement of anesthesia often preclude its use in routine clinical practice. As such, clinicians can utilize a multifaceted approach incorporating patient history, physical examination, serum biochemistry (amylase, lipase, and canine pancreatic lipase immunoreactivity [cPLI]), and abdominal ultrasound (Dröes & Tappin, 2017).

Dogs presenting with acute pancreatitis frequently suffer from concurrent comorbidities, such as hyperadrenocorticism (HAC), urinary tract infections, and diabetes mellitus (DM) (Cook et al., 1993; Dröes & Tappin, 2017; Feldman & Nelson, 2003; Trivedi et al., 2011). Recent research has specifically highlighted a strong association between pancreatitis and the development of DM, emphasizing the need for veterinarians to pursue proactive diagnostic screening for early detection (Heeley et al., 2020). When DM is present, it profoundly impacts lipid metabolism; these patients most commonly present with secondary HTG, though hypercholesterolemia is also frequently observed (Barrie et al., 1993; Feldman & Nelson, 2003; Johnson, 2005; Rogers et al., 1975b; Whitney, 1992; Wilson et al., 1986). Notably, because this hyperlipidemia is a secondary complication, the HTG typically resolves once the underlying DM is successfully managed (Gleeson et al., 1990; Whitney, 1992).

Canine DM is a complex disease and has been associated with environmental and genetic factors (Mattin et al., 2014). Unlike humans, dogs are not classified as type 1 or 2 diabetic, but instead as insulin dependent or non-insulin dependent, based on the dog's need for insulin (Zicker et al., 2010). The majority of dogs present as insulin dependent, similar to type 1 DM in humans (Zicker et al., 2010). Clinical signs of canine DM include polyuria, polydipsia, polyphagia, weight loss, and lethargy (ESVE, 2015; Nelson, 2015; Zicker et al., 2010). As the disease progresses, patients may develop more severe or atypical signs, such as blindness (often secondary to cataract formation), anorexia, vomiting, and diarrhea (Zicker et al., 2010). Beyond outward physical symptoms, DM presents with distinct clinicopathologic abnormalities on routine blood work. Most notably, serum biochemistry often reveals elevated concentrations of cholesterol, triglycerides, and blood urea nitrogen (Choudhary et al., 2021). In complicated cases, such as diabetic ketoacidosis (DKA), the excessive loss of fluid through urination

(osmotic diuresis) causes profound systemic dehydration. On diagnostic blood panels, this severe fluid loss concentrates the blood, resulting in an elevated packed cell volume (PCV) (Zicker et al., 2010). Other potential, though less common, serum biochemistry elevations include alanine aminotransferase (ALT) and alkaline phosphatase (ALP) activities, cPLI, bile acids, and total bilirubin (Zicker et al., 2010).

Another significant endocrinopathy associated with both DM and secondary lipid disorders is HAC (Bauer, 2004; Cook et al., 1993; Feldman & Nelson, 2003; Johnson, 2005; Miceli et al., 2017; Rogers, 1977; Rogers et al., 1975b; Whitney, 1992). In canine patients, this disease frequently presents with concurrent lipid abnormalities, including both hypercholesterolemia and HTG (Barrie et al., 1993; Bauer, 2004; Feldman & Nelson, 2003; Huang et al., 1999; Johnson, 2005; Ling et al., 1979; Whitney, 1992), with HTG being the most predominant lipid abnormality (Bauer, 2004; Feldman & Nelson, 2003; Johnson, 2005).

The etiology of naturally occurring HAC is divided into two primary categories. The vast majority of cases (80–85%) are pituitary-dependent, driven by the excessive secretion of adrenocorticotropic hormone (ACTH) and the remaining 15–20% of cases result from cortisol-secreting adrenocortical tumors, which may be benign or malignant (Labelle et al., 2004; McGreevy et al., 2016; Rijnberk & Kooistra, 2010; Sanders et al., 2018).

Clinical diagnosis of HAC can be challenging due to its varied and sometimes nonspecific manifestations. Hallmark clinical signs include polyuria, polydipsia, polyphagia, lethargy, excessive panting, and muscle atrophy leading to a "potbellied" appearance (Sanders et al., 2018). Dermatologic and systemic complications such as truncal alopecia, hepatomegaly, systemic hypertension, and recurrent urinary tract infections are also prevalent (Behrend et al., 2013; Galac et al., 2014; McGreevy et al., 2016). On routine serum biochemistry, common

alterations include elevations in ALP and ALT activities, alongside hypercholesterolemia, hyperglycemia, and decreased blood urea nitrogen (BUN) (Feldman & Nelson, 1996; Herrtage & Ramsey, 2012; Kintzer et al., 1999; Peterson, 1984). Notably, while these metabolic and lipid shifts are profound, current literature has not established a causal link between dietary nutrition and the primary development of HAC in dogs (Fascetti & Delaney, 2023).

Despite the lack of nutritional etiology, the management of HAC, DM, and pancreatitis often necessitates a similar multimodal approach centered on dietary intervention. Current therapeutic strategies are largely predicated on the evidence that high fat foods can induce insulin resistance (Massillon et al., 1997). Experimental models have shown that high fat intake in healthy dogs mimics the metabolic shifts seen in natural aging, including increased adiposity, dyslipidemia, and impaired insulin sensitivity (Hoffman et al., 2021; Kawasumi et al., 2014; Lawler et al., 2008; McKenzie et al., 2024; Turner et al., 2020). However, the specific impact of dietary fat in naturally occurring disease remains a subject of debate; for instance, it has been suggested that the role of fat restriction in the management of pancreatitis may be overemphasized (Cridge et al., 2024).

Recent data further clarify these lipid driven metabolic changes. McKenzie et al. (2024) demonstrated that a high fat food (74% metabolizable energy from fat) induced significant increases in fasting insulin and insulin resistance in dogs without causing clinically significant weight gain. The study highlighted a correlation between hyperinsulinemia and elevations in leptin, cholesterol, and triglycerides, suggesting that dyslipidemia is a primary driver of insulin resistance (McKenzie et al., 2024). These food induced alterations can parallel age related pathologies and potentially shorten the canine lifespan (Lawler et al., 2008). Similar metabolic responses have been observed in felines, where low carbohydrate, high fat foods significantly

increased fat metabolite concentrations (plasma triglycerides, free fatty acid (FFA), beta-hydroxybutyrate, and cholesterol) (Thiess et al., 2004).

Based on these physiological responses, clinicians have historically recommended low-fat foods, defined as providing less than 25 g of fat per 1,000 kcal or less than 12% fat on a dry matter basis, to manage metabolic endocrinopathies (Elliott, 2005; Ford & Ludlow, 2010b; Ford, 1996; Johnson, 2005). Many commercially available maintenance diets meet these criteria. Additionally, most treats and table scraps should be strictly avoided (Elliott, 2005). Following the initiation of a low-fat food, serum lipid concentrations should be re-evaluated after approximately 4 to 8 weeks (Ford, 1996). If serum triglyceride concentrations decrease, dietary therapy may be continued indefinitely, with ongoing monitoring every 6 to 12 months (Ford, 1996). For dogs that fail to sufficiently respond to standard low-fat foods, an ultra low-fat therapeutic option (e.g., 10 to 12 g of fat per 1,000 kcal) may be implemented, or pharmacological treatment can be initiated (Elliott, 2005). Furthermore, ultra low-fat therapeutic foods can incorporate additional nutritional modifications, such as polyunsaturated fatty acids to lower triglycerides and increased dietary fiber to reduce cholesterol. When dietary intervention alone is inadequate, supplemental therapies including fish oils, gemfibrozil, and occasionally niacin may be utilized (Kashyap et al., 2002; Logas et al., 1991; Spencer & Barradell, 1996; Stalenhoef et al., 2000).

Fish oils, which are rich in omega-3 fatty acids, can be administered as an exogenous supplement or incorporated directly into therapeutic foods. Fish oils have been shown to effectively decrease the production of triglyceride-rich very-low-density lipoproteins (VLDL)(Ford & Ludlow, 2010b). One study identified a significant reduction in serum triglycerides in healthy dogs supplemented with dietary supplementation with 7 g of menhaden

fish oil (1.65% on a dry-matter basis [DMB]) (LeBlanc et al., 2005). However, established dosage guidelines remain highly inconsistent. Recommended dosage of fish oils can range from as low as 1g/4.55 kg of body weight daily (Bauer, 1995) to a markedly higher dose of up to 220 mg/kg of body weight (Carroll, 2022; Ford & Ludlow, 2010b). This discrepancy highlights the need for further clinical evaluation to determine the most efficacious and safe therapeutic protocol.

Fibric acid derivatives (fibrates), such as fenofibrate, and gemfibrozil, have been reported to effectively reduce serum triglyceride concentrations, reduce lipemia, and improve lipid profiles in cholestatic dogs (Gori et al., 2025; Spencer & Barradell, 1996; Stalenhoef et al., 2000). These pharmacological agents are typically introduced in combination with dietary therapy when nutritional management alone fails to adequately lower serum triglycerides (Bauer, 1995; Whitney, 1992). In canine patients, therapeutic dosages typically include 200 mg/day for gemfibrozil and 4-10 mg/kg once daily for fenofibrate, which function primarily by reducing VLDL secretion (Bauer, 1995; Carroll, 2022; Gori et al., 2025).

Niacin is a B vitamin that can be incorporated into the formulation of therapeutic foods or administered as an oral supplement to support dogs with hyperlipidemia. Although research evaluating its direct effects on hypertriglyceridemia in dogs remains limited, niacin has been successfully utilized to treat dyslipidemia in human medicine (Kashyap et al., 2002). Early research suggests that these positive lipid-lowering effects may translate to canine patients with dosing as high as 100 mg/day, however side effects of erythema and pruritus have been reported (Bauer, 1995; Johnson, 2005; Whitney, 1992). When used as a therapeutic intervention, niacin is generally recommended at a dosage of 50 to 200 mg/day, and can be divided into two daily doses (Carroll, 2022).

Despite the clinical efficacy of these interventions, implementing strict dietary fat restrictions poses a practical challenge due to innate canine palatability preferences. Dogs naturally demonstrate a strong taste preference for fat over protein or carbohydrates (Hall et al., 2018; Hewson-Hughes et al., 2013; Tôrres et al., 2003). Furthermore, one study specifically showed that canine taste preference was higher for high fat foods compared to high protein or high carbohydrate options (Hall et al., 2018). This preference for high fat options can render therapeutic low-fat foods unpalatable, ultimately compromising owner compliance and the efficacy of the nutritional treatment plan.

Given the limitations of low-fat foods regarding palatability and compliance, alternative nutritional strategies involving the manipulation of macronutrient ratios, specifically ketogenic foods, have gained traction in veterinary research. Ketogenic foods replace digestible carbohydrates with protein and fat to create foods that are low in carbohydrates and support a state of nutritional ketosis (Zilberter & Zilberter, 2018). Reducing dietary carbohydrates necessitates a concurrent increase in dietary fat and/or protein to meet energy requirements. Specifically, while ketogenic foods do restrict carbohydrates (< 10% of total energy) they are primarily defined by the high fat content and strict adherence to a ketogenic ratio (Accurso et al., 2008; Feinman et al., 2015; Volek et al., 2008; Westman et al., 2008). Accordingly the ketogenic ratio (KR) is a ratio of the sum of ketogenic factors to the sum of antiketogenic factors: KR = (0.46 x grams of protein + 0.9 x grams of fat)/(0.58 x grams of protein + 0.1 x grams of fat + grams of carbohydrate)(Zilberter & Zilberter, 2018). First indicated for the management of human epilepsy in 1921, additional studies in humans continue to investigate applications including: obesity, metabolic syndrome, and type 2 diabetes, cancer metabolism, Parkinson disease and Alzheimer disease (Chamma et al., 2025; Choi et al., 2020; Jagadish et al., 2019;

Kim, 2017a, 2017b; Luong et al., 2025; Mohorko et al., 2019; Mooli & Ramakrishnan, 2022). More recent evidence suggests ketogenic formulations may offer therapeutic benefits for a broader range of endocrine and metabolic disorders, including diabetes mellitus, obesity, and inflammatory conditions in dogs (Jackson, 2022; Jackson & Waldy, 2021; Tavener et al., 2024; Vendramini et al., 2024). Recent studies demonstrate that ketogenic foods can induce a "metabolic switch" in canines, shifting the body from a glucose-dependent state to one reliant on fat catabolism (Jackson, 2022, 2024; Jackson & Waldy, 2021; Tavener et al., 2024). This shift promotes the production of ketone bodies like β-hydroxybutyrate (B-HBA), which are associated with significant anti-inflammatory and immunomodulatory effects, including the targeted downregulation of proinflammatory cytokines (Jackson, 2022; Karagiannis et al., 2022; Tavener et al., 2024; Vandenberghe et al., 2020). Furthermore, the degree of this metabolic switch is macronutrient dependent; carbohydrate restriction accompanied by fat replacement induces nutritional ketosis to a much greater extent than carbohydrate replacement with protein alone (Jackson, 2022). The clinical viability of these high fat foods is further supported by data regarding their digestibility and acceptance. Contrary to concerns that high lipid loads might compromise gastrointestinal function, one study indicated that increasing dietary fat significantly improved the apparent total tract digestibility of dry matter, organic matter, and gross energy in healthy dogs without detrimental effects on stool quality (Kilburn et al., 2020).

Despite the emerging physiological evidence supporting carbohydrate restriction, the regulatory framework for labeling such commercial diets remains strict. According to the 2025 Association of American Feed Control Officials Official Publication, Regulation PF10 prohibits the use of absolute claims such as “low carbohydrate”, “low dietary starch”, or “low sugars” on pet food labels (AAFCO, 2025). However, manufacturers are permitted to use comparative

descriptive terms such as "less" or "reduced" carbohydrates, provided the label explicitly states the percentage of reduction and identifies the product of comparison, ensuring that therapeutic claims are substantiated within the regulatory guidelines (AAFCO, 2025).

Previous comparative research has demonstrated the metabolic efficacy of ketogenic foods relative to standard high carbohydrate dietary options (Jackson, 2022, 2024; Jackson & Waldy, 2021; Tavener et al., 2024). Across three independent crossover studies involving healthy dogs, ketogenic foods were compared to foods with significantly higher carbohydrate content (Gupta-Saraf et al., 2022; Jackson, 2022; Jackson & Waldy, 2021). In these trials, subjects exhibited a significant reduction in serum triglycerides (TG) after transitioning to the ketogenic food from higher carbohydrate formulations (Jackson, 2022; Jackson & Waldy, 2021). This lipid lowering effect occurred concurrently with a significant elevation in B-HBA, confirming that the replacement of digestible carbohydrates with dietary fat and protein successfully induces a metabolic shift toward ketosis (Gupta-Saraf et al., 2022; Jackson, 2022). Furthermore, this macronutrient profile appears to alleviate hepatic stress, as evidenced by significantly lower ALP activity in dogs fed a fat based ketogenic food compared to those fed protein based or high carbohydrate alternatives (Jackson & Waldy, 2021).

Beyond primary lipid management, a ketogenic nutritional approach maintains intake and favorable metabolic regulation. Palatability and caloric sufficiency were demonstrated by consistent food intakes and metabolizable energy consumption across feeding trials of a ketogenic food, supporting the maintenance of body weight (Gupta-Saraf et al., 2022; Jackson, 2022; Jackson & Waldy, 2021). Despite the increased fat content, pancreatic health was preserved, as cPLI levels remained stable and did not elevate in dogs consuming the ketogenic food (Gupta-Saraf et al., 2022). In terms of metabolic signaling, the food modulated key

hormones by increasing both leptin and ghrelin; notably, the fat based ketogenic formulation specifically decreased the leptin:ghrelin ratio, a change proposed to provide metabolic benefits (Jackson & Waldy, 2021). Collectively, these findings support the utility of the ketogenic food when fed to healthy dogs and highlight the need for further testing in dogs with hypertriglyceridemia to confirm its effectiveness in managing the condition while maintaining metabolic health. Therefore, the objective of this research was to evaluate the efficacy of a ketogenic food, in which digestible carbohydrates are replaced with fat and protein for reducing serum triglyceride levels in dogs with confirmed hypertriglyceridemia.

Table 1.1. Functions of Major Regulatory and Industry Bodies in Commercial Pet Food (Roudebush et al., 2010; Zicker, 2008).

Agency/Organization	Function	Reference
US Food & Drug Administration/Center for Veterinary Medicine (FDA CVM)	• Specifies some label requirements • Regulates health claims • Ensures food safety	fda.gov/cvm
Association of American Feed Control Officials (AAFCO)	• Not a regulatory enforcement agency • Sets nutrient standards for substantiation of some claims • Provides model regulations for adoption by U.S. states and territories • Plays a role in defining, standardizing, and reviewing ingredients • Provides labeling guidelines	aafco.org
US Department of Agriculture (USDA)	• Regulates some pet food ingredients • Inspects animal research facilities • No regulatory authority over domestic pet food, except for that under voluntary inspection program	usda.gov
Federal Trade Commission	• Regulates advertising of pet foods	ftc.gov

National Research Council (NRC)	• Not a government agency, but advises government on matters related to science • Evaluates and compiles nutrition research • Makes nutrient recommendations	animalnutrition.org/nrc_reports
State Department of Agriculture	• Adopts and enforces animal food regulations	nasda.org/about-nasda/state-agriculture-departments/ *Varies per state
American Feed Industry Association (AFIA)	• Trade organization that represents major feed and pet food manufacturers in the United States	https://www.afia.org/
Pet Food Institute (PFI)	• Trade organization that represents major pet food manufacturers in the United States	petfoodinstitute.org
European Council of Ministers	• Approves directives and regulations • Creates basic laws	consilium.europa.eu
European Federation of the Pet Food Industry (FEDIAF)	• Trade organization that represents major pet food manufacturers in Europe	europeanpetfood.org
National Government (Ministry of Agriculture)	• Implements European legislation and controls its application • Houses national experts	commission.europa.eu
Confederation of the Food and Drink Industries of the EU (CIAA)	• Trade organization that represents human food manufacturers in Europe • Works closely with FEDIAF on matters of mutual interest	fooddrinkeurope.eu

Adapted from Zicker (2008) and Roudebush et al. (2010).

Table 1.2. Main primary and secondary lipid disorders and respective lipid and lipoprotein abnormalities in dogs.

Causes of hyperlipidemia	Lipid(s) affected (increased)	Lipoprotein class mainly affected (increased)
Primary		
Primarily triglyceride defect		
Miniature Schnauzers	Triglycerides, maybe cholesterol	VLDL, chylomicrons
Brittany Spaniels	Triglycerides	VLDL, chylomicrons

Causes of hyperlipidemia	Lipid(s) affected (increased)	Lipoprotein class mainly affected (increased)
Primarily cholesterol defect		
Briards	Cholesterol	HDL
Rough collies	Cholesterol	HDL, VLDL, LDL
Shetland sheepdogs	Cholesterol, maybe triglycerides	HDL, LDL
Doberman Pinschers	Cholesterol	Unknown
Rottweilers	Cholesterol	Unknown
Mixed defect		
Beagles	Triglycerides, cholesterol	HDL, LDL
Secondary		
Pancreatitis	Triglycerides, maybe cholesterol	VLDL, chylomicrons, LDL, HDL
Hypothyroidism	Triglycerides, cholesterol	HDL, VLDL, LDL
Hyperadrenocorticism	Triglycerides, cholesterol	VLDL, LDL
Diabetes mellitus	Triglycerides, cholesterol	VLDL
Protein losing nephropathy	Cholesterol, maybe triglycerides	Unknown
Cholestasis	Cholesterol, triglycerides	LDL
Obesity	Triglycerides, cholesterol	VLDL, LDL, HDL
High fat diets	Triglycerides, cholesterol	HDL

HDL: high density lipoproteins, LDL: low density lipoproteins, VLDL: very low density lipoproteins.

From Xenoulis and Steiner (2010).

Chapter 2 - Clinical Evaluation of a Low Carbohydrate Compared to a Low-Fat Food in Dogs with Confirmed Hypertriglyceridemia

Abstract

Introduction: Canine hypertriglyceridemia (HTG) is traditionally managed using low-fat foods. However, a dog's innate preference for dietary fat can compromise palatability and compliance with strict low-fat foods. This study evaluated an alternative nutritional strategy: replacing dietary carbohydrates with protein and fat as a highly palatable therapeutic option for dogs with confirmed HTG.

Methods: Nineteen adult dogs with a confirmed history of HTG completed the study. All dogs completed a 4-week prefeed period on a standard maintenance food and. Dogs were then randomly assigned to receive either a Control food (Royal Canin Veterinary Diet Adult Gastrointestinal Low-Fat Dry Dog Food.) or an experimental Test food (ketogenic formulation) for a 10-week treatment period. Clinical assessments monitored throughout the study included daily food intake, body weight, coat quality, fecal characteristics (color, odor, size, casing, and presence of blood, mucus, segmentation, or unusual texture), fecal consistency, fecal pH, and moisture.

Results: Dogs fed the Control food exhibited transient decreases in daily caloric intake (Kcal/day, $P = 0.05$), intake normalized by the study's conclusion ($P = 0.11$), and dogs maintained body weight with no significant loss ($P > 0.05$) documented through the end of the study. Dogs fed the Test food maintained adequate average consumption and exhibited no change ($P = 0.23$) in body weight. While no adverse changes in fecal characteristics were observed in either group, dogs fed the Test food demonstrated higher fecal pH ($P < 0.01$) and

overall fecal moisture ($P < 0.01$) compared to the Control group. Overall coat quality did not differ ($P = 0.18$) between the treatment groups by the end of study.

Discussion: These findings indicate that a food with a ketogenic ratio > 1.5 represents a highly palatable alternative to traditional low-fat formulations for dogs presenting with an endocrine disease and concurrent inappetence. By maintaining intake and body weight without eliciting adverse gastrointestinal or dermatological effects, these data provide the first preliminary evidence supporting this novel food when fed to HTG dogs. Additional research is warranted to confirm these initial findings and fully establish its therapeutic potential.

Introduction

Hyperlipidemia is characterized by elevated blood concentrations of triglycerides (HTG), cholesterol (hypercholesterolemia), or both (Barrie et al., 1993; Ford, 1996; Johnson, 2005). The condition can present as a primary, or idiopathic disorder (Xenoulis & Steiner, 2015). More commonly, however, it occurs secondary to underlying conditions such as endocrinopathies (e.g., hypothyroidism, diabetes mellitus, hyperadrenocorticism, pancreatitis), obesity, protein-losing nephropathy, cholestasis, or the consumption of high fat foods (Ford & Ludlow, 2010b; Miceli et al., 2017; Miceli et al., 2021; Xenoulis & Steiner, 2010). Although the clinicopathological presentation of these diverse etiologies is relatively uniform (Xenoulis et al., 2020a), primary hypertriglyceridemia (HTG) is strongly breed-predisposed, most notably affecting up to 56% of otherwise apparently healthy Miniature Schnauzers (Smith et al., 2017; Xenoulis & Steiner, 2015; Xenoulis et al., 2007). Although HTG is an increasingly recognized condition in dogs, broad studies examining its overall incidence and prevalence are lacking (Miceli et al., 2021). Dogs with primary HTG are often asymptomatic for extended periods; however, severe or prolonged HTG can precipitate secondary diseases that necessitate medical intervention

(Xenoulis & Steiner, 2010, 2015). Findings from Xenoulis et al. (2020b) contradict previous hypothesis that HTG causes pancreatitis, by demonstrating >70% of dogs with pancreatitis had serum triglycerides and cholesterol concentrations within the normal reference range (26-108 mg/dL and 124-335 mg/dL, respectively). Additionally noting that overall prevalence of hyperlipidemia increased up to 43% in dogs with concurrent diabetes mellitus (Xenoulis et al., 2020b). Confirming this clinical link, Kim et al. (2019) demonstrated that dogs with pancreatitis are 12.4 times more likely to have diabetes mellitus compared to healthy dogs (Kim et al., 2019). These findings demonstrate that HTG is a significant biological mediator in the association between diabetes mellitus and pancreatitis (Kim et al., 2019; Xenoulis et al., 2020b). Therefore treatment to resolve hyperlipidemia could help lower the chances of onset of diabetes mellitus. Diagnosis relies on serum biochemistry; however, there are notable variations in reference intervals for triglycerides between laboratories (Xenoulis & Steiner, 2015). Upper limits of reference intervals can range from 109 mg/dL up to 151 md/dL (Kim et al., 2019; Xenoulis & Steiner, 2015; Xenoulis et al., 2007, 2008). Such variations in reference intervals can significantly impact how clinicians interpret the severity of triglyceride concentration elevations and subsequently manage patients. Effective management of HTG requires differentiating between primary and secondary forms, as treating the underlying cause often resolves secondary HTG (Ford, 1996; Johnson, 2005; Rogers, 1977; Whitney, 1992). For primary HTG, dietary modification is the recommended initial intervention. Current best practices suggest feeding a low-fat food restricted to less than 25 grams of fat per 1,000 kcal on a DMB, to help reduce the amount of fat metabolized in the intestines (Elliott, 2005; Ford, 1996; Johnson, 2005; Xenoulis & Steiner, 2010). Dietary fats are absorbed in the intestines and packaged into chylomicrons, which are lipoproteins responsible for transporting triglycerides and cholesterol throughout the

body (Karam et al., 2017; Xenoulis & Steiner, 2010). As such the reduction in chylomicrons can help to manage blood lipid concentrations (Bauer, 1995; Karam et al., 2017; Xenoulis & Steiner, 2010).

Beyond strict fat restriction, the specific type of dietary fat incorporated into the food is critical. Despite maintaining a low total fat content, therapeutic foods often utilize targeted nutrition by including polyunsaturated omega-3 fatty acids to actively reduce circulating serum triglycerides and cholesterol. Omega-3 fatty acids, derived from fish oils, help lower serum lipids by reducing the hepatic production of very-low-density lipoproteins (VLDL), which are responsible for transporting triglycerides into the bloodstream (LeBlanc et al., 2005). Furthermore, omega-3s limit overall triglyceride production because they serve as poor substrates for triglyceride synthesizing enzymes (Elliott, 2001; LeBlanc et al., 2005). Consistent with these mechanisms, supplementing canine foods with marine fish oils (a source of n-3 fatty acids), with or without vitamin E (fish oils 1.65% DMB and 0.17 g of α-tocopherol acetate), significantly reduces serum triglyceride and cholesterol concentrations (LeBlanc et al., 2005). Additionally, Helland et al. (2025) completed a meta-analysis comprising 12 articles and 243 dogs which concluded that intake of marine oils can result in a lower total cholesterol concentration.

Dietary fiber is thought to be an essential component of HTG management, leading some commercial low-fat foods to feature increased fiber content (Ford & Ludlow, 2010b). Soluble fibers, such as psyllium, oat bran, guar gum, and pectin, reduce cholesterol when included at dietary concentrations of 3% to 10% (Anderson et al., 2000; Ford & Ludlow, 2010b; Hunninghake et al., 1994). Soluble fibers help reduce serum cholesterol uptake due to their viscosity and fermentabilities (Nakashima et al., 2018). The increased viscosity slows the rate of

flow, which impairs the digestion of lipids by bile salts; leading to a reduction in total cholesterol (TC) and low-density lipoprotein cholesterol (LDL) without negatively affecting high-density lipoproteins (HDL) (Nakashima et al., 2018; Phungviwatnikul, 2022). Specifically, β-glucans derived from oats and barley are commonly utilized for this purpose (Cloetens et al., 2012; Reyna-Villasmil et al., 2007; Smith et al., 2008). Furthermore, Peña et al. (2014), demonstrated that feeding obese, otherwise healthy dogs, a targeted low-fat, high fiber food (34.6% protein, 8.2% fat, 38.2% carbohydrate, and 25.7% total dietary fiber; DMB) helped reduce both serum triglycerides and total cholesterol. Finally, niacin supplementation offers an additional nutritional strategy shown to successfully lower triglyceride concentrations in both humans and dogs (Bauer, 1995; Johnson, 2005; Kashyap et al., 2002; Whitney, 1992).

Given the innate canine preference for dietary fat, replacing dietary carbohydrates with protein and fat represents a promising alternative nutritional strategy. In human medicine, low-carbohydrate foods have been shown to sustain long-term weight loss more effectively than low-fat foods, while simultaneously lowering serum triglycerides and increasing HDL cholesterol (Brehm et al., 2003; Clifton & Keogh, 2007; Dansinger et al., 2005; Foster et al., 2003; Samaha et al., 2003; Stern et al., 2004). The evaluation of substituting carbohydrates with protein and fat to positively influence canine health has only recently been investigated (Jackson, 2024). Co-evolution between humans and dogs has been hypothesized as the reason canine glycemic responses to dietary starches largely parallel those of humans (Briens et al., 2021; Pajic et al., 2019; Wang et al., 2013), making these macronutrient shifts highly relevant to canine health. Reducing dietary carbohydrates necessitates a concurrent increase in dietary fat and/or protein to meet energy requirements. Specifically, while ketogenic foods do restrict carbohydrates, comprising < 10% of total energy, they are primarily defined by the high fat content and strict

adherence to a ketogenic ratio (Accurso et al., 2008; Feinman et al., 2015; Volek et al., 2008; Westman et al., 2008; Zilberter & Zilberter, 2018).Whereas a non-ketogenic, low carbohydrate food is less restrictive, allowing carbohydrates to make up to 26% of total energy (Accurso et al., 2008; Feinman et al., 2015; Volek et al., 2008; Westman et al., 2008). The strict macronutrient shift from ketogenic foods can induce a state of nutritional ketosis, characterized by elevated β-hydroxybutyrate (B-HBA) (Gershuni et al., 2018; Hume et al., 2006; Wallenius et al., 2022; Zhu et al., 2022). Notably, while dogs achieve ketosis more slowly and exhibit lower peak B-HBA concentrations than humans (Crandall, 1941), this metabolic shift remains clinically beneficial. In humans, the ketogenic ratio (KR) is utilized to predict nutritional ketosis via an empirically derived formula based on macronutrient composition (Wilder & Winter, 1922). The KR is calculated as $KR = \frac{(0.46 \times \text{grams of protein}) + (0.9 \times \text{grams of fat})}{((0.58 \times \text{grams of protein}) + (0.1 \times \text{grams of fat}) + \text{grams of carbohydrate})}$ (Zilberter & Zilberter, 2018). For human foods, a KR greater than 1.5 is generally recognized as the threshold to induce ketosis (Zilberter & Zilberter, 2018).

Recent canine studies confirm the physiological advantages of carbohydrate restriction that maintain this threshold (KR ≥ 1.5), as demonstrated by elevated B-HBA. Feeding dogs low-carbohydrate, ketogenic foods yields measurable improvements in overall metabolism (Jackson, 2022, 2024; Jackson & Waldy, 2021; Tavener et al., 2024) and more efficiently reduces fat mass while preserving lean muscle mass compared to high carbohydrate foods (Bierer & Bui, 2004). Furthermore, dietary management can be enhanced by combining high protein with high fiber (high protein [103 g/1,000 kcal ME] high fiber [60 g/1,000 kcal ME]), which has been shown to significantly increase satiety and reduce voluntary food intake in dogs (Weber et al., 2007). Ultimately, these high-fat, high-protein, and low-carbohydrate macronutrient profiles align seamlessly with innate canine taste preferences (Hewson-Hughes et al., 2013), offering a highly

palatable therapeutic option that successfully circumvents the compliance issues traditionally associated with strict low-fat foods.

This study evaluated the impact of feeding an experimental food that replaced carbohydrates with protein and fat compared to a food with moderate protein, and low-fat in dogs with confirmed hypertriglyceridemia. It was hypothesized that the experimental food would maintain food intake, body composition and fecal consistency compared to the nutritional standard of care. To assess outcomes related to body composition, fecal consistency and food intake, body weight and fecal consistency were recorded at week 4 of the prefeed period and weeks 2, 5, 6, 8, and 10 of the treatment period, alongside daily food intake monitoring. Additional exploratory endpoints reviewed coat quality, fecal moisture, and fecal pH.

Methods

Study Design

This was a randomized, stratified study design with repeated measures of 20 adult male and female dogs. Dogs were randomly assigned to one of two treatment foods: Test or Control. The study consisted of a 4 week prefeed and a 10-week treatment period. The study protocol (CP1048a) was reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) at Hill's Pet Nutrition, Inc. (Topeka, KS, USA).

Animal Management and Welfare

All dogs included in the study were housed in a colony and provided care by Ontario Nutri-Lab Inc. (Fergus, Ontario, Canada) and were maintained and treated in accordance with the Hill's Global Animal Welfare Policy. Dogs were group housed for the majority of the testing period in spacious indoor rooms, with the exception of feeding times and biological collections.

Windows within the dogs' rooms provided a natural light cycle, supplemented by full-spectrum LED lights.

All dogs were allowed normal socialization with other dogs and had daily opportunities for behavioral and physical enrichment through access to care personnel. Toys were provided to encourage play and exercise. Dogs were fed once daily in a 30 minute time period, to maintain their initial body weight. Food offerings were based on each dog's calculated metabolizable energy requirement (MER)(Cline et al., 2021). To maintain body weight, food offerings were reviewed and adjusted weekly based on recorded body weights. Food offerings were increased only if a dog was consuming its entire daily allocation but still losing weight; conversely, food amounts were not altered if a dog consistently left food uneaten. Additionally, dogs had ad libitum access to fresh, clean drinking water, and received routine immunizations. Dogs were also monitored for parasites and received routine heartworm, hookworm, and roundworm prevention. The conduct of the study did not interfere with the animals' normal daily routine. The study protocol was reviewed and approved by the Institutional Animal Care and Use Committee, Hill's Pet Nutrition, Inc., in Topeka, Kansas. Procedures were designed to avoid or minimize pain, discomfort, or distress, and dogs were monitored for any signs of disease. At no time during the study were dogs subjected to any procedures expected to cause pain or distress. If an adverse event occurred, the dog's health always took precedence over continuation in the trial.

Study Population

The study enrolled 20 adult dogs with confirmed hypertriglyceridemia housed in a colony setting. Dogs were included in the study if they had a demonstrated history of two or more fasted sampling (>15 hours) blood collections that showed increased serum concentrations above the

normal interval for either triglycerides (>1.71 mmol/L or 151 mg/dL) cholesterol (>8.9 mmol/L or 343.6 mg/dL), or had a positive lipemia index, or a combination of all 3. Other inclusion criteria included dogs with a history of good appetite, willing to eat dry food only, and a history of providing successful stool samples during the day.

Dogs were excluded from the study if they were participating in another clinical trial or exhibited severe concurrent systemic or organ disease, such as chronic kidney disease. Dogs presenting with injuries causing significant swelling or edema, or showing obvious signs of aging (e.g., muscle wasting), were also deemed ineligible. Additionally, dogs were excluded if they had received oral antibiotics or anti-inflammatory medications (e.g., NSAIDs, corticosteroids) within the two months preceding the study. Finally, animals were excluded if they were fractious, had a history of poor eating behavior, or demonstrated difficulty in providing stool or blood samples. After confirming inclusion criteria, remaining subjects were then randomized onto the Test or Control Food. For randomization dogs were stratified based on age and weight.

Study Foods

All foods administered were nutritionally complete and balanced dry formulations and contained only ingredients approved by the Association of American Feed Control Officials. Dogs were subjected to a four-week prefeed period in which they consumed a maintenance food (Purina Dog Chow Complete Adult).

The Test food is an experimental food made by extrusion processing at the Hill's experimental food laboratory following Good Manufacturing Practice (GMP) guidelines. The Test food included chicken, beef tallow, egg product, spray dried pork stock, powdered cellulose, flaxseed, pork gelatin, chicken liver flavor, dried hydrolyzed casein, dried hydrolyzed whey,

hydrolyzed chicken liver, calcium sulfate, lactic acid, potassium citrate, pork liver flavor, dried beet pulp, dried citrus pulp, psyllium seed husk, dried apple pomace, oat bran, choline chloride, vitamins [vitamin e supplement, L-ascorbyl-2-polyphosphate (source of vitamin c), niacin supplement, thiamine mononitrate, vitamin a supplement, calcium pantothenate, riboflavin supplement, biotin, vitamin b12 supplement, pyridoxine hydrochloride, folic acid, vitamin d3 supplement], taurine, minerals (ferrous sulfate, zinc oxide, copper sulfate, manganous oxide, calcium iodate, sodium selenite), L-carnitine, L-tryptophan, mixed tocopherols for freshness, magnesium oxide, natural flavors, beta-carotene. The Control food (Royal Canin Veterinary Diet Adult Gastrointestinal Low-Fat Dry Dog Food) was selected as it met the current nutritional guidelines for supporting dogs with an endocrinopathy (Ford & Ludlow, 2010b). The Control food consisted of brewers rice, chicken by-product meal, barley, natural flavors, pork digest, pea fiber, dried plain beet pulp, chicken fat, monocalcium phosphate, salt, potassium chloride, calcium carbonate, powdered psyllium seed husk, sodium aluminosilicate, fish oil, fructooligosaccharides, choline chloride, vitamins[dl-alpha tocopherol acetate (source of vitamin e), l-ascorbyl-2-polyphosphate (source of vitamin c), biotin, d-calcium pantothenate, vitamin a acetate, riboflavin supplement, niacin supplement, vitamin b12 supplement, pyridoxine hydrochloride (vitamin b6), thiamine mononitrate (vitamin b1), vitamin d3 supplement, folic acid], rosemary extract, preserved with mixed tocopherols and citric acid, hydrolyzed yeast, dl-methionine, marigold extract (tagetes erecta l.), trace minerals [zinc proteinate, zinc oxide, ferrous sulfate, manganese proteinate, manganous oxide, copper sulfate, sodium selenite, calcium iodate, copper proteinate], taurine.

Nutrient analysis of the study foods was performed at Eurofins Nutrition Analysis Center (Des Moines, IA, USA) using standard Association of Official Agricultural Chemists (AOAC)

methods (AOAC International, 2023). Key nutrient levels of the Control food and Test food are summarized in Table 1.1, on a caloric and dry matter basis. The Control food contained 68.4 g/1,000 kcal crude protein, 23.2 g/1,000 kcal crude fat, 4.6 g/1,000 kcal crude fiber, and 166.6 g/1,000 kcal nitrogen-free extract (NFE) on caloric basis. The Test food contained 100.4 g/1,000 kcal crude protein, 67.5 g/1,000 kcal crude fat, 9.0 g/1,000 kcal crude fiber, and 17.7 g/1,000 kcal NFE on a caloric basis. Nitrogen-free extract (NFE) was calculated whereby the percentages of crude protein, fat, crude fiber, moisture and ash are subtracted from 100%. The Test food had a higher energy density (4,679.1 kcal/kg) compared to the Control food (3,770.6 kcal/kg), on a dry matter basis. ME was calculated according to the National Research Council (NRC et al., 2006) using total dietary fiber (TDF).The foods consisted of the following macronutrient compositions as a percentage of energy (protein/fat/carbohydrate Figure 1): Test (37/56/7); Control (24/18/58). The KR was used to evaluate micronutrition profile on a ketogenic shift. The KR was calculated according to the Zilberter and Zilberter (2018) equation (KR = (0.46 X grams of Protien + 0.9 X grams of Fat)/(0.58 X grams of Protein + 0.1 X grams of Fat + grams of NFE). Based on the KR calculation, the Test food had a KR of 1.6, while the Control food had a KR of 0.3.

Study Assessments

Body weight was measured on week 4 of the prefeed period and weeks 2, 5, 6, 8, and 10 of the treatment period and calories offered were adjusted weekly to ensure dogs maintained an ideal body weight. Daily food intake was recorded by an automated feeding system. A veterinarian or colony staff assessed body condition score (BCS) in week 4 of the prefeed period and weeks 5 and 10 of the treatment period using a 5-point scale (1 = thin, 3 = ideal, 5 = obese).

Stool consistency was evaluated by a veterinarian or colony staff on week 4 of the prefeed period and weeks 2, 5, 6, 8, and 10 of the treatment period using a 5-point scale (1 = liquid to 5 = firm). Stool characteristics (presence of mucus, blood, unusual odor, color, texture, or size) were recorded on week 4 of the prefeed period and weeks 5 and 10 of the treatment period. Fecal pH and moisture were assessed on week 4 of the prefeed period and weeks 4, and 10 of the treatment period. To explore the tertiary endpoint, coat quality was evaluated weekly by a veterinarian or colony staff using a non-validated, ordinal rating scale. Dogs were not bathed prior to these assessments to ensure an accurate representation of their coat. Across all parameters, lower scores indicated superior coat condition. The evaluated parameters included: luster (1 = extremely shiny to 7 = extremely dull), grooming (1 = excellent to 4 = poor), texture (1 = extremely soft to 7 = extremely greasy/scruffy), dander (1 = none to 5 = excessive), shedding (1 = none to 5 = excessive), and overall appearance (1 = excellent to 7 = poor).

Fresh fecal samples were collected under fasting conditions at the end of the prefeed period, and treatment weeks 5 and 10; however, if the initial collections were unsuccessful, dogs were provided their food to stimulate a gastro-colic response. Feces subsequently collected up to 30 minutes post-feeding. Dogs were placed in individual housing during stooling so that positive identification was documented for each dog and the sample was assuredly assigned to a single subject. Whole feces were collected within 30 min of defecation. Fecal samples were then homogenized until visually uniform with pH and moisture measured immediately after homogenization, snap-frozen in liquid nitrogen, and stored at −70°C until needed for further analysis. To determine moisture, approximately 5 g of fresh feces were placed in a thin layer in an aluminum pan and dried at 104°C for 4 hours (modified AOAC 935.29). Fresh fecal pH

measurements were analyzed using a SevenExcellence S500 pH meter (Mettler-Toledo, LLC, Columbus, OH, USA) equipped with an InLab Expert Pro electrode (Material No. 30014096).

Dogs were removed from the study if they lost 15% of their body weight, did not eat for 2–3 consecutive days, were diagnosed with a new condition, or required prohibited medications (e.g., anti-inflammatories, antibiotics, antiallergenic agents, or drugs affecting study outcomes). Removal also occurred if a dog did not tolerate the study food or if the veterinarian determined removal was in the dog's best interest. In all instances, the principal investigator was notified, and removal was determined in consultation with the colony veterinarian.

Statistical Analysis

Intake, body weight, BCS, fecal consistency, fecal pH, fecal moisture, and coat quality were analyzed using a linear-mixed model with Diet, Day, and the Diet x Day interaction as fixed-effects. An appropriate variance-covariance structure to account for the correlation between the repeated measurements was selected using the Akaike information criterion (AIC) fit statistic. The Kenward-Roger adjustment was used to estimate the denominator degrees of freedom in F-Tests and standard errors for the means. The two foods were compared to each other at each time point using SLICEDIFF=Day, adjust=SIMULATE to control for multiplicity. Within each diet, the post baseline timepoints were compared to baseline using the SLICEDIFF=Diet adjust=DUNNETT.

With categorical data, including fecal characteristics (color, odor, size, casing, and presence of blood, mucus, segmentation, or unusual texture), two different analyses were used. If the data had ordinal-categorical response categories, the Cochran-Mantel-Haenszel Row Mean Score Differ test with modified RIDIT scores was used. If the assessment was binary (yes/no or presence/absence), Fisher’s exact test was used. Separate analyses were performed for each time

point. With demographic data, the two foods were compared for differences in age and weight using a t-test. Gender distribution was analyzed using Fisher's exact test. A *P*-value of ≤ 0.05 was considered statistically significant and $0.05 < P \leq 0.10$ was considered a tendency. All analyses were performed using SAS version 9.4.

Results

Disposition and Baseline Characteristics

Of the 20 dogs enrolled in the study, 19 completed the study (Table 2.1). One dog assigned to the Control food was withdrawn during week 4 of the prefeed period due to a non-trial-related health issue. Specifically, the animal developed a rapidly growing, invasive mass in the tracheal region that required immediate removal from the study for medical management.

There were no significant differences ($P > 0.05$) in mean age or body weight between the treatment groups, at the beginning of the study. The mean (± SD) age was 10.73 (± 1.0) years for the Control group and 11.12 (±1.1) years for the Test group. Mean body weight was 12.64 (± 2.73) kg and 12.83 (± 2.47) kg, respectively (Table 2). The Control group consisted of 3 females (27.3%) and 8 males (72.7%), while the Test group consisted of 6 females (75.0%) and 2 males (25.0%). There was a tendency towards a difference ($P = 0.07$) in gender distribution between the two groups. The majority of dogs included in the study were neutered Beagles (n=18, Brussels Griffon cross n=2) and received heartworm, hookworm, and roundworm preventive medications.

Food Intake and Body Weight

All dogs were fed to maintain body weight and, as such, there was no diet x day interaction for body weight or body condition score ($P > 0.05$, Figure 2.2b-c). However, there was a significant diet x day interaction effect for food intake (g/day, $P = 0.02$) and caloric intake

(Kcal/day, $P = 0.03$, Figure 2.2a). On a dry matter basis, dogs fed the Control food consumed an average of 702 kcal/day and the dogs fed the Test food consumed an average 765 kcal/day. There was a decrease ($P < 0.05$) in daily caloric intake (Kcal/day) for the Control group beginning on weeks 2, 5, and 8-9, which did not persist ($P = 0.11$) through the end of the study, when compared to baseline. Additionally, dogs on the Test food demonstrated an isolated increase ($P < 0.01$) in caloric intake (Kcal/day) on week 1, when compared to baseline. Alternatively, looking at food intake (g/day), dogs fed the Control food ate more on weeks 4, 8, 9, and 10 ($P \leq 0.05$), with a tendency for higher intake observed on weeks 1, 3, 6, and 7 ($P < 0.10$), consuming an average 216 g/day for the Control group and 173.9 g/day for the Test group.

Fecal Evaluations

There was a diet x day interaction ($P < 0.01$) for fecal consistency. Mean fecal consistency scores of the Control and Test groups were 4.1 $\pm$ 0.22 and 3.5 $\pm$ 0.20, respectively. When compared to baseline, increases ($P = 0.01$) in fecal consistency were observed from week 1 and tended to increase ($P = 0.09$, at each timepoint) on weeks 4 and 5 for dogs fed the Control food, and increased ($P = 0.05$) on week 1 for dogs fed the Test food (Figure 2.3). No differences ($P > 0.05$) in fecal color, odor, size, casing, segmentation, texture, or the presence of mucus or blood in stool were observed at any point during the study in the overall population or among dogs in either group.

Fecal pH demonstrated a diet x day interaction ($P < 0.01$) between the Test and Control food. Mean fecal pH for the Control group demonstrated no change ($P = 0.18$) throughout the study period with a mean score of 5.7 $\pm$ 0.08 week 1 and 5.6 $\pm$ 0.06 week 10, when compared to baseline. The Test group showed a mean fecal pH of 5.7 $\pm$ 0.07 on week 1, increasing to 6.4 $\pm$ 0.05 on week 10 ($P < 0.01$), compared to baseline. Fecal moisture was impacted ($P < 0.01$) by

diet. Dogs fed the Test food demonstrated a tendency to increase (Week 0: 74.4 ± 1.15, Week 10: 76.4 ± 0.58; $P = 0.07$) and the Control food demonstrated increased (Week 0: 69.0 ± 1.21, Week 10: 71.3 ± 0.62; $P = 0.05$) in fecal moisture, when compared to baseline. Nevertheless, a between group comparison revealed that dogs fed the Test food had significantly increased ($P < 0.01$) fecal moisture overall when compared to dogs fed the Control food. Fecal pH and moisture are represented in Table 2.3.

Coat Quality

A significant effect of the diet was observed for shedding ($P = 0.03$) and overall coat quality ($P = 0.04$), with a trending effect noted for grooming ($P = 0.08$). Additionally, coat luster was significantly impacted by a diet x day interaction ($P = 0.02$). No effects ($P > 0.05$) were observed for dander or texture between the groups. At baseline, dogs fed the Test food had lower scores for overall coat quality ($P = 0.02$; Test: 2.1 ± 0.14, vs. Control: 2.6 ± 0.15), grooming ($P = 0.02$; Test: 2.0 ± 0.12, vs. Control: 2.4 ± 0.13), luster ($P = 0.01$; Test: 3.4 ± 0.23, vs. Control: 4.3 ± 0.25), alongside a higher score for shedding ($P = 0.04$; Test: 3.0 ± 0.13, vs. Control: 2.6 ± 0.12), when compared to the Control group. However, these initial differences did not persist; by the end of the study, there were no differences between the two treatment groups regarding overall coat quality (P = 0.17), grooming (P = 0.17), luster (P = 0.41), or shedding (P = 0.52). Additionally, dogs fed the Test food demonstrated an improvement in shedding (P = 0.03) from baseline to week 10. Conversely, dogs fed the Control food demonstrated an improvement in both coat luster ($P = 0.02$), and shedding ($P < 0.01$), over the same period.

Discussion

Canine HTG is a complex metabolic condition conventionally managed through strict dietary fat restriction. However, therapeutic success relies heavily on patient compliance, which

can be compromised by the reduced palatability of low-fat foods. As such, the present study evaluated an alternative nutritional strategy: replacing dietary carbohydrates with protein and fat to create a highly palatable therapeutic option for dogs with confirmed HTG. The results of this study demonstrate that the Test food successfully maintains body weight and adequate caloric intake without inducing adverse gastrointestinal effects, thereby offering a viable, long-term management option for dogs with HTG.

In the present study the maintenance of the observed coat quality assessments, including dander, grooming, texture, and overall coat quality, may be explained by the immunomodulatory effects of the Test food (KR = 1.6), as shown by Tavener et al. (2022) using a variant of a ketogenic formulation (KR > 1.5), which supports canine skin health through its elevated fat content and the immunomodulatory effects of added omega-3 and omega-6 fatty acids. Canine atopic dermatitis (CAD) and related dermatological conditions are driven by complex inflammatory cascades, notably involving Th1, Th2, Th17, and Th22 lymphocytes and their associated cytokines (Majewska et al., 2016; Mazrier et al., 2022; Nuttall et al., 2002). Prior research indicates that feeding ketogenic foods can downregulate key pro-inflammatory mediators implicated in compromised skin barrier function (Tavener et al., 2024). For instance, feeding ketogenic foods to dogs, has been shown to significantly reduce circulating concentrations of inflammatory markers including complement component 5 (C5), granulocyte colony-stimulating factor (G-CSF or CSF3), interferon-gamma (IFN-γ), interleukin-5 (IL-5), interleukin-33 (IL-33), interleukin-3 (IL-3), interleukin-10 receptor beta (IL-10RB), interleukin-17C (IL-17C), tumor necrosis factor superfamily (TNFSF), tumor necrosis factor superfamily member 13 (TNFSF13), tumor necrosis factor superfamily member 13B (TNFSF13B), and tumor necrosis factor superfamily member 14 (TNFSF14), all commonly associated with canine

dermatitis and allergic responses (Greenfeder et al., 2001; Tavener et al., 2024; Tavener et al., 2022). Collectively, the suppression of the inflammatory markers (IL-5, IL-33, IFN- γ, CSF3, and C5) in the present study provides a rationale for why systemic carbohydrate reduction does not negatively impact, and may inherently support coat quality and dermatological health in dogs.

A clinical challenge in the nutritional management of canine endocrinopathies and lipid disorders is overcoming innate taste preferences. Dogs naturally demonstrate a strong preference for dietary fat over carbohydrates or protein (Rankovic et al., 2019). Furthermore, previous evaluations of the ketogenic food demonstrate that healthy dogs voluntarily increase total daily fat intake when compared to feeding regimens without controlled carbohydrates (Jackson, 2022; Tavener et al., 2024). The findings of the present study corroborate this innate preference; dogs fed the low-fat Control food exhibited a significant reduction in voluntary daily caloric intake, which ultimately resulted in measurable body weight loss by the conclusion of the trial.

The successful weight maintenance observed in both treatment groups, despite slight fluctuations in weekly intake noted for the Control group, can be largely attributed to the energy-dense formulation of the Test food (4679.1kcal/kg compared to 3770.6 kcal/kg in the Control food). The elevated caloric density of the ketogenic test food, allowed the dogs to meet their metabolic energy requirements with a smaller volume of food (173 average grams per day consumed compared to 216 average grams per day per kg consumed by the Control group). Ketogenic foods with restricted caloric content, have been shown to alter the hormonal regulation of energy balance, which subsequently drives an increase in overall energy expenditure (Bistrian, 2019; Ebbeling et al., 2020; Ludwig et al., 2021; Ludwig & Ebbeling, 2018). In humans, there has been a reported increase in energy expenditure when ketogenic

foods with restricted carbohydrate versus high carbohydrate foods are consumed (Cahill Jr, 1970; Guyenet & Hall, 2021; Hall, 2019; Hall et al., 2019; Jefferson et al., 1974). Despite these findings, it remains controversial whether the increased energy expenditure observed by consumption of ketogenic foods is a short-term phenomenon (Hall, 2019) or if it results in a clinically significant impact on long-term weight management. Consistent with this uncertainty, dogs fed the Test food in the present study exhibited no significant changes in body weight. However, long-term evaluations are necessary to fully understand how body weight might be affected over time when feeding a ketogenic food. This is particularly relevant because, as the current study progressed toward its conclusion, the average caloric intake of dogs on the Test food began to gradually decrease, eventually leveling out with the intake of the Control group. This decrease in intake could be attributed to the energy dense nature of the Test food, causing them to eat less than their energy requirement. In dogs, several studies have demonstrated that feeding a high protein, low carbohydrate food can aid in management of body weight (Bierer & Bui, 2004; Phungviwatnikul, 2022; Russell et al., 2011; Weber et al., 2007). Furthermore, previous research has established that high protein, low carbohydrate foods are highly effective at preserving lean muscle mass while facilitating the loss of fat mass in canines (Bierer & Bui, 2004). Recent studies further support these benefits, demonstrating high protein low carbohydrate foods (KR > 0.95) increase postprandial energy expenditure and fat oxidation in dogs (Banton, Pezzali, et al., 2025; Banton, Raheb, et al., 2025). Additionally high protein, low carbohydrate foods (KR > 0.95) can lead to prolonged elevations in postprandial glucagon, a hormone associated with appetite suppression and weight loss (Banton, Raheb, et al., 2025). The restricted carbohydrate profile of the ketogenic Test food suggests that replacing dietary

carbohydrates with protein and fat is an effective strategy for maintaining a healthy body weight and optimal body composition.

In addition to weight maintenance, the macronutrient shift in the Test food has specific implications for nutritional ketosis. A recent study evaluating similar low carbohydrate/high fat and high carbohydrate/low-fat foods investigated how replacing dietary carbohydrate energy with protein versus fat impacts nutritional ketosis in dogs (Jackson, 2022). Several studies have demonstrated that feeding a food with a ketogenic ratio (KR) above 1.5 resulted in higher levels of circulating β-hydroxybutyrate (B-HBA) compared to a food with a lower KR, highlighting the value of using the KR to predict the ability of canine foods to induce ketosis (Jackson, 2022; Tavener et al., 2024). To successfully induce nutritional ketosis in humans, a formulation must achieve a ketogenic ratio (KR) >1.5 (Zilberter & Zilberter, 2018). Although specific KR thresholds are only beginning to be investigated for dogs (Jackson, 2022, 2024; Jackson & Waldy, 2021; Tavener et al., 2024), and macronutrient parameter estimates may differ between species, the available data support a similar predictive framework. Prior to the initiation of the present study, the KR was calculated for both study foods; the Test food yielded a KR of 1.6, whereas the Control food yielded a KR of 0.3. This indicates that the Test food was appropriately formulated to promote a state of nutritional ketosis. In human and canine models, it has been shown that achieving ketosis has significant clinical implications for lowering serum triglycerides (Bray et al., 2018; Jackson, 2022; Sievenpiper, 2020; Yancy et al., 2004), a metabolic benefit related to hypertriglyceridemic dogs to be further explored in the following chapter.

When introducing a high fat food to manage lipid disorders, veterinarians must closely monitor gastrointestinal tolerance. Notably, none of the dogs evaluated in the present study

exhibited adverse gastrointestinal signs, such as vomiting, diarrhea, or the presence of mucus, blood, or abnormal segmentation in the feces. To comprehensively assess gastrointestinal health, fecal consistency, fecal characteristics, fecal pH, and fecal moisture were utilized as measurable parameters. Throughout the treatment period, fecal consistency scores remained largely similar between the two groups, with differences observed only during weeks 5 and 6. Of clinical significance, both Test and Control groups maintained optimal fecal consistency scores of 3 to 5 (based on a 6-point scale). The fecal consistency score results observed in the present study align with recent work that showed optimal fecal consistency when dogs were fed a food that had similar macronutrient composition to that of the Test food in the present study (Tavener et al., 2024).

Despite the stable clinical presentation, quantitative differences in fecal parameters were observed between the treatment groups. Dogs fed the Test food demonstrated significantly higher overall fecal moisture and a higher fecal pH compared to the Control food. At baseline, fecal pH values were similar between the Test and Control groups (5.75 and 5.73, respectively). By the conclusion of the study, however, the mean fecal pH had significantly decreased to 5.58 in the Control group and increased to 6.40 in the Test group. According to Godglück et al. (2025) the recommended physiological canine fecal pH range is 6.0 to 7.0. Fecal pH is heavily influenced by dietary macronutrient composition; specifically, an increased proportion of dietary protein alters colonic microbial fermentation, which can induce a higher fecal pH (Zentek et al., 2003). This elevation occurs because the increased availability of protein in the large intestine , which shifts the microbiota away from saccharolytic fermentation and toward proteolytic metabolism (Jackson & Jewell, 2019; Pinna et al., 2018). Consequently, this metabolic shift decreases the production of short chain fatty acids (SCFA), and increases branched chain fatty,

driving the fecal pH upward (Ephraim et al., 2020). Consistent with the findings of the current study, previous research has similarly established that protein rich foods, such as hydrolyzed protein, result in a higher fecal pH, regardless of fiber addition, when compared to grain rich foods (Hang et al., 2013; Herstad et al., 2017). Conversely, a reduction in fecal pH often indicates increased carbohydrate fermentation in the colon (Flickinger et al., 2003). This process results in the accumulation of SCFA (Twomey et al., 2003), which are associated with normal large bowel function and the prevention of colonic pathology (Topping & Clifton, 2001). Given that an elevated fecal pH is indicative of a shift toward proteolytic putrefaction, high protein foods have been shown to negatively shift the gut microbiota, leading to poor stool quality (Hang et al., 2013; Nery et al., 2012). Specifically poorly digestible protein, can cause increased fermentation in the colon leading to decreased stool quality (Nery et al., 2012). However, one study demonstrated that increasing dietary fiber from 8.3 to 10.5% on a dry matter basis can effectively mitigate these effects and maintain fecal consistency (Hernot, 2005). This could explain the clinical observation in the present study, where although the Test food increased fecal pH, fecal consistency remained, as the Test food consisted of 10.9% total dietary fiber on a dry matter basis. Given that the Test food increased fecal pH, potentially indicating a shift away from SCFA producing fermentation, further research is warranted to fully understand its impact on the gut microbiome and overall gastrointestinal function.

Our study has limitations. First, the trial utilized dogs with mild cases of HTG. We also did not screen dogs to see if they had any underlying secondary conditions causing the hyperlipidemia or evaluate parameters related to the function of the GI microbiome. Moreover, the dogs included in this study were colony-housed and were almost entirely Beagles, which limits the ability to extrapolate these results to a more diverse population of pet dogs.

Importantly, the population utilized in the present study also restricts the ability to extrapolate these results to breeds specifically predisposed to primary hypertriglyceridemia.

Overall, this study is, to the authors knowledge, the first study to evaluate the effects of a low carbohydrate, high fat food on HTG dogs. These data demonstrate that the Test food is a highly palatable, clinically viable alternative to traditional low-fat foods for the management of canine hypertriglyceridemia. Dogs fed the Test food exhibited an initial increase in intake before stabilizing, demonstrating effective dietary compliance while safely maintaining their body weight throughout the study. Furthermore, current literature supports that this macronutrient shift promotes a beneficial state of nutritional ketosis, potentially yielding systemic anti-inflammatory effects (Gupta-Saraf et al., 2022; Jackson, 2022, 2024; Jackson et al., 2021; Jackson & Waldy, 2021; Tavener et al., 2024), though not directly assessed in the present study. While the Test food resulted in expected physiological shifts in fecal pH and moisture, it did not cause adverse gastrointestinal signs or negatively affect coat quality, ultimately making the Test food a practical nutritional option for management of hypertriglyceridemia in dogs.

Table 2.1. Calculated nutrient content of the Test and Control foods on a dry matter basis and energy basis (grams/1,000 kcal of metabolizable energy).

Nutrient	**Prefeed Food[1]**	**Control Food[2]**	**Test Food[3]**	**Unit**
ME (Calculated)[4]	4477.0	3770.6	4679.1	Kcal/kg
Protein (Crude)	26.9	24.1	48.3	% (DMB)
Protein (Crude)	63.1	68.4	100.4	g/1000 Kcal
Fat (Crude)	15.3	8.2	32.4	% (DMB)
Fat (Crude)	35.9	23.2	67.5	g/1000 Kcal
Ash	7.6	7.4	6.5	% (DMB)
Ash	18.0	21.1	13.5	g/1000 Kcal
TDF	9.0	10.0	10.9	% (DMB)
TDF	21.0	28.5	22.6	g/1000 Kcal
Fiber (Crude)	1.7	1.6	4.3	% (DMB)
Fiber (Crude)	4.1	4.6	9.0	g/1000 Kcal
NFE	57.6	58.7	0.8	% (DMB)
NFE	135.4	166.6	17.7	g/1000 Kcal
Linoleic Acid	2.0	1.6	2.5	% (DMB)

Linoleic Acid	4.6	4.5	5.2	g/1000 Kcal
Lysine	1.3	1.4	3.2	% (DMB)
Lysine	3.1	4.1	6.6	g/1000 Kcal
Methionine	0.4	0.7	1.1	% (DMB)
Methionine	1.0	2.0	2.2	g/1000 Kcal
Taurine	0.0	0.2	0.4	% (DMB)
Taurine	0.1	0.7	0.8	g/1000 Kcal
Omega 3 Sum	0.1	0.3	0.9	% (DMB)
Omega 3 Sum	0.3	0.8	1.9	g/1000 Kcal
Omega 6 Sum	2.1	1.7	2.8	% (DMB)
Omega 6 Sum	4.9	4.8	5.7	g/1000 Kcal
N6:N3 Ratio	21:1	5.7:1	3:1	% (DMB)
N6:N3 Ratio	16:1	6:1	3:1	g/1000 Kcal
EPA	0.0	0.1	0.0	% (DMB)
EPA	0.1	0.2	0.1	g/1000 Kcal
DHA	0.0	0.1	0.0	% (DMB)
DHA	0.1	0.2	0.1	g/1000 Kcal
Niacin	16.4	9.9	444.9	% (DMB)
Niacin	3.8	2.8	92.5	g/1000 Kcal
Phosphorus	1.1	1.0	0.6	% (DMB)
Phosphorus	2.6	2.9	1.3	g/1000 Kcal
Ketogenic Ratio[5]	0.3	0.3	1.6	

Docosahexaenoic acid, DHA; eicosapentaenoic acid, EPA; Metabolizable energy, ME; NFE, nitrogen-free extract; TDF, total dietary fiber.

[1]Prefeed food, Purina Dog Chow, whole grain corn, meat and bone meal, corn gluten meal, beef fat naturally preserved with mixed-tocopherols, soybean meal, poultry by-product meal, chicken, egg and chicken flavor, whole grain wheat, animal digest, salt, calcium carbonate, potassium chloride, mono and dicalcium phosphate, L-lysine monohydrochloride, choline chloride, minerals [zinc sulfate, ferrous sulfate, manganese sulfate, copper sulfate, calcium iodate, sodium selenite], vitamins [vitamin E supplement, niacin (vitamin B-3), vitamin A supplement, calcium pantothenate (vitamin B-5), pyridoxine hydrochloride (vitamin B-6), vitamin B-12 supplement, thiamine mononitrate (vitamin B-1), vitamin D-3 supplement, riboflavin supplement (vitamin B-2), menadione sodium bisulfite complex (vitamin K), folic acid (vitamin B-9), biotin (vitamin B-7)], yellow 6, yellow 5, red 40, blue 2, garlic oil. E-4101.

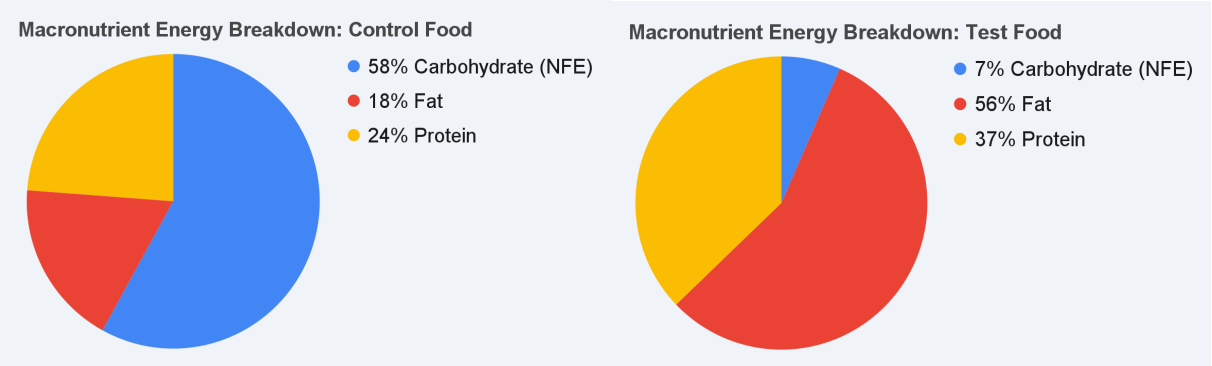
[2]Control food, Royal Canin Veterinary Diet Adult Gastrointestinal Low Fat Dry Dog Food, brewers rice, chicken by-product meal, barley, natural flavors, pork digest, pea fiber, dried plain beet pulp, chicken fat, monocalcium phosphate, salt, potassium chloride, calcium carbonate, powdered psyllium seed husk, sodium aluminosilicate, fish oil, fructooligosaccharides, choline chloride, vitamins [DL-alpha tocopherol acetate (source of vitamin E), L-ascorbyl-2-polyphosphate (source of vitamin C), biotin, D-calcium pantothenate, vitamin A acetate, riboflavin supplement, niacin supplement, vitamin B12 supplement, pyridoxine hydrochloride (vitamin B6), thiamine mononitrate (vitamin B1), vitamin D3 supplement, folic acid], rosemary extract, preserved with mixed tocopherols and citric acid, hydrolyzed yeast, DL-methionine, marigold extract (Tagetes erecta L.), trace minerals [zinc proteinate, zinc oxide, ferrous sulfate, manganese proteinate, manganous oxide, copper sulfate, sodium selenite, calcium iodate, copper proteinate], taurine.

[3]Test food, experimental food, chicken, beef tallow, egg product, spray dried pork stock, powdered cellulose, flaxseed, pork gelatin, chicken liver flavor, dried hydrolyzed casein, dried hydrolyzed whey, hydrolyzed chicken liver, calcium sulfate, lactic acid, potassium citrate, pork liver flavor, dried beet pulp, dried citrus pulp, psyllium seed husk, dried apple pomace, oat bran, choline chloride, vitamins [(vitamin E supplement, L-ascorbyl-2-polyphosphate (source of vitamin C), niacin supplement, thiamine mononitrate, vitamin A supplement, calcium pantothenate, riboflavin supplement, biotin, vitamin B12 supplement, pyridoxine hydrochloride, folic acid, vitamin D3 supplement]), taurine, minerals (ferrous sulfate, zinc oxide, copper sulfate, manganous oxide, calcium iodate, sodium selenite), L-carnitine, L-tryptophan, mixed tocopherols for freshness, magnesium oxide, natural flavors, beta-carotene.

[4]Nitrogen-free extract (NFE) was calculated whereby the percentages of crude protein, fat, crude fiber, moisture and ash are subtracted from 100%. Gross Energy (GE) was predicted using the NRC et al. (2006) equation, $GE\ \left(\frac{kcal}{kg}\right) = (57 \times Protein\%) + (94 \times Fat\%) + 41 \times (NFE\% + Crude\ Fiber\%)$. To use the GE calculation in the metabolizable energy (ME) equation, results were converted to kcal per 100g by dividing by 10. The calculated ME utilized equation recommended in the NRC et al. (2006) using the total dietary fiber (TDF) equation, $ME(Kcal/kg) = GE\ (Kcal/100) \times \left(\frac{96.6 - [0.95 \times TDF\%]}{100}\right) - (1.04 \times Crude\ Protein\%)$. Results were converted to Kcal per kg by multiplying by 10.

[5]Ketogenic ratio (KR) was calculated using the equation adapted from Zilberter and Zilberter (2018), $KR = \frac{((0.9 \times Fat\%) + (0.46 \times Crude\ Protein\%))}{NFE\% \times ((0.58 \times Crude\ Protein\%) + (0.1 \times Fat\%))}$.

Figure 2.1. Caloric distribution of the Test and Control Foods in dogs (n=19) with hypertriglyceridemia.



Percentage of calories from carbohydrates, fat and protein. Carbohydrate content was estimated by calculating the nitrogen-free extract (NFE), whereby the percentages of crude protein, fat, crude fiber, moisture and ash are subtracted from 100%.

Table 2.2. Baseline characteristics of dogs (n=19) with hypertriglyceridemia fed foods differing in macronutrients.

Item	**Control Food[1]**	**Test food[2]**	***P*-value**
Animals, n	9	10	-
Female, n (%)	3 (33.3)	8 (66.7)	0.07
Male, n (%)	9 (80.0)	2 (20.0)	
Age, yrs	10.7 ± 0.98	11.1 ± 1.07	0.43

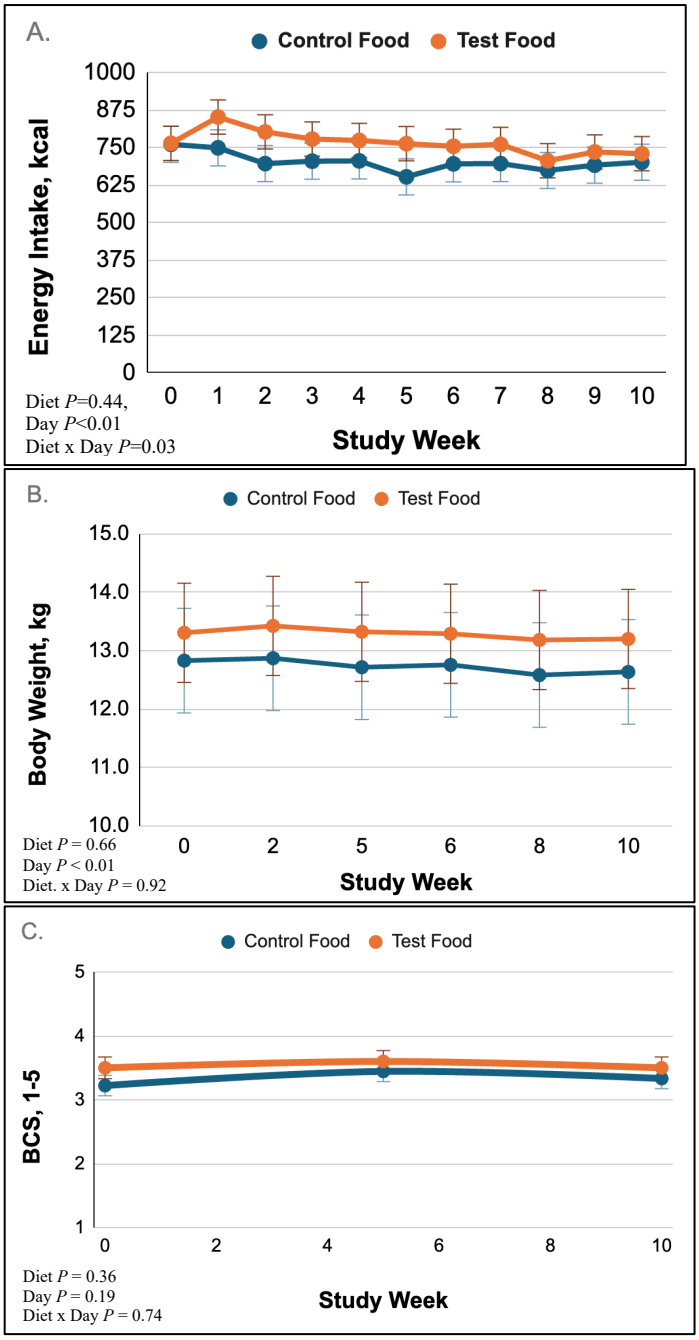
Data represent n (%) or mean ± standard error.
*P ≤ 0.05 between Control and Test at baseline.
0.05 < P ≤ 0.10 between Control and Test at baseline.
[1]Control food characteristics of % of calories from Carbohydrate, Fat & Protein (58% / 18% / 24%).
[2]Test food characteristics of % of calories from Carbohydrate, Fat & Protein (7% / 56% / 37%).

Table 2.3. Effect of two foods differing in macronutrients on selected fecal parameters in dogs (n=19) with hypertriglyceridemia.

	Week 0		Week 5		Week 10		*P*-value			
Item	**Control[1] Food**	**Test[1] Food**	**Control[1] Food**	**Test[1] Food**	**Control Food**	**Test Food**	**SEM**	**Diet**	**Week**	**Diet x Day**
Fecal pH	5.7	5.7	5.7	6.3	5.6	6.4	0.09	<0.01	<0.01	<0.01
Fecal Moisture,%	69.0	74.4	-	-	71.3	76.4	0.85	<0.01	0.01	0.82

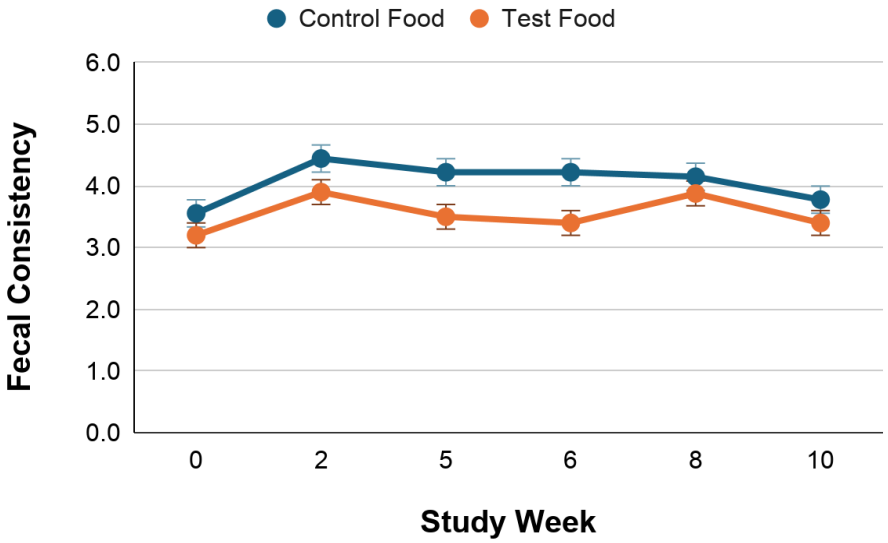
[1]The number of animals in each treatment was 9 and 10 dogs for Control food and Test food, respectively. Characteristics of % of calories from Carbohydrate, Fat & Protein (58% / 18% / 24%; and 7% / 56% / 37%) for the Control food and Test food respectively.

Figure 2.2. Average caloric intake (a), body weight (b) and body condition score (c) in dogs (n=19) assigned to foods differing in macronutrients.



BCS, body condition score on a scale of 1 to 5 with 1 being very thin, 3 being ideal and 5 being obese.
Characteristics of % of calories from Carbohydrate, Fat & Protein (58% / 18% / 24%; and 7% / 56% / 37%) for the Control food and Test food respectively.
The number of animals in each treatment was 9 and 10 dogs for Control food and Test food, respectively.

Figure 2.3. Average fecal consistency by study week in dogs (n=19) assigned to foods differing in macronutrients.



Characteristics of % of calories from Carbohydrate, Fat & Protein (58% / 18% / 24%; and 7% / 56% / 37%) for the Control food and Test food respectively.
The number of animals in each treatment was 9 and 10 dogs for Control food and Test food, respectively.
Diet $P<0.01$
Day $P<0.01$
Diet x Day $P=0.72$

Chapter 3 - Metabolic and Hematological Evaluation of Low Carbohydrate Food Compared to a Low-Fat Food in Dogs with Confirmed Hypertriglycerdemia

Abstract

Introduction: Canine hypertriglyceridemia (HTG) is a metabolic disorder that is traditionally managed with low-fat foods. However, recent evidence suggests that ketogenic, low carbohydrate foods effectively lower circulating triglycerides in healthy dogs. This study evaluated the clinical and metabolic impacts of feeding a novel ketogenic Test food compared to a traditional low-fat Control food to dogs with confirmed HTG.

Methods: After completing the prefeed, nineteen dogs were randomly assigned to either the ketogenic experimental dog food [Test food; ketogenic ratio (KR: 1.6)] or Royal Canin Veterinary Diet Adult Gastrointestinal Low-Fat Dry Dog Food. [Control food; (KR: 0.3)]. Following a 4-week prefeed period, subjects completed a 10 week treatment period where fasted blood and fecal samples were collected throughout the study to evaluate complete blood counts, serum chemistry, comprehensive lipid profiles, metabolic hormones, and fecal short-chain fatty acids (SCFAs).

Results: Overall, there was a significant effect of day (P =0.02) in serum triglyceride concentrations. Dogs fed both the Test and Control food behaved similarly demonstrating a mean reduction of -161 mg/dL and -112 mg/dL, respectively. This improvement from baseline, in lipid metabolism, was accompanied by no significant change ($P = 0.21$) in pancreatic lipase immunoreactivity (cPLI) and significant increases in β-hydroxybutyrate (B-HBA, $P < 0.01$), confirming nutritional ketosis. Fecal analysis revealed the Test food demonstrated an inverse

shift in altered microbial fermentation compared to the Control food. The Test food increased proteolytic short-chain fatty acids (SCFAs), including 2-methylbutyric acid ($P = 0.03$) and isobutyric acid ($P < 0.01$, and decreased saccharolytic SCFAs including propionic acid ($P < 0.01$), when compared to baseline. Conversely, the Control food demonstrated a significant increase in saccharolytic SCFAs including propionic acid ($P < 0.01$) and acetic acid ($P < 0.01$), and led to no changes in proteolytic SCFAs ($P > 0.05$) when compared to baseline. Additionally, when compared to baseline, both foods modulated hormones involved in appetite regulation; both the Test and Control foods decreased ($P < 0.01$, $P = 0.02$; respectively) leptin concentrations. However, while the Test food increased ghrelin concentrations ($P = 0.02$), the Control food resulted in no change ($P = 0.18$) in ghrelin concentrations, when compared to baseline.

Discussion: This study is the first to evaluate the effects of replacing dietary carbohydrates with protein and fat to achieve a ketogenic ratio > 1.5 as an effective nutritional management strategy for HTG. Feeding the ketogenic Test food to dogs with HTG results in triglyceride improvements and alters microbial fermentation. However, further research is needed to understand the long term effects on microbial fermentation in the gut and the efficacy of the Test food as a dietary alternative for improving systemic lipid metabolism.

Introduction

Lipids are essential for energy storage and cellular structure (Bishop et al., 2000; Karam et al., 2017). However, lipids are hydrophobic which require them to be packaged into spherical transport vehicles called lipoproteins to travel through the aqueous bloodstream (Bauer, 1996, 2004; Ginsberg, 1998; Johnson, 2005; Watson & Barrie, 1993; Whitney, 1992; Xenoulis & Steiner, 2010). The major serum lipoprotein fractions in dogs include chylomicrons, very low

density lipoproteins (VLDL), low density lipoproteins (LDL), and high density lipoproteins (HDL) (Karam et al., 2017). Fat metabolism in dogs operates via two distinct pathways the exogenous pathway and endogenous pathway (Bauer, 2004; Ginsberg, 1998). The exogenous pathway utilizes chylomicrons to transport and metabolize dietary fats from the intestines (Karam et al., 2017). The endogenous pathway metabolizes endogenously produced lipids, by using VLDL, HDL and LDL, secreted by the liver to distribute lipids to the body (Bauer, 1996; Karam et al., 2017). Triglyceride rich lipoproteins, chylomicrons and VLDL, primarily deliver energy-rich triglycerides to cells in the body (Xenoulis & Steiner, 2010). Whereas HDL and LDL predominantly transport cholesterol. Cholesterol assists in the metabolism of bile acids and steroid hormones and synthesis of vitamin D (Ginsberg, 1998). Additionally, the quantity of cholesterol transported by LDL from the liver to peripheral tissues exceeds its catabolism, and the excess cholesterol is returned back to the liver by HDL (Karam et al., 2017). Consequently, these major serum lipoprotein fractions are implicated in several disease conditions (Jeusette et al., 2008; Pasquini et al., 2008; Rogers et al., 1975b; Whitney et al., 1987; Whitney et al., 1993).

Hyperlipidemia in dogs encompasses a group of metabolic disorders of diverse etiology (Xenoulis & Steiner, 2015). These disorders are frequently secondary to underlying endocrine disorder, such as hypothyroidism, diabetes mellitus, or hyperadrenocorticism (Bauer, 2004; Feldman et al., 2014; Johnson, 2005; Rogers, 1977; Rogers et al., 1975b; Whitney, 1992; Xenoulis & Steiner, 2010). Despite this etiologic diversity, routine clinicopathological tests identify a uniform phenotypic presentation, typically defined by elevated serum triglyceride concentrations (hypertriglyceridemia; HTG), cholesterol (hypercholesterolemia; HC), or both (Karam et al., 2017). In humans hyperlipidemia is associated with elevated LDL and triglycerides (Karam et al., 2017). HTG represents a condition of major clinical significance in

veterinary medicine. Although many affected animals remain asymptomatic, HTG is a well-established risk factor for numerous secondary disorders (Aguirre et al., 2007; Furrow et al., 2016; Furrow et al., 2017; Violette & Ledbetter, 2019; Xenoulis et al., 2011b; Xenoulis & Steiner, 2010, 2015; Xenoulis et al., 2008). Idiopathic HTG is most prevalent in Miniature Schnauzers (Xenoulis & Steiner, 2015). Prevalence and severity in the breed increase with age, affecting 75% of the breed by 10 years of age (Tate, 2022; Xenoulis et al., 2007). This breed specific condition is characterized by varying degrees of increased very low-density lipoproteins (VLDL) and chylomicrons, which may occur with or without concurrent HC (Whitney et al., 1993; Xenoulis et al., 2013; Xenoulis et al., 2007). Although the cause of this increase in lipoproteins was hypothesized to have a monogenic basis due to its breed predisposition, recent research evaluating candidate genes failed to identify a single causal mutation (Tate, 2022) Instead, data suggest that HTG in Miniature Schnauzers is likely a complex, polygenic trait driven by the cumulative effects of multiple genetic variants combined with environmental factors (Tate, 2022).

Diagnostics of HTG rely heavily on blood biochemistry. Additionally, a recent study emphasized the importance of utilizing multiple fasted blood collections, as serum triglyceride concentrations can vary significantly over time within individual dogs, even when consuming the same food (Xenoulis et al., 2020a). Furthermore, the upper limits of reference intervals for fasting triglycerides have been noted to range from 109 up to 151 mg/dL (Kim et al., 2019; Xenoulis & Steiner, 2015; Xenoulis et al., 2007, 2008). It is important to note that these specific intervals are not universally standardized; rather, reference intervals can fluctuate significantly based on the analytical methodologies and validation protocols employed by individual diagnostic laboratories.

Historically, methodologies such as electrophoresis, sequential density gradient centrifugation, and size exclusion chromatography have been unable to fully capture the continuous density distribution of these lipoprotein fractions (Xenoulis et al., 2013). Conversely, continuous lipoprotein density profiling (CLPDP) provides a more comprehensive identification of lipoprotein subclasses, offering significant diagnostic value (Larner et al., 2011; Xenoulis et al., 2013). Notably, CLPDP is an effective screening tool capable for detecting critical lipoprotein profile differences, even in dogs presenting with normal serum triglyceride and cholesterol concentrations (Xenoulis et al., 2013).

Although the functional characteristics and composition of most canine lipoprotein subfractions remain largely unknown, they are nominally assigned to traditional functional classes based strictly on their density (Xenoulis et al., 2020b). Based on established density (d) characteristics, these subfraction classifications include triglyceride rich lipoproteins (TRL; chylomicrons and VLDL; d < 1.017 g/mL), LDL1 (d = 1.019–1.023 g/mL), LDL2 (d = 1.023–1.029 g/mL), LDL3 (d = 1.029–1.039 g/mL), LDL4 (d = 1.039–1.050 g/mL), LDL5 (d = 1.050–1.063 g/mL), HDL2b (d = 1.063–1.091 g/mL), HDL2a (d = 1.091–1.110 g/mL), HDL3a (d = 1.110–1.133 g/mL), HDL3b (d = 1.133–1.156 g/mL), and HDL3c (d = 1.156–1.179 g/mL) (Guérin et al., 1996; Xenoulis et al., 2013). The clinical utility of this advanced profiling, CLPDP, is highlighted by its ability to differentiate healthy dogs from those with pancreatitis with up to 89% accuracy (Xenoulis et al., 2020b). However, in that same study, overall serum triglyceride concentrations exhibited a tendency to be higher in dogs with pancreatitis compared to healthy controls (Xenoulis et al., 2020b). Importantly, multiple studies have established that HTG acts as a primary precipitating factor for pancreatitis, rather than occurring merely as a

consequence of the disease (Bass et al., 1976; CHIKAMUNE et al., 1998; Whitney et al., 1987; Xenoulis et al., 2020b).

The most widely recommended initial approach for managing HTG is the use of a therapeutic low-fat food (Ford & Ludlow, 2010b; Xenoulis & Steiner, 2015). Due to this endogenous production, recent research has increasingly investigated the metabolic effects of replacing dietary carbohydrates with protein and/or fat to support canine health, noting several compelling metabolic shifts that warrant further evaluation (Clifton & Keogh, 2007; Gupta-Saraf et al., 2022; Jackson, 2022; Jackson & Waldy, 2021; Phungviwatnikul, 2022; Tavener et al., 2024). As noted by Clifton and Keogh (2007), reducing carbohydrate intake drives a more dramatic decrease in serum triglyceride concentration. This metabolic shift associated with reduced carbohydrate intake has been supported by six independent crossover studies evaluating healthy and chronic gastroenteritis (CGE) dogs (Gupta-Saraf et al., 2022; Jackson, 2022, 2024; Jackson et al., 2021; Jackson & Waldy, 2021; Tavener et al., 2024). In these trials, transitioning dogs from high carbohydrate foods (> 57% carbohydrate on an energy basis; KR < 0.46) to low carbohydrate (< 10% carbohydrate on an energy basis; KR > 0.90) formulations designed to induce ketosis consistently resulted in significant reductions in circulating triglycerides within 5 weeks (Jackson, 2022, 2024; Jackson & Waldy, 2021), decreases in alkaline phosphatase (ALP) (Jackson et al., 2021; Jackson & Waldy, 2021), stable pancreatic lipase levels (Jackson et al., 2021), decreased cytokines indicative of anti-inflammatory effects (Gupta-Saraf et al., 2022; Tavener et al., 2024), and overall improvements in metabolic health (Jackson, 2022, 2024; Jackson & Waldy, 2021).

Consequently, the aim of the present pilot study was to evaluate the clinical and metabolic impacts of feeding a novel experimental food, formulated by replacing carbohydrates

with protein and fat (KR = 1.6), compared to a low-fat Control food (KR = 0.3) in dogs with confirmed HTG. We hypothesized that altering the dietary macronutrient profile to meet a KR of 1.6 would improve systemic lipid metabolism, specifically a significant reduction in serum triglycerides.

Methods

Ethics

The study protocol (CP1048a) was reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) at Hill's Pet Nutrition, Inc. (Topeka, KS, USA). Canine participants were housed in a colony and provided care by Ontario Nutri-Lab Inc. (Fergus, Ontario, Canada). All dogs were maintained and treated in strict accordance with the Hill's Global Animal Welfare Policy. Experimental procedures were designed to mitigate pain, discomfort, and distress, and dogs were continuously monitored for clinical signs of disease. At no point during the study were dogs subjected to procedures anticipated to induce distress. In the event of an adverse health observation, the welfare of the dog consistently superseded continued participation in the study.

Animal Management and Housing

Canine participants were group housed in a colony setting within spacious indoor enclosures, with the exception of feeding times and biological sample collection. Natural lighting provided by windows was supplemented with full spectrum LED illumination to maintain a standard light cycle. Behavioral enrichment was provided through conspecific socialization, interactive play with caretakers, daily access to outdoor exercise areas, and the provision of toys. Dogs were fed once daily in a 30 minute time period, to maintain their initial body weight and provided ad libitum access to fresh, clean drinking water. Food offerings were based on each

dog's calculated by metabolizable energy requirement (MER). To maintain body weight food offerings were reviewed and adjusted weekly based on recorded body weights. Food offerings were increased only if a dog was consuming its entire daily allocation but still losing weight; conversely, food amounts were not altered if a dog consistently left food uneaten. Routine veterinary care was maintained throughout the study, including standard immunizations and the regular administration of heartworm, hookworm, and roundworm preventatives. All study procedures were integrated into the animals' normal daily routines to minimize disruption and stress.

Study Population

The study initially enrolled 20 adult dogs (≥ 12 months of age) with a confirmed diagnosis of HTG. Inclusion criteria required a documented history of two or more fasted sampling (>15 hours) blood collections of which demonstrated elevations in serum lipid parameters above normal reference intervals. Specifically, dogs were required to exhibit triglyceride concentration >1.71 mmol/L or 151 mg/dL, cholesterol concentration >8.9 mmol/L or 343.6 mg/dL, a positive lipemia index, or a combination of these markers. Additional inclusion requirements necessitated a history of good appetite, a willingness to exclusively consume dry food, and a demonstrated ability to consistently provide stool samples during daytime hours.

Dogs were excluded from the study if they were concurrently participating in other clinical studies or diagnosed with severe systemic or organ disease, such as chronic kidney disease. Further exclusion criteria included injuries resulting in significant swelling or edema, overt signs of advanced aging (e.g., muscle wasting), and the administration of oral antibiotics or anti-inflammatory medications (e.g., non-steroidal anti-inflammatory drugs [NSAIDs],

corticosteroid) within the two months prior to study commencement. Dogs demonstrating fractious behavior, poor appetite, or difficulty in providing blood or fecal samples were also deemed ineligible.

Study Design

This was a randomized, stratified study design with repeated measures, 20 adult male and female dogs. Enrolled dogs entered a 4 week prefeed phase to attempt establishment of a uniform metabolic baseline across the cohort. One dog was removed during the prefeed for proper medical treatment of a non-study related health issue. Nineteen dogs successfully completed the prefeed period. The remaining subjects were randomized onto the Test or Control Food for a 10 week experimental period. For randomization dogs were stratified based on age and weight.

Study Foods

All foods were nutritionally complete and balanced dry formulations for adult dogs, containing only ingredients approved by the Association of American Feed Control Officials (AAFCO). Prior to the treatment phase, all dogs completed a 4 week prefeed period during which they consumed a commercial adult maintenance food (Purina Dog Chow Complete Adult). Following the prefeed period, dogs were transitioned to their assigned treatment foods. The Control food consisted of Royal Canin Veterinary Diet Adult Gastrointestinal Low-Fat Dry Dog Food. The Control food consisted of brewers rice, chicken by-product meal, barley, natural flavors, pork digest, pea fiber, dried plain beet pulp, chicken fat, monocalcium phosphate, salt, potassium chloride, calcium carbonate, powdered psyllium seed husk, sodium aluminosilicate, fish oil, fructooligosaccharides, choline chloride, vitamins [DL-alpha tocopherol acetate (source of vitamin E), L-ascorbyl-2-polyphosphate (source of vitamin C), biotin, D-calcium pantothenate, vitamin A acetate, riboflavin supplement, niacin supplement, vitamin B12

supplement, pyridoxine hydrochloride (vitamin B6), thiamine mononitrate (vitamin B1), vitamin D3 supplement, folic acid], rosemary extract, preserved with mixed tocopherols and citric acid, hydrolyzed yeast, DL-methionine, marigold extract (Tagetes erecta L.), trace minerals [zinc proteinate, zinc oxide, ferrous sulfate, manganese proteinate, manganous oxide, copper sulfate, sodium selenite, calcium iodate, copper proteinate], taurine. The Control food was selected because it meets the current nutritional guidelines for supporting dogs with an endocrinopathy (Ford & Ludlow, 2010b).

The Test food was an experimental ketogenic formulation (Table 3.1). The experimental Test food was manufactured via extrusion processing at the Hill's experimental food laboratory in strict adherence to Good Manufacturing Practice (GMP) guidelines. The Test food included chicken, beef tallow, egg product, spray dried pork stock, powdered cellulose, flaxseed, pork gelatin, chicken liver flavor, dried hydrolyzed casein, dried hydrolyzed whey, hydrolyzed chicken liver, calcium sulfate, lactic acid, potassium citrate, pork liver flavor, dried beet pulp, dried citrus pulp, psyllium seed husk, dried apple pomace, oat bran, choline chloride, vitamins [(vitamin E supplement, L-ascorbyl-2-polyphosphate (source of vitamin C), niacin supplement, thiamine mononitrate, vitamin A supplement, calcium pantothenate, riboflavin supplement, biotin, vitamin B12 supplement, pyridoxine hydrochloride, folic acid, vitamin D3 supplement]), taurine, minerals (ferrous sulfate, zinc oxide, copper sulfate, manganous oxide, calcium iodate, sodium selenite), L-carnitine, L-tryptophan, mixed tocopherols for freshness, magnesium oxide, natural flavors, beta-carotene.

To evaluate the ketogenic potential of the foods, the KR was utilized. The KR describes the specific proportion of macronutrients formulated to induce nutritional ketosis; in humans, a KR greater than 1.5 is generally recognized as the clinical threshold necessary to achieve ketosis

(Wilder & Winter, 1922; Zilberter & Zilberter, 2018). The KR is calculated using the following equation: KR = (0.46 x grams of Protein + 0.9 x grams of Fat) / (0.58 x grams of Protein + 0.1 x grams of Fat + grams of NFE) (Zilberter & Zilberter, 2018). Nitrogen-free extract (NFE) was calculated whereby the percentages of crude protein, fat, crude fiber, moisture and ash are subtracted from 100%. Prior to the initiation of the present study, the KR was calculated for both study foods. The Test food yielded a KR of 1.6, satisfying the threshold for ketosis, whereas the Control food yielded a KR of 0.3. Comprehensive nutrient analysis of all study foods was subsequently performed by the Eurofins Nutrition Analysis Center (Des Moines, IA, USA) utilizing standard Association of Official Agricultural Chemists (AOAC) methodologies (AOAC International, 2023). Key nutrient levels of the Control food and Test food are summarized in Table 1.1, on a caloric and dry matter basis. The Control food contained 68.4 g/1,000 kcal crude protein, 23.2 g/1,000 kcal crude fat, 4.6 g/1,000 kcal crude fiber, and 166.6 g/1,000 kcal nitrogen-free extract (NFE) on a caloric basis. The Test food contained 100.4 g/1,000 kcal crude protein, 67.5 g/1,000 kcal crude fat, 9.0 g/1,000 kcal crude fiber, and 17.7 g/1,000 kcal NFE on a caloric basis.

Study Assessments

Daily food allotments were initially calculated based on the metabolizable energy requirement (MER) for each dog (Cline et al., 2021). To maintain an ideal and stable body weight throughout the trial, feeding amounts were closely monitored and adjusted on a weekly basis according to routine body weight measurements.

Blood samples were under fasting conditions at the conclusion of the prefeed phase (week 0), as well as during treatment weeks 2, 5, 6, 8, and 10. Whole blood was processed immediately post collection. For complete blood counts (CBC), 2 mL of whole blood was

collected into ethylenediaminetetraacetic acid (EDTA) tubes, gently inverted 3 to 4 times, chilled and shipped on ice to Hill's Pet Nutrition Center (Topeka, KS, USA). CBC was analyzed using an automated analyzer (Sysmex XN 1000-V, Sysmex America, Inc., Lincolnshire, IL, USA).

Serum chemistry and lipid profiles, 4 mL of blood was collected into gold-top serum separator tubes (SST) at all time points. Samples were allowed to clot for 20 to 30 minutes prior to centrifugation at 3,000 rmp (2,000 X g) for 10 minutes. Serum was separated into 0.75 mL aliquots for clinical chemistry and 1.0 mL aliquot for lipid panels. Serum chemistry profiles were analyzed within 24 hours of collection (Cobas c501, Roche Diagnostics, Indianapolis, IN, USA).

At baseline, week 5 and week 10, additional blood was collected for specialized panels. The metabolic panel required 4 mL of blood collected into red top tubes (RTT; clot activator, no gel), clotted for 20 to 30 minutes, and centrifuged at 3,000 rpm (2,000 X g) for 10 minutes to yield a 1.5mL serum aliquot. Samples were shipped on ice to Hill's Pet Nutrition Center (Topeka, KS, USA) and stored at −80°C for subsequent analyses. To evaluate the metabolic effects of the foods, circulating B-HBA, serving as a primary marker of nutritional ketosis, was assessed via an enzymatic reaction assay (IDEXX BioAnalytics Inc., Columbia, MO, USA).

Satiety hormones were measured in plasma samples collected before the provision of the day's meal. A total of 2 mL of blood was collected into grey top BD P800 protease inhibitor tubes, inverted 3 to 4 times, and centrifuged at 3,000 rpm (2,000 X g) for 10 minutes, yielding a 0.75 mL plasma aliquot. All separated serum and plasma aliquots were immediately stored in cryovials at −70°C for subsequent analyses. Satiety hormones were evaluated using an ELISA kit (Milliplex Map Canine Gut Hormone Magnetic Bead Panel—Endocrine Multiplex Assay; Millipore Sigma, Burlington, MA, USA), and measured on a Luminex 200 (Luminex Inc.,

Austin, TX, USA). Fecal SCFAs were analyzed independently by Metabolon, Inc. (Morrisville, NC, USA).

Fresh fecal samples were collected under fasting conditions; however, if the initial collections were unsuccessful, dogs were provided their food to stimulate a gastro-colic response. Feces subsequently collected up to 30 minutes post-feeding. Dogs were placed in individual housing during stooling so that positive identification was documented for each dog and the sample was assuredly assigned to a single subject. For the study, no stool collections were completed in a group-housed environment. Within 30 minutes of collection, stools were processed and homogenized using a THINKY mixer inside a primary homogenization bag. The homogenized feces were transferred into a 35 mL catheter syringe to facilitate precise distribution into 2 mL cryovials. The prepared cryovials were capped tightly and snap-frozen in liquid nitrogen within 45 minutes of defecation, before being transferred to −70°C storage. Aliquots ranging from 0.5 to 1.0 mL were prepared for short chain fatty acid (SCFA) analysis. Fecal SCFAs were analyzed independently by Metabolon, Inc. (Morrisville, NC, USA).

Predetermined criteria for subject withdrawal included a body weight loss exceeding 15%, anorexia persisting for 2 to 3 consecutive days, the diagnosis of a novel medical condition, or the requisite administration of prohibited pharmacological agents (e.g., anti-inflammatories, antibiotics, antiallergenic medications, or other drugs known to confound study outcomes). Dogs were also subject to removal if they exhibited intolerance to the assigned study food or if the attending veterinarian determined that continued participation compromised the animal's welfare.

Statistical Analysis

Biological samples, including pancreatic and metabolic biomarkers complete blood count (CBC), fecal SCFA, and satiety hormones, were analyzed using a linear mixed-model with Diet,

Day, and the Diet x Day interaction as fixed effects. An appropriate variance covariance structure to account for the correlation between the repeated measurements was selected using the Akaike information criterion (AICC) fit statistic. The Kenward-Roger adjustment was used to estimate the denominator degrees of freedom in F-Tests and standard errors for the means. The two foods were compared to each other at each time point using SLICEDIFF=Day, adjust=SIMULATE to control for multiplicity. Within each food, the post baseline timepoints were compared to baseline using the SLICEDIFF=Diet adjust=DUNNETT. A P-value ≤ 0.05 was considered statistically significant and $0.05 < P \leq 0.10$ was considered a tendency. All analyses were performed using SAS version 9.4.

Results

Baseline Characteristics

A total of 20 dogs were initially enrolled in the study; of these, 19 successfully completed the trial. One dog assigned to the Control group was prematurely withdrawn prior to the commencement of the treatment phase. This withdrawal constituted the sole adverse event reported during the trial and was determined to be entirely unrelated to the study food. Prior to treatment assignment, subjects were stratified based on body weight and age. As a result, the treatment groups were well balanced, exhibiting no significant differences ($P \geq 0.05$) in mean age or body weight at baseline. However, there was a tendency toward a difference (P = 0.07) in gender distribution between the two groups. The study population primarily consisted of neutered Beagles (n = 18), alongside two Brussels Griffon mixed breed dogs (n = 2). Throughout the duration of the study, the only pharmacological agents administered to the participants were routine heartworm, hookworm, and roundworm preventive medications.

Complete Blood Count and Serum Chemistry

None of the CBC analytes, with the exception of mean corpuscular volume (MVC), hemoglobin, mean corpuscular hemoglobin (MCH), and red blood cell (RBC) had a diet x day interaction, as shown in Table 3.2A ($P > 0.05$). Additionally, the serum chemistry analytes, albumin, albumin:globulin ratio, alkaline phosphatase (ALP), ammonium, blood urea nitrogen (BUN), BUN:creatine ratio, calcium, immunoglobulin, glucose, iron, magnesium, osmolality, phosphorus, potassium, hemolysis, sodium, potassium ratio had a diet x day interaction, as shown in Table 3.2B ($P < 0.05$). Diet effects were noted among the following analytes in the CBC and serum chemistry: HGB, total protein, homocysteine, platelet count (PLT), and Hematocrit (HCT) ($P < 0.05$). When compared between treatment groups, dogs fed the Test food demonstrated a lower ($P = 0.02$) total circulating immunoglobulins than dogs fed the Control food (Test: 2.3 $\pm$ 0.08 vs. Control: 2.6 $\pm$ 0.09), at study end. Furthermore, dogs fed the Control food exhibited a significant decrease in the albumin to globulin ratio (1.4 $\pm$ 0.08; $P < 0.01$) and albumin (3.6 $\pm$ 1.15; $P < 0.01$) on week 10 when compared baseline, whereas dogs fed the Test food showed a significant increase during weeks 2 through 8 (1.7 $\pm$ 0.78; $P < 0.05$) in albumin to globulin ratio and no changes (3.6 $\pm$ 0.14; $P = 0.72$) in albumin.

Compared to baseline, ALP levels in dogs fed the Test food decreased ($P = 0.04$) to a mean of 107.1$\pm$ 103.95 U/L, dropping from above the normal reference range down to within normal limits (17.4 - 135.3 U/L; Figure 3.2). After reduction on week 2, ALP levels remained stable for the remainder of the study. Additionally, the Test food induced a decrease in serum calcium ($P = 0.01$), phosphorus ($P < 0.01$), and uric acid ($P = 0.01$), when compared to baseline, by end of study. Dogs fed the Test food demonstrated shifts in BUN and BUN to creatinine ratio. Specifically, BUN increased at week 2,6-8 ($P < 0.05$), while remaining within the normal

reference range (15.3 - 31.4 mg/dL). Although this increase was not maintained through the end of study, when compared to baseline. Furthermore, the BUN to creatine ratio significantly increased ($P = 0.01$) from weeks 2 through 8, exceeding the normal reference range (12.42 - 25.3 Ratio). Conversely, dogs fed the Control food exhibited a significant increase (844.2 ± 174.47; $P < 0.01$) in ALP, elevating serum concentrations 708.7 U/L above normal limits (17.4 - 135.3 U/L). Dogs on the Control food demonstrated no change in serum calcium ($P = 0.33$) and phosphorus ($P = 0.29$) alongside significant decreases in BUN ($P = 0.04$) and the BUN to creatinine ratio ($P < 0.01$), when compared to baseline. Furthermore, the effect on uric acid did not differ significantly between the two diets ($P = 0.50$). By the end of the study, uric acid concentrations had significantly decreased from baseline in the Test group ($P < 0.01$) and showed a trending decrease in the Control group ($P = 0.06$).

Pancreatic and Metabolic Biomarkers

Serum triglyceride concentrations demonstrated no significant diet x day interaction (P = 0.62) or effect of diet ($P = 0.78$), although a significant effect of day ($P = 0.02$) was observed. While an overall effect of time was observed across the study, within group analyses revealed that only dogs fed the Test food experienced a decrease in circulating triglycerides from 321.1 ± 72.70 at baseline, to 176.0 ± 48.05 on week 2 ($P = 0.08$), to 123.8 ± 32.49 on week 5 ($P < 0.01$), to 168.3 ± 44.82 on week 6 ($P < 0.01$), to 160.9 ± 30.37 on week 10 ($P = 0.08$; Figure 3.1). This reduction shifted the cohort's mean triglycerides closer to the reference interval (>151 mg/dL), achieving a mean reduction of 161 mg/dL. In contrast, dogs fed the Control food exhibited no significant decrease in circulating triglycerides from 291.0 ± 77.44 at baseline, to 178.56 ± 32.00 end of study ($P = 0.31$).

Additional biomarkers of fat digestion and metabolic stress were evaluated between treatment groups. For cPLI, and bile acids, there were no significant diet x day interaction, diet, or day effect ($P > 0.05$). Cortisol concentrations similarly showed no diet x day interaction or effect of diet ($P > 0.05$), although a significant effect of day ($P = 0.03$) was observed. Additionally, B-HBA demonstrated a significant diet x day interaction ($P < 0.01$), alongside an of effect of both diet ($P < 0.01$), and day ($P < 0.01$). Compared to baseline, both the Test and Control foods resulted in no change ($P = 0.83$, $P = 0.87$; respectively) in serum cortisol concentrations by study end. Furthermore, evaluation of B-HBA concentrations on week 5 revealed that while the dogs fed the Control food experienced no significant change ($P = 0.80$), dogs fed the Test food demonstrated a significant increase ($P < 0.01$) when compared to baseline, consistent with the successful induction of nutritional ketosis (Table 3.3).

For total cholesterol, and LDL, there was a significant diet x day interaction, alongside effects of diet, and day ($P < 0.05$). HDL concentrations showed no effect of diet x day interaction ($P = 0.014$) and significant effect of day ($P = 0.01$) and diet ($P = 0.05$. The Test food yielded a significant increase ($P < 0.01$) in total cholesterol (TC) of +51 mg/dL, whereas the Control food demonstrated a significant decrease ($P = 0.05$) of -25 mg/dL by study end, when compared to baseline (Figure 3.3). However, when evaluating the ratio of TC to high density lipoprotein (TC:HDL), the Test food induced a marginal ratio increase of +0.19, resulting in a final TC:HDL ratio of 1.5. Additionally, the Test food demonstrated no change ($P = 0.24$) in HDL concentrations by study end, when compared to baseline. In contrast, the Control food significantly decreased ($P = 0.02$) HDL concentrations by the end of the study when compared to baseline, yielding a final TC:HDL ratio of 1.3.

Satiety Hormones

As detailed in Table 3.4, satiety hormones were evaluated to determine the impact of the dietary interventions over time. Analysis of circulating ghrelin revealed a significant diet x day interaction ($P = 0.04$), as well as a significant effect of day ($P = 0.01$). Final ghrelin concentrations were not significantly different ($P = 0.18$) in dogs fed the Test food compared to those fed the Control food. Furthermore, the Test food induced a significant increase in ghrelin from baseline to study end ($P = 0.01$), whereas the Control food yielded no change ($P = 0.78$). In contrast, leptin and PYY were influenced solely by the passage of time; both demonstrating no effect of a diet x dat ($P > 0.05$) interaction and a significant effects of day ($P < 0.05$) observed. Finally, circulating concentrations of GIP and PP remained stable throughout the study, showing no significant effect of a diet x day interaction, nor any effect of diet, or day ($P > 0.05$). Additionally both foods induced a induced a decrease in circulating leptin from baseline (Control: -5092, $P = 0.02$; Test: -6280; $P = 0.01$), by the end of the study.

Fecal SCFA

Fecal analysis revealed the Test food demonstrated an inverse shift in altered microbial fermentation compared to the Control food (Figure 3.4 and 3.5). Among proteolytic SCFAs, isobutyric acid demonstrated significant diet x day ($P = 0.02$) interaction, alongside an effects of both diet ($P < 0.01$) and day ($P < 0.01$). 2-methylbutyric acid exhibited no effect on diet X day ($P = 0.12$) interaction and a significant effect of diet ($P < 0.01$) and day ($P = 0.01$). Finally, isovaleric acid demonstrated only an effect of diet ($P = 0.04$). Conversely, the evaluation of saccharolytic SCFAs highlighted a diet x day interaction ($P < 0.01$), alongside significant effects of diet ($P < 0.01$) and day ($P = 0.02$) for propionic acid. Acetic and butyric acids demonstrated

no significant diet x day ($P > 0.05$) interactions or effect of effects of diet ($P > 0.05$), though both demonstrated and effect of day ($P < 0.05$).

The Test food increased proteolytic short-chain fatty acids (SCFAs) including 2-methylbutyric acid ($P = 0.03$) and isobutyric acid ($P < 0.01$) and led to no change ($P = 0.13$) in isovaleric acid production. Additionally, the Test food decreased saccharolytic SCFAs including propionic acid ($P < 0.01$) and led to no change ($P = 0.12$) in butyric acid, and acetic acid ($P > 0.05$). Conversely, the Control food demonstrated a significant increase in saccharolytic SCFAs, including propionic acid ($P < 0.01$) and acetic acid ($P < 0.01$), and no changes ($P = 0.28$) in butyric acid at study end, when compared to baseline. Additionally, the Control food did not change ($P > 0.05$) in proteolytic SCFAs production including 2-methylbutyric acid, isobutyric acid, and isovaleric acid.

Discussion

This study provides a comprehensive metabolic and hematological evaluation of a low carbohydrate, high fat Test food (KR = 1.6) compared to a traditional low-fat Control food in dogs with confirmed hypertriglyceridemia (HTG). The primary finding demonstrated that the Test food behaved similarly to the nutritional standard of care, Control food, which led to a reduction in circulating triglyceride concentration. Overall, the Test food achieved a mean concentration of 161 mg/dL and the Control food demonstrated a mean concentration of 179 mg/dL, shifting the cohort closer to the reference interval. These findings align with previous research demonstrating that ketogenic foods, formulated with protein and fat replacing standard carbohydrate inclusions (< 78.8% of metabolizable energy) (Karr-Lilienthal et al., 2002) and a KR > 0.9, effectively lower triglycerides (Jackson & Waldy, 2021). For example, feeding ketogenic foods with KRs of 0.97 and 1.63 previously led to a 30% decrease in triglyceride

concentrations (Jackson & Waldy, 2021). Similar triglyceride lowering effects noted in healthy dogs have been identified in human weight loss trials utilizing carbohydrate restriction (< 30g) (Foster et al., 2003; Samaha et al., 2003). While previous research has established low-fat foods as an effective approach for reducing triglyceride concentrations in dogs with HTG within 8 weeks (Xenoulis et al., 2020a), the nonsignificant improvement in the Control group may be attributed to triglyceride concentration variability, which is discussed further in the limitations.

The analysis of serum B-HBA further elucidated the physiological impact of the macronutrient shift of replacing carbohydrates with protein and fat. Dogs fed the Test food experienced an increase in circulating B-HBA a ketone, when compared to baseline. This increase in B-HBA indicates a state of nutritional ketosis, whereas the Control food maintained baseline B-HBA concentrations. Ketogenesis operates similarly in dogs and humans (Jackson, 2022). Dogs exhibit characteristics of ketosis comparable to humans, including their acetoacetate:B-HBA ratio and their ability to utilize ketones as a primary energy source (Crandall, 1941; Jackson, 2022). The current study's findings are consistent with recent literature, which demonstrated that feeding a ketogenic food (KR > 1.0) led to greater increases in B-HBA, albumin, blood urea nitrogen (BUN), triglycerides, and cholesterol compared to a high carbohydrate (HiCHO, KR=0.46) food (Jackson, 2024).

Total cholesterol (TC) and lipoprotein fractions were evaluated to understand the effect of feeding a ketogenic food (KR=1.6). Previous studies have shown TC can increase by up to 30% when dogs are fed a ketogenic food (KR > 0.9) compared to a HiCHO food (KR > 0.46) (Jackson & Waldy, 2021), with similar elevations noted in other evaluations of ketogenic foods (KR > 0.9) (Jackson, 2022, 2024). To appropriately contextualize the rise in TC observed in dogs fed the Test food, the ratio of TC:HDL was calculated. As demonstrated by Choi (2024), the

TC:HDL ratio serves as a robust prognostic biomarker for metabolic health, with a cutoff value of 3.0 differentiating healthy conditions from disease states. Additionally, lower HDL concentrations are strongly correlated with hyperlipidemia (Choi, 2024). While TC increased for dogs fed the Test food, both groups remained below the reference range (> 344 mg/dL) of TC concentrations. Additionally, the TC:HDL ratio remained well below the prognostic threshold of 3.0, yielding a final ratio of 1.5. Furthermore, by study end, the Test food decreased the HDL:LDL ratio, similar to how the Control food performed, which aligns with current nutrition recommendations for dogs with hyperlipidemia. Previous studies indicate that dogs with hypercholesterolemia or pancreatitis typically present with increased LDL and decreased HDL fractions, resulting in a lower HDL:LDL ratio compared to healthy dogs (Bass et al., 1976; CHIKAMUNE et al., 1998; Oda et al., 2017; Whitney et al., 1987; Xenoulis et al., 2020b). Therefore, the favorable TC:HDL ratio and comparable shift in HDL:LDL ratio observed in the Test group to the Control group suggest that the elevation of TC requires more research to understand the clinical implications. Additionally, increased fat intake is known to elevate both HDL and LDL, often raising cardiovascular concerns in humans, However, dogs uniquely metabolize fat as HDL-dominant mammals with less incidence of atherosclerosis occurring (Hess et al., 2003; Mansoor et al., 2016; Mori et al., 2011). Furthermore, the specific functional characteristics of canine lipoprotein density subfractions remain largely unknown, as such, human-centric classifications of HDL and LDL can only be applied nominally (Oda et al., 2017; Tate, 2022). As such the clinical consequences of dietary-induced cholesterol elevations in dogs cannot be directly assumed from human medicine alone (Hess et al., 2003; Tate, 2022; Xenoulis, 2015). This is due to the distinct metabolic physiology of canines, coupled with limited research correlating specific lipoprotein changes to cardiovascular disease or hyperlipidemia

complications (Choi, 2024). Importantly, maintaining these ratios suggests the diet alone may not shift lipid profiles toward specific patterns typically associated with hyperlipidemia and pancreatitis, though long-term studies are warranted.

A historical clinical concern for dogs with HTG is the development of pancreatitis (Xenoulis, 2015; Xenoulis et al., 2013; Xenoulis et al., 2020b). However, this presumed causal relationship between HTG and pancreatitis has been challenged as a recent study identified over 70% of dogs with pancreatitis present with triglyceride and cholesterol concentrations within normal reference intervals (Xenoulis et al., 2020b). To ensure the Test food did not exacerbate pancreatic stress, cPLI was evaluated. Both dietary groups behaved similarly, which resulted in numerical decreases in cPLI. Throughout the 10 week period, cPLI concentrations for all dogs remained below the ≥ 400 µg/L diagnostic threshold for clinical pancreatitis (Serrano et al., 2021). Furthermore by end of the study, neither group demonstrated a cPLI concentration (Test: 93.1 ± 36.38, Control: 171.3 ± 38.35) that would place the dogs within the 201–400 µg/L equivocal range requiring a recheck in 3-4 weeks (Serrano et al., 2021). These findings help alleviate concerns that feeding a ketogenic food increases inherently risks compromising pancreatic health in dogs with a history of HTG.

Beyond pancreatic health, serum chemistry analysis revealed favorable hepatic enzymatic shifts in the Test group. The Test food induced decreases in liver enzymes, including ALP, suggesting an alleviation of hepatic stress which can be associated with HTG (Assawarachan et al., 2021; Fernandez & Kidney, 2007; Verma et al., 2024). Notably, ALP decreased from above the reference range (17.4 - 135.2 U/L) to within normal limits, mirroring findings by Jackson and Waldy (2021) who observed significantly lower ALP concentrations in dogs fed a ketogenic food (KR = 1.63). In contrast, the Control food resulted in significant increases in ALP,

alongside significant decreases in albumin, the albumin:globulin (A:G) ratio, and total protein. These shifts in dogs fed the Control food mirror patterns observed in dogs with hepatic dysfunction, which typically present with increased ALT, AST, GGT, and ALP, and decreased total protein, albumin, and A:G ratios (Verma et al., 2024). While none of the dogs in the present study had known secondary conditions, hepatic biomarkers and lipoprotein profiles observed in the present study suggest that future studies should implement stringent screening for secondary endocrine diseases.

Fecal analysis highlighted production of SCFAs consistent with the macronutrient profile of the Test food (0.8% carbohydrate as NFE, DMB; KR = 1.6). The Test food increased proteolytic SCFAs, including 2-methylbutyric acid, and isobutyric acid, while significantly decreasing saccharolytic SCFA including propionic acid. The change in SCFA production reflects the expected shift from carbohydrate fermentation towards protein and fat degradation. Conversely, the carbohydrate dense Control food increased production of saccharolytic SCFAs, including propionic acid and acetic acid demonstrated no change in proteolytic SCFAs including 2-methylbutyric acid, isovaleric acid, and isobutyric acid. Saccharolytic SCFAs are essential for maintaining intestinal homeostasis, fueling epithelial cells, and strengthening gut barrier function; reduced levels are occasionally associated with intestinal disorders (Parada Venegas et al., 2019). Branched-chain fatty acids (BCFAs) such as 2-methylpropionate, 2-methylbutyrate, and 3-methylbutyrate, are byproducts of proteolytic fermentation (Jackson & Jewell, 2019). Ultimately, the present study supports the conclusion by Ephraim et al. (2020) that lower concentrations of SCFAs and higher BCFAs concentrations may be associated with increased protein consumption in dogs. Although both foods provided comparable levels of insoluble fiber (Test: 5.2 vs. Control: 6.8 g/1,000 Kcal) and soluble fiber (Test: 17.5 vs. Control 21.7 g/1,000

Kcal), the elevated level of protein in the Test food is the most likely driver of the observed fermentation shift. The exact mechanism behind the shift in SCFAs, such as potential difference in protein digestibility, require further investigation. Additionally SCFA produced in the gut are rapidly absorbed by the colon, and should not be used as sole indication of SCFA status (McNeil et al., 1978). Given these physiological variables, the elevated protein content and differences in nutritional formulation of the Test food could be the driver of the observed fermentation shift; however, further research is needed to definitively confirm true cause.

Some literature associates BCFAs with harmful compounds like uremic toxins (Jackson & Jewell, 2019), other reports highlight beneficial properties, including the suppression of pro-inflammatory markers, and gastrointestinal support (Ran-Ressler et al., 2011; Yan et al., 2017). As noted in the preceding chapter, the Test food (KR = 1.6) increased fecal pH, reflecting its reduced carbohydrate and fiber content. This outcome is the expected inverse of previous findings, which demonstrate that dietary inclusions of prebiotic fiber typically lower fecal pH by increasing saccharolytic and decreasing proteolytic SCFA production (Jackson & Jewell, 2019; McGrath et al., 2024). Therefore, further research is required to fully understand the long-term colonic health implications of decreased SCFA and increased BCFA production in HTG dogs.

The evaluation of circulating satiety hormones revealed distinct regulatory effects. Biologically, ghrelin operates as an appetite stimulating hormone that drives food intake (Cummings, 2001; Yokoyama et al., 2005), whereas leptin acts as a satiety signal that suppresses feeding (Ishioka et al., 2002; IWASE et al., 2000; Jeusette et al., 2006). Therefore, evaluation of these hormones can help understand how effective a food may be at regulating satiety and balancing metabolic demands. Both foods successfully induced a decrease in circulating leptin from baseline. However, in alignment with findings reported by Jackson (2024), the Test food

triggered an increase in circulating ghrelin, while the Control food yielded no significant increase. The elevated ghrelin activity is consistent with findings reported by Jackson (2024), suggesting that replacing carbohydrates with protein and fat fundamentally alters postprandial hormonal signaling. Through the interaction of ghrelin and leptin, the endocrine system regulates hunger and energy balance (Cortese et al., 2019; Yeung & Tadi, 2020). Similar hormonal relationships have been observed in canine weight loss studies, indicating that ghrelin and leptin influence how dogs adjust to metabolic shifts much like humans do (Jeusette et al., 2006). Consistent with the hormonal changes and findings outlined in the previous chapter, dogs fed the Test food successfully maintained body weight throughout the study.

This study has several limitations. The sample size was relatively small (n=19) and consisted primarily of colony housed Beagles. Because primary HTG is most prevalent in Miniature Schnauzers, extrapolating these results to the most highly affected demographic is restricted. The study also exclusively assessed dogs with moderate, primary HTG; the efficacy of a ketogenic food in managing severe cases (> 400 mg/dL) or those with secondary comorbidities remains unknown. Furthermore, serum triglycerides can be highly variable in response to dietary changes and this study did not utilize multiple baseline collections to average out intra-individual variation. This variability may explain why the low-fat Control food did not demonstrate a significant reduction in serum triglyceride concentrations. Future studies should utilize multiple baseline collections and broader lipoprotein panels to more accurately track hematological shifts.

The objective of this study was to comprehensively evaluate the metabolic and hematological effects of a low carbohydrate, high fat food in dogs with confirmed HTG. To the authors' knowledge, this is the first study to evaluate the effects of feeding a ketogenic food (KR = 1.6) specifically to primary HTG dogs. Ultimately, this study demonstrates that a targeted

macronutrient formulation replacing dietary carbohydrates with protein and fat effectively supports dogs with primary HTG without inducing pancreatitis, as evidenced by cPLI concentrations remaining well below diagnostic thresholds. This nutritional approach could offer veterinarians a novel option for managing canine primary HTG.

Table 3.1. Calculated nutrient content of the Test and Control foods on a dry matter basis and energy basis (grams/1,000 kcal of metabolizable energy).

Nutrient	Prefeed Food[1]	Control Food[2]	Test Food[3]	Unit
ME (Calculated)[4]	4477.0	3770.6	4679.1	Kcal/kg
Protein (Crude)	26.9	24.1	48.3	% (DMB)
Protein (Crude)	63.1	68.4	100.4	g/1000 Kcal
Fat (Crude)	15.3	8.2	32.4	% (DMB)
Fat (Crude)	35.9	23.2	67.5	g/1000 Kcal
Ash	7.6	7.4	6.5	% (DMB)
Ash	18.0	21.1	13.5	g/1000 Kcal
TDF	9.0	10.0	10.9	% (DMB)
TDF	21.0	28.5	22.6	g/1000 Kcal
Fiber (Crude)	1.7	1.6	4.3	% (DMB)
Fiber (Crude)	4.1	4.6	9.0	g/1000 Kcal
NFE	57.6	58.7	0.8	% (DMB)
NFE	135.4	166.6	17.7	g/1000 Kcal
Linoleic Acid	2.0	1.6	2.5	% (DMB)
Linoleic Acid	4.6	4.5	5.2	g/1000 Kcal
Lysine	1.3	1.4	3.2	% (DMB)
Lysine	3.1	4.1	6.6	g/1000 Kcal
Methionine	0.4	0.7	1.1	% (DMB)
Methionine	1.0	2.0	2.2	g/1000 Kcal
Taurine	0.0	0.2	0.4	% (DMB)
Taurine	0.1	0.7	0.8	g/1000 Kcal
Omega 3 Sum	0.1	0.3	0.9	% (DMB)
Omega 3 Sum	0.3	0.8	1.9	g/1000 Kcal
Omega 6 Sum	2.1	1.7	2.8	% (DMB)
Omega 6 Sum	4.9	4.8	5.7	g/1000 Kcal
N6:N3 Ratio	21:1	5.7:1	3:1	% (DMB)
N6:N3 Ratio	16:1	6:1	3:1	g/1000 Kcal
EPA	0.0	0.1	0.0	% (DMB)
EPA	0.1	0.2	0.1	g/1000 Kcal
DHA	0.0	0.1	0.0	% (DMB)
DHA	0.1	0.2	0.1	g/1000 Kcal
Niacin	16.4	9.9	444.9	% (DMB)
Niacin	3.8	2.8	92.5	g/1000 Kcal
Phosphorus	1.1	1.0	0.6	% (DMB)
Phosphorus	2.6	2.9	1.3	g/1000 Kcal
Ketogenic Ratio[5]	0.3	0.3	1.6	

Docosahexaenoic acid, DHA; eicosapentaenoic acid, EPA; Metabolizable energy, ME; NFE, nitrogen-free extract; TDF, total dietary fiber.

[1]Prefeed food, Purina Dog Chow, whole grain corn, meat and bone meal, corn gluten meal, beef fat naturally preserved with mixed-tocopherols, soybean meal, poultry by-product meal, chicken, egg and chicken flavor, whole grain wheat, animal digest, salt, calcium carbonate, potassium chloride, mono and dicalcium phosphate, L-lysine monohydrochloride, choline chloride, minerals [zinc sulfate, ferrous sulfate, manganese sulfate, copper sulfate, calcium iodate, sodium selenite], vitamins [vitamin E supplement, niacin (vitamin B-3), vitamin A supplement, calcium pantothenate (vitamin B-5), pyridoxine hydrochloride (vitamin B-6), vitamin B-12 supplement, thiamine

mononitrate (vitamin B-1), vitamin D-3 supplement, riboflavin supplement (vitamin B-2), menadione sodium bisulfite complex (vitamin K), folic acid (vitamin B-9), biotin (vitamin B-7)], yellow 6, yellow 5, red 40, blue 2, garlic oil. E-4101.

[2]Control food, Royal Canin Veterinary Diet Adult Gastrointestinal Low Fat Dry Dog Food, brewers rice, chicken by-product meal, barley, natural flavors, pork digest, pea fiber, dried plain beet pulp, chicken fat, monocalcium phosphate, salt, potassium chloride, calcium carbonate, powdered psyllium seed husk, sodium aluminosilicate, fish oil, fructooligosaccharides, choline chloride, vitamins [DL-alpha tocopherol acetate (source of vitamin E), L-ascorbyl-2-polyphosphate (source of vitamin C), biotin, D-calcium pantothenate, vitamin A acetate, riboflavin supplement, niacin supplement, vitamin B12 supplement, pyridoxine hydrochloride (vitamin B6), thiamine mononitrate (vitamin B1), vitamin D3 supplement, folic acid], rosemary extract, preserved with mixed tocopherols and citric acid, hydrolyzed yeast, DL-methionine, marigold extract (Tagetes erecta L.), trace minerals [zinc proteinate, zinc oxide, ferrous sulfate, manganese proteinate, manganous oxide, copper sulfate, sodium selenite, calcium iodate, copper proteinate], taurine.

[3]Test food, experimental food, chicken, beef tallow, egg product, spray dried pork stock, powdered cellulose, flaxseed, pork gelatin, chicken liver flavor, dried hydrolyzed casein, dried hydrolyzed whey, hydrolyzed chicken liver, calcium sulfate, lactic acid, potassium citrate, pork liver flavor, dried beet pulp, dried citrus pulp, psyllium seed husk, dried apple pomace, oat bran, choline chloride, vitamins [(vitamin E supplement, L-ascorbyl-2-polyphosphate (source of vitamin C), niacin supplement, thiamine mononitrate, vitamin A supplement, calcium pantothenate, riboflavin supplement, biotin, vitamin B12 supplement, pyridoxine hydrochloride, folic acid, vitamin D3 supplement]), taurine, minerals (ferrous sulfate, zinc oxide, copper sulfate, manganous oxide, calcium iodate, sodium selenite), L-carnitine, L-tryptophan, mixed tocopherols for freshness, magnesium oxide, natural flavors, beta-carotene.

[4]Nitrogen-free extract (NFE) was calculated whereby the percentages of crude protein, fat, crude fiber, moisture and ash are subtracted from 100%. Gross Energy (GE) was predicted using the NRC et al. (2006) equation, $GE\ \left(\frac{kcal}{kg}\right) = (57 \times Protein\%) + (94 \times Fat\%) + 41 \times (NFE\% + Crude\ Fiber\%)$. To use the GE calculation in the metabolizable energy (ME) equation, results were converted to kcal per 100g by dividing by 10. The calculated ME utilized equation recommended in the NRC et al. (2006) using the total dietary fiber (TDF) equation, $ME(Kcal/kg) = GE\ (Kcal/100) \times \left(\frac{96.6 - [0.95 \times TDF\%]}{100}\right) - (1.04 \times Crude\ Protein\%)$. Results were converted to Kcal per kg by multiplying by 10.

[5]Ketogenic ratio (KR) was calculated using the equation adapted from Zilberter and Zilberter (2018), $KR = \frac{((0.9 \times Fat\%) + (0.46 \times Crude\ Protein\%))}{NFE\% \times ((0.58 \times Crude\ Protein\%) + (0.1 \times Fat\%))}$.

Table 3.2. Effect of food on blood parameters measured by complete blood count (A) and serum chemistry (B) in dogs (n=19) for 10 weeks fed the Test and Control foods.

		Week 0		**Week 2**		**Week 5**		**Week 6**		**Week 8**		**Week 10**		***P*-value**			
Item	RI[1]	Control Food[2]	Test Food[3]	Control Food	Test Food	Control Food	Test Food	Control Food	Test Food	Control Food	Test Food	Control Food	Test Food	SEM	Diet	Week	Diet x Day
(A) CBC																	
HGB, pg	23.2-27.1	15.8	16.7	15.9	16.5	15.9	15.4	15.7	16.6	15.4	16.8	15.6	17.0	0.57	0.02	0.36	0.41
PLT, k/ul	112.3-485.4	264.0	169.7	266.0	198.7	276.2	206.8	229.9	134.2	305.4	251.4	245.7	166.6	40.3	0.08	0.22	0.72
HCT, M/uL	32.7-56.1	49.1	51.1	49.7	50.3	49.6	53.5	49.5	50.8	48.0	51.1	48.4	52.3	44.7	0.05	0.19	0.16
MCV, fL	66.2-76.2	73.6	73.8	73.8	73.6	74.4	73.6	75.8	74.6	74.6	73.0	74.4	72.2	0.85	0.41	<0.01	<0.01
MCH, pg	22.3-25.2	23.7	24.0	23.7	24.0	23.9	23.8	24.1	24.3	24.0	24.0	24.0	23.5	0.42	0.91	0.22	0.05
RBC, M/uL	4.9-7.9	6.7	6.9	6.8	6.9	6.7	7.3	6.5	6.8	6.4	7.0	6.5	7.2	0.18	0.05	0.06	0.02

Lymphocytes, %	n/a	27.0	26.5	32.2	28.8	31.5	26.8	29.6	25.7	31.5	27.7	28.8	24.6	1.98	0.17	<0.01	0.26
Lymphocytes, k/µL	0.75–2.45	2.5	2.0	2.7	2.0	2.5	1.9	2.5	1.8	2.5	1.9	2.3	1.7	0.24	0.07	<0.01	0.64
Monocytes, %	n/a	4.2	4.0	4.4	4.4	3.9	3.9	3.9	4.1	4.1	3.8	4.1	3.8	0.26	0.75	0.01	0.56
Monocytes, k/µL	0.13–0.7	0.3	0.4	0.3	0.4	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.04	0.19	<0.01	0.69
Neutrophils, %	n/a	65.7	66.6	59.7	63.6	60.3	66.6	63.1	67.4	60.8	65.9	63.9	69.4	2.1	0.10	<0.01	0.20
Neutrophils, k/µL	2.23–6.32	6.4	5.3	5.1	4.4	4.7	4.5	5.3	4.6	4.9	4.6	5.0	4.7	0.84	0.36	0.09	0.71
WBC, k/µL	3.31–9.49	9.7	7.9	8.5	7.0	7.8	6.8	8.4	6.9	8.0	6.9	7.9	6.8	0.99	0.15	0.01	0.74
(B) Serum Chemistry																	
Albumin/ Globulin ratio	1.1–2.4	1.5	1.6	1.5	1.6	1.5	1.7	1.5	1.7	1.5	1.7	1.4	1.6	1.55	0.24	<0.01	0.01
Albumin, g/dL	2.8–4.0	3.8	3.6	3.8	3.6	3.8	3.7	3.7	3.6	3.7	3.6	3.6	3.6	0.15	0.55	<0.01	0.02
ALP, U/L	17–134	425	215	650	107	759	113	735	105	728	117	844	118	174.50	<0.01	0.15	0.02
ALT, U/L	17–55	57	103	92	57	117	88	112	82	88	84	122	80	33.10	0.54	0.08	0.36
BUN, mg/dL	7.6–19.3	13.5	15.6	12.4	19.2	11.7	16.4	12.1	20.6	11.9	20.9	11.3	15.4	1.36	<0.01	<0.01	<0.01
BUN/ Creatinine ratio	11.3–26.4	23.9	27.3	20.2	32.4	19.7	29.8	19.9	35.9	20.1	33.5	19.2	29.3	1.95	<0.01	0.01	<0.01
Calcium, mg/dL	9.0–10.8	11	10.9	11.2	10.7	11.3	10.6	11.1	10.6	11	10.6	11.1	10.6	0.14	0.02	0.06	<0.01
Ig, g/dL	1.5–2.6	2.5	2.4	2.5	2.3	2.6	2.2	2.5	2.2	2.5	2.2	2.6	2.3	0.09	0.02	<0.01	0.05
Glucose, mg/dL	79–116	73.2	70.9	70.7	81	66.5	72.1	74.6	78.3	78.5	81.1	75.1	83.5	3.88	0.11	<0.01	0.09
Osmolality, mOsm/kg	291-307	298.7	298.0	300.9	303.0	300.7	300.6	302.4	304.9	301.1	305.4	300.2	301.0	0.87	0.15	<0.01	<0.01
Phosphorus, mg/dL	2.3–4.7	4.4	4.5	4.5	3.8	4.4	3.6	4.7	4.1	4.5	4.2	4.6	3.8	0.23	0.04	0.10	0.05
Magnesium, mg / dL	1.6-2.3	2.0	1.9	1.9	1.9	2.0	2.0	1.9	2.0	1.9	2.0	1.9	1.9	0.06	0.67	0.14	0.07
Sodium, mmol/L	142.3-149	146.2	145.4	147.2	147.2	147.8	146.9	148.3	148.1	147.6	148.4	147.3	147.0	0.37	0.51	<0.01	0.05
Potassium Ratio	28.3-37.7	30.6	31.7	29.8	31.7	30.8	32.1	30.0	32.5	30.0	32.4	30.7	32.3	0.64	0.05	0.03	<0.01

ALP, alkaline phosphatase; ALT, alanine transaminase; BUN, blood urea nitrogen; CBC, complete blood count; HCT, hematocrit; HGB, hemoglobin; Ig, immunoglobulin; MCH, mean corpuscular hemoglobin; MVC, mean corpuscular volume; n/a, not applicable; PLT, platelet count; RBC, red blood cell; RI, reference interval; SE, standard error; WBC, white blood cells.

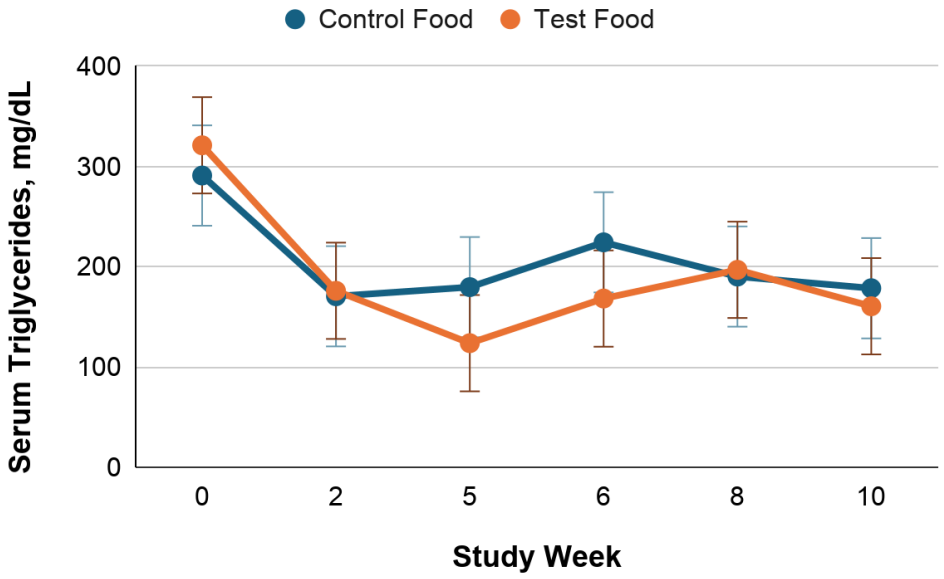
The number of animals in each treatment was 9 and 10 dogs for Control food and Test food, respectively.

[1] IDEXX BioAnalytics, Columbia, MO, USA. Values established using clinically healthy, adult dogs of various breeds.

[2]Control food characteristics of % of calories from Carbohydrate, Fat & Protein (58% / 18% / 24%).

[3]Test food characteristics of % of calories from Carbohydrate, Fat & Protein (7% / 56% / 37%).

Figure 3.1. Average serum triglyceride concentrations (mg/dL) by study week in dogs (n=19) assigned to foods differing in macronutrients.



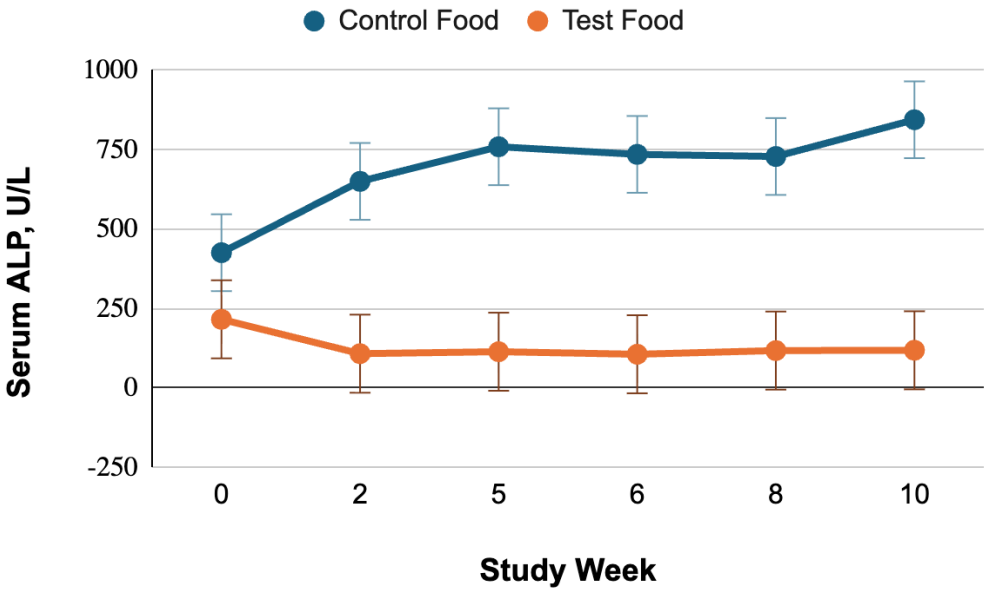
Characteristics of % of calories from Carbohydrate, Fat & Protein (58% / 18% / 24%; and 7% / 56%/ 37%) for the Control food and Test food respectively.
The number of animals in each treatment was 9 and 10 dogs for Control food and Test food, respectively.
Diet *P*=0.80
Week *P*=0.02
Diet x Day *P*=0.62

Table 3.3. Mean of lipids profiles and biomarkers of fat digestion by study week in dogs (n=19) assigned to foods differing in macronutrients.

	Test Food			Control Food			**P-value**			
Variable	Week 0	Week 5	Week 10	Week 0	Week 5	Week 10	SEM	Diet	Week	Diet x Day
cPLI, ug/L	259.0	168.0	93.0	388.0	278.0	171.0	154.25	0.41	0.13	0.96
Bile acids, umol/L	10.3	5.9	8.0	6.4	3.8	4.8	5.18	0.34	0.26	0.86
Cortisol, ug/dL	2.1	1.8	2.0	2.0	1.6	1.8	0.30	0.44	0.03	0.98
B-HBA, mg/dL	0.9	1.4	1.4	0.9	0.9	0.9	0.09	<0.01	<0.01	<0.01

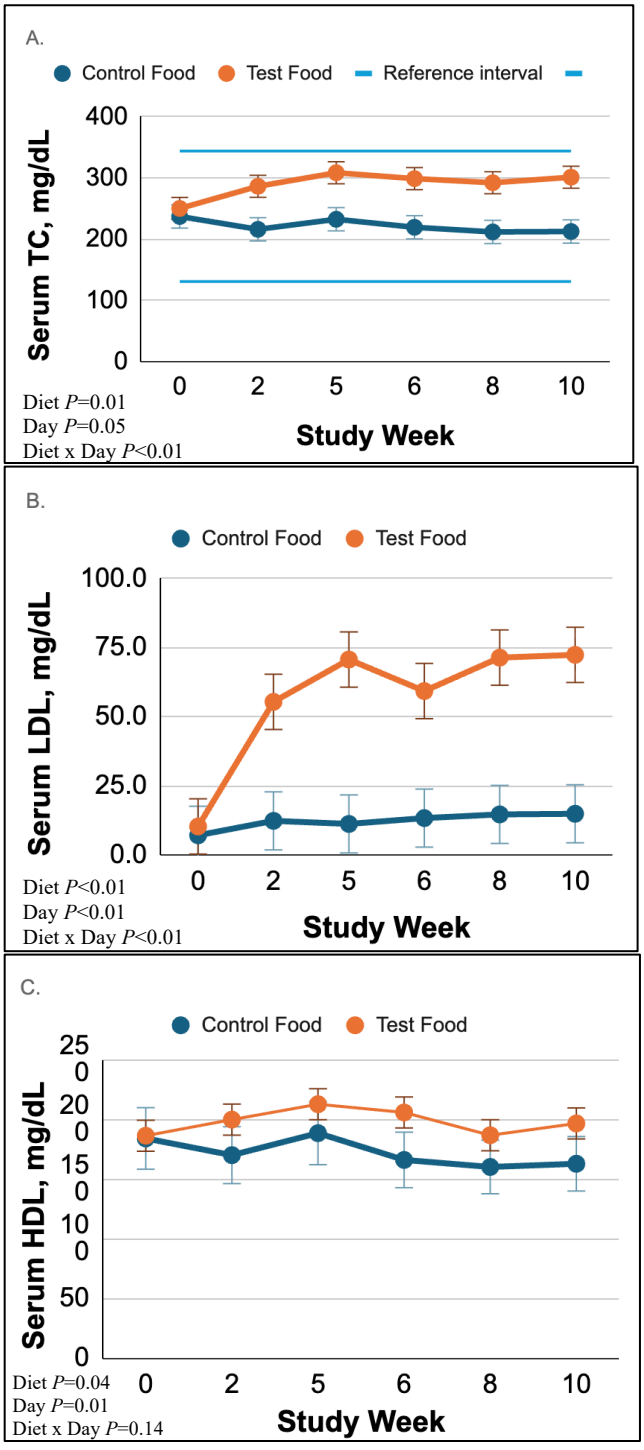
B-HBA, β-hydroxybutyrate; cPLI, canine pancreatic lipase immunoreactivity; GGT, gamma-glutamyl transferase; SE, standard error.
The number of animals in each treatment was 9 and 10 dogs for Control food and Test food, respectively.
Characteristics of % of calories from Carbohydrate, Fat & Protein (58% / 18% / 24%; and 7% / 56% / 37%) for the Control food and Test food respectively.

Figure 3.2. Average serum alkaline phosphatase concentrations (U/L) by study week in dogs (n=19) assigned to foods differing in macronutrients



Characteristics of % of calories from Carbohydrate, Fat & Protein (58% / 18% / 24%; and 7% / 56%/ 37%) for the Control food and Test food respectively.
The number of animals in each treatment was 9 and 10 dogs for Control food and Test food, respectively.
Diet $P<0.01$
Week $P=0.15$
Diet x Day $P=0.02$

Figure 3.3. Average serum total cholesterol (A), low density lipoprotein (B), and high density lipoprotein (C) by study week in dogs (n=19) assigned to foods differing in macronutrients.



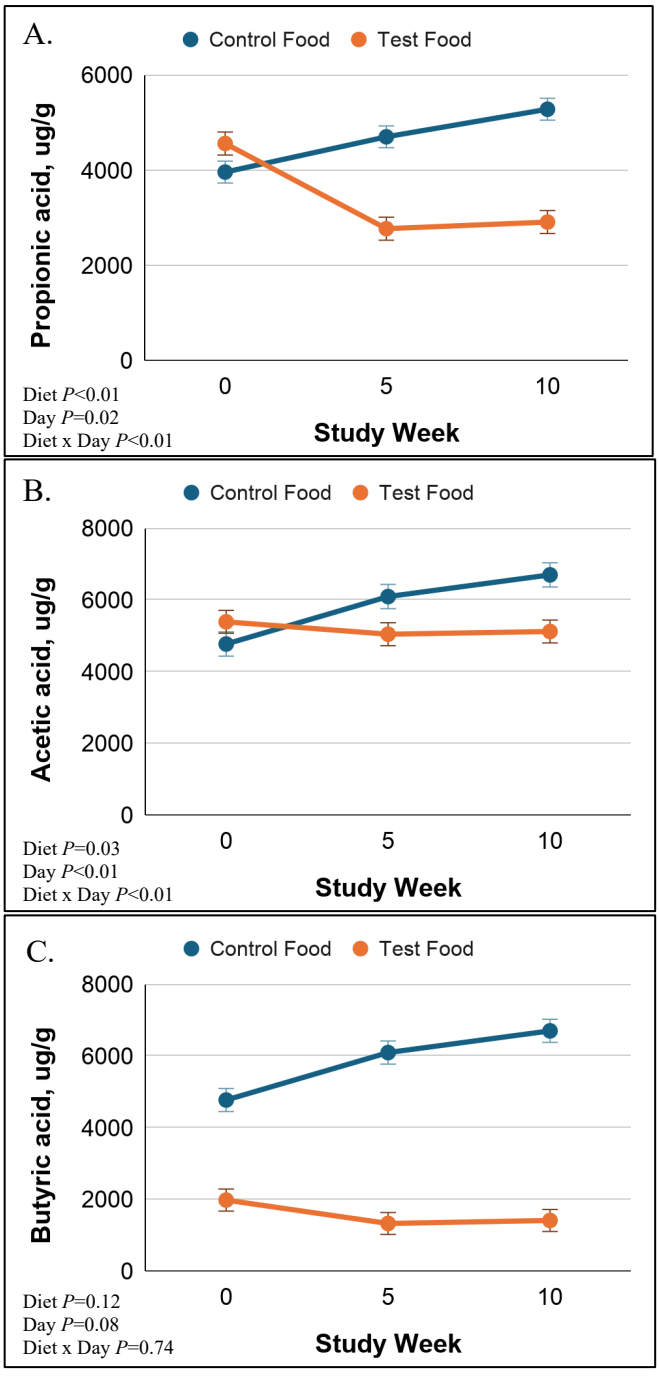
HDL, high density lipoproteins; LDL, low density lipoproteins; TC, Total cholesterol
IDEXX BioAnalytics, Columbia, MO, USA. Reference interval indicated by the blue line, were established using clinically healthy, adult dogs of various breeds.
Characteristics of % of calories from Carbohydrate, Fat & Protein (58% / 18% / 24%; and 7% / 56%/ 37%) for the Control food and Test food respectively.
The number of animals in each treatment was 9 and 10 dogs for Control food and Test food, respectively.

Table 3.4. Mean circulating satiety, regulatory, and counter-regulatory hormones by study week in dogs (n=19) assigned to foods differing in macronutrients.

	Test Food			Control Food			**P-value**			
Variable	Week 0	Week 5	Week 10	Week 0	Week 5	Week 10	SEM	Diet	Week	Diet x Day
GIP, pg/mL	12.8	10.1	12.1	14.4	12.1	11,9	2.90	0.7	0.58	0.9
PP, pg/mL	145.0	136.0	129.0	206.0	157.0	147.0	63.82	0.53	0.49	0.83
PYY, pg/mL	435.0	322.0	451.0	476.0	356.0	459.0	122.61	0.87	0.01	0.92
Leptin, pg/mL	11232.0	6257	6140	17171	12671	10891	4259.60	0.17	<0.01	0.26
Ghrelin, pg/mL	148.0	109	109	84	284	379	62.70	0.28	0.01	0.04

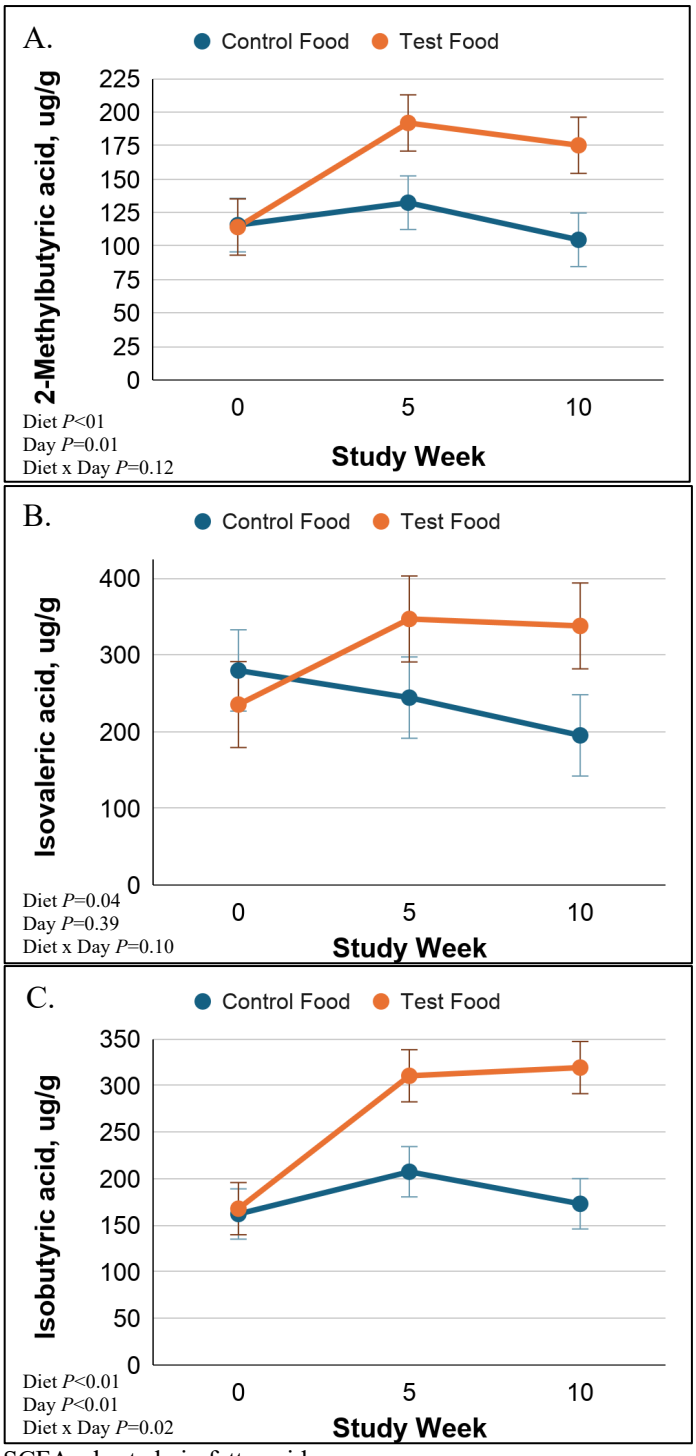
GIP, Glucose-dependent Insulinotropic Polypeptide.; PP, pancreatic polypeptide; PYY, peptide YY.
Data is presented as mean ± SE.
Characteristics of % of calories from Carbohydrate, Fat & Protein (58% / 18% / 24%; and 7% / 56%/ 37%) for the Control food and Test food respectively.
The number of animals in each treatment was 9 and 10 dogs for Control food and Test food, respectively.

Figure 3.4. Average fecal (ug/g) saccharolytic SCFA represented by propionic acid (A), acetic acid (B), and butyric acid (C) by study week in dogs (n=19) assigned to foods differing in macronutrients.



SCFA, short chain fatty acid
Characteristics of % of calories from Carbohydrate, Fat & Protein (58% / 18% / 24%; and 7% / 56%/ 37%) for the Control food and Test food respectively.
The number of animals in each treatment was 9 and 10 dogs for Control food and Test food, respectively.

Figure 3.5. Average fecal (ug/g) proteolytic SCFA represented by 2-methylbutyric_acid (A), isobutyric acid (B), and Isovaleric acid (C) by study week in dogs (n=19) assigned to foods differing in macronutrients.



SCFA, short chain fatty acid

Characteristics of % of calories from Carbohydrate, Fat & Protein (58% / 18% / 24%; and 7% / 56%/ 37%) for the Control food and Test food respectively.

The number of animals in each treatment was 9 and 10 dogs for Control food and Test food, respectively.

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