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Impact of fatty acid on markers of exocrine pancreatic stimulation

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To the Graduate Council:

I am submitting herewith a thesis written by Yunyi Zhang entitled "Impact of fatty acid on markers of exocrine pancreatic stimulation." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Comparative and Experimental Medicine.

Angela Rollins, Major Professor

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Impact of fatty acid composition on markers of exocrine pancreatic stimulation

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ABSTRACT

Chronic pancreatitis in dogs is typically managed with a low-fat diet. Human research suggests consuming medium-chain triglycerides (MCT) may lower pancreatic enzyme release compared to consuming long chain fatty acids (LCFA). Twelve healthy adult colony dogs were fed a meal of cod and rice with either 3% metabolizable energy (ME) fat (control), high MCT (25% ME MCT oil, 25% ME butter), high saturated LCFA (50% ME butter), or high unsaturated LCFA (50% ME canola oil) in a 4-period by 4-treatment crossover design. Serum concentrations of canine pancreatic lipase immunoreactivity, gastrin, amylase, cholecystokinin (CCK), cholesterol, triglycerides and serum activities of DGGR lipase were measured at times 0 (fasted), 30, 120 and 180 minutes post-prandial. Following a 3-or 4-day wash-out period, each dog was assigned a new diet and the process was repeated for all treatments.

Data was analyzed as a repeated-measures mixed model ANOVA. Post-hoc pairwise comparisons were run using Tukey-Kramer adjusted p-values. Shapiro-Wilk tests were used to evaluate residual normality. All statistical assumptions were sufficiently met. Statistical significance was defined as $P<0.05$. Of the markers tested, only serum triglyceride concentrations were affected by treatment, with consumption of high MCT resulting in lower triglycerides than both LCFA groups at times 120 and 180 minutes ($P<0.0001$). As expected, the high MCT group also had higher triglycerides compared to the control ($P<0.0001$). The type of dietary fat consumed had little acute impact on most markers of exocrine pancreatic stimulation in healthy dogs.

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Physiology of the exocrine pancreas

The pancreas has both endocrine and exocrine functions. The exocrine pancreas secretes bicarbonate and digestive enzymes for food digestion. Normal pancreatic secretion activity ensures proper delivery of digestive enzymes, which coordinate food emptying by regulating stimulatory and inhibitory pancreatic secretion[1]. The exocrine pancreas is composed of acinar cells and ductal cells[2]. The main function of the ductal cells is for bicarbonate fluid secretion[3]. Acinar cells constitute more than 80% of the pancreatic exocrine secretion[3]; they receive both hormone and neurotransmitter signals that stimulate the release of digestive enzymes and the precursor forms of digestive enzymes, known as zymogens, into the intestinal lumen for the digestion of nutrients[2]. There are four digestive phases before and during food intake that lead to pancreatic secretion. They include the cephalic phase, gastric phase, intestinal phase and absorptive phase. Differentiation between the gastric phase and the intestinal phase can be complex, therefore they are frequently discussed as one intestinal phase.[1] The cephalic phase occurs before food intake, triggered by sensory input such as the smell and sight of food; the gastric phase occurs when food enters the stomach, which initiates pancreatic secretion; the intestinal phase is initiated by acidic chyme entering the duodenum, which triggers the intestinal phase of pancreatic secretion[1]. The major pancreatic secretion response occurs in the intestinal phase, in which stimulation is initiated by hormones and neurotransmitters such as acetylcholine, serotonin and other neuropeptides[1].

Anticipation of intake food, related to the sight, taste, and smell of food, triggers the vagus nerve to initiate pancreatic secretion, which can account for up to 20-25% of exocrine secretion related to a meal and may last up to 4 hours in dogs. As food enters the stomach, gastric secretion of gastrin and gastric acids are initiated and partially digested food is termed chyme. Gastric chyme is then slowly released into the duodenum for further digestion and absorption. The presence of nutrients and the drop in intraluminal

pH in the duodenum also stimulates pancreatic enzyme release[4]. Secretin is released from the basolateral side of the S cells of the proximal duodenum and into the bloodstream when acidic chyme first enters the intestine. The S cell is located at the mucosal epithelium on the villi of the duodenum; it is named S cell because of its small size[5]. Pancreatic duct cells release bicarbonate rich fluid in response to the secretin to buffer duodenal pH following the influx of acidic gastric chyme[1]. Secretin has also been proposed to stimulate acinar cells to secrete lipase[3]. Previous research on the infusion of secretin into rats tail veins by surgical cannulation has demonstrated that it induced an increase in lipases levels synthesized in the pancreatic lobules after the pancreas was removed and homogenized[6]. Cholecystokinin (CCK) is another peptide hormone released from the basolateral side of the I-cell lining the small intestinal epithelium in response to a meal. Proteins, peptides, amino acids, and lipids act as secretagogues for CCK with the response to individual nutrients varying by species containing peptides and lipids. The pathway of CCK on pancreatic enzyme release is discussed in more detail below.

During the phases of food digestion, the pancreas releases digestive enzymes in both active and inactive forms, called proenzymes. The pancreas can release some active enzymes that target carbohydrate, lipid and nucleotide digestion such as amylase, lipase, and ribonuclease[7]. The inactive form of enzymes are called proenzymes, which are stored in zymogen granules. Key proteolytic enzymes, or proteases include trypsinogen and chymotrypsinogen.

Zymogen granules are specialized organelles that package and secrete exocrine pancreatic enzymes[8]. Zymogens are important components within the pancreatic secretion system, as they provide a protective storage mechanism that prevents active digestive enzymes from causing cellular damage and autodigestion of the pancreas. Digestive enzymes remain as proenzymes within in the zymogen granules until they are released from the pancreas. One example is trypsinogen, the proenzyme of trypsin, is critical to digestion, and is packed within zymogen granules[9]. Before activation, trypsin activity is inhibited by the pancreatic secretory trypsin inhibitor (PSTI) that binds on the trypsin enzyme active site in pancreatic acinar cells. Enterokinase is a protease that is secreted from small intestinal mucosa cells. Once trypsinogen and other zymogens reach

the duodenum, trypsinogen is converted to trypsin by enterokinase[10]. This is the beginning of the enzyme activation process[10,11]. Trypsin activates the remaining of the proenzymes released from zymogen granules. These activated enzymes are required for nutrient digestion[10]. Normally, the pancreas secretes an adequate amount of digestive enzymes to facilitate digestion, and the process is regulated by a negative feedback[4]. The negative feedback for pancreatic secretion occurs after the activated enzymes are consumed with dietary protein within the small intestine. The decreased availability of active enzymes promotes pancreatic enzyme release. Once the meal is mostly digested and there are fewer proteins available to occupy the proteases, the intestinal surface detects the rise in protease enzymes and initiates a negative feedback signal to the pancreas to cease the enzyme release.

Canine pancreatitis

Pancreatitis is the most common disease of the exocrine pancreas in dogs[12], which results from the abnormal and premature release of pancreatic enzymes. Pancreatitis can be classified as acute or chronic. Acute pancreatitis occurs due to inappropriate activation of enzymes near or within the pancreas before zymogens reach the small intestine. Early activation of proteases can cause autodigestion of the pancreatic acinar cells and lead to inflammation and/or necrosis of the pancreas and /or nearby tissues[13]. Clinical signs of acute pancreatitis in dogs include cranial abdominal pain, vomiting, depression, anorexia, and weakness[13,14]. Severe cases may involve cardiovascular shock, disseminated intravascular coagulation (DIC), multi-organ failure, and death[14].

Chronic pancreatitis is defined as continuous or recurring inflammation of the pancreas with irreversible morphological changes that cause pain or permanent loss of function[15] in both exocrine and endocrine aspects. Chronic and ongoing inflammation and fibroses may eventually lead to exocrine pancreatic insufficiency (EPI) or diabetes mellitus (DM)[15]. Dogs with chronic pancreatitis often present with intermittent, mild to moderate gastrointestinal signs, episodic anorexia and/or food aversion. Dogs with chronic pancreatitis can also have acute flares with vomiting, diarrhea, and lethargy similar to acute pancreatitis[15].

In humans, acute or chronic pancreatitis cases are caused by complex interactions between genetic and environmental factors[16]; however, the etiology of pancreatitis is often unclear in dogs[16]. Risk factors for developing pancreatitis can be present in any age, breed, or sex of the animal. Some dog breeds are more likely to develop chronic pancreatitis. A post-mortem study of canine pancreatitis from the UK found that Cavalier King Charles Spaniels, English Cocker Spaniels, Boxers, and Collies were highly represented in the study[17,18]. Some case studies found several USA breeds who appear at greater risk for chronic pancreatitis, such as the Yorkshire terrier and miniature schnauzer.[19-21] Beyond ages and breeds, certain health conditions are also cited risk factors. For example, dogs with concurrent endocrine disease, hypercalcemia, and obesity are at risk of developing chronic pancreatitis[22]. Other risk factors include previous surgeries, having been neutered or certain medications, or dietary indiscretion spayed[20,21,14].

Diagnosis

Clinical diagnosis of canine chronic pancreatitis can be challenging. Dogs with pancreatitis may present with a variety of non-specific clinical signs such as anorexia, weakness, vomiting, diarrhea, depression and/or abdominal pain[14,22].

Historically, the serums amylase and lipase were used to diagnose pancreatitis in dogs[22], but these tests haven fallen out of favor because they lack sensitivity and specificity. Recently, pancreatic lipase immunoreactivity (PLI) has been used more widely for clinical diagnosis[22]. A study published by Cridge et al. found that, in comparison to histopathology samples, serum amylase was a less sensitive marker for diagnosing pancreatitis than canine specific pancreatic lipase immunoreactivity (Spec cPLI or cPLI)[23]. By utilizing 22 samples from the histopathology lab, Texas A&M University found that serum amylase has a 18.2% sensitivity for detecting pancreatitis, which is significantly lower than the Spec cPLI test result of 63.6% sensitivity[24]. Another study reported by McCord et al. found that in 84 dogs with clinical pancreatitis, the serum amylase had a specificity of 76.7%-80.6%, while the Spec cPLI test had between 80.5%-88.0% specificity[25]. Therefore, the serum amylase has no longer been considered

as a routine diagnostic method for canine pancreatitis when compared to cPLI[23]. Details of cPLI will be discussed further below.

Diagnostic imaging in the form of abdominal ultrasound and radiography are other tools used to aid in the diagnosis of both acute and chronic canine pancreatitis[23]. Abdominal radiography can also help rule out other acute abdominal diseases during examinations[26]. Some other imaging methods are contrast-enhanced ultrasonography, elastography, computed tomography angiography and magnetic resonance imaging[23]. But Cridge et al (2020) has recommended performing quantitative pancreatic lipase assay in addition to abdominal ultrasound[27].

Nutritional Management of Chronic Pancreatitis

Proper nutritional support is key for reducing the frequency and/or severity of chronic pancreatitis. Feeding a low-fat diet is recommended to human patients with chronic pancreatitis[28]. Previous research in dogs also suggests feeding a low-fat diet reduces post-prandial pain associated with pancreatitis[17]. This reduction in pain may be the result of less enzyme release from the pancreas since fat is a potent stimulator of CCK[28]. The mechanistic benefit for feeding a low-fat diet is to reduce CCK stimulation of the inflamed pancreas. Because dietary fatty acids are potent secretagogues of CCK secretion in dogs, low-fat diets minimize CCK secretion and subsequent pancreatic stimulation and further enzyme release. Initially, the pain of chronic pancreatitis results from the exocrine pancreatic secretion activity and hyperstimulation, leading to the increased pressure of pancreatic duct and tissue[29,30]. In addition, hyperlipidemia is a known risk-factor for pancreatitis and feeding a low-fat diet can decrease serum lipid levels [31].

Protein peptides and free amino acids are also important stimuli of pancreatic enzyme secretion and carbohydrates have a negligible effect. Therefore, avoiding foods with excess protein levels and providing moderate carbohydrate intake is desired. In dogs with chronic pancreatitis, dry matter (DM) protein composition for dogs should be around 15-30% in calories densities[32]. It is critical to balance the protein composition to other nutrients in the diet. Protein is one of the three essential nutrients (carbohydrate,

lipids, protein). Protein can stimulate protease enzyme secretion, and protease is the most responsible for autodigestion of the pancreas and the inflammatory process[33]. However, if the diet has very minimal protein content, it may cause deficiencies of other nutrients. For example, vitamin B12 deficiency[34].

As dietary protein enters into the stomach it triggers the release of gastrin to stimulate the release of gastric acid. Gastrin also stimulates the exocrine pancreas to release proteases in anticipation of chyme entering the duodenum. In the duodenum, CCK is a major hormone promoting more protease secretion from the pancreas. Gastric distension also stimulates mechanoreceptors to provide neuronal signals to the vagal nerve to stimulate parietal cells, resulting in exocrine pancreatic secretion as well[35].

Markers of exocrine pancreatic stimulation

Cholecystokinin (CCK)

Cholecystokinin is a peptide hormone mainly produced by I-cells in the lining of the duodenum and proximal jejunum. After a meal containing fatty acids and proteins lipids, peptides and amino acids reach the small intestine and act as a cellular signal for I-cells to synthesize and release CCK. CCK is released from the basal side of the I-cell into the bloodstream[36]. In the peripheral circulation, CCK interacts with CCK-A receptor at the gallbladder smooth muscle cells, the binding process is transported by G-protein coupled receptors with in the cytosol[37]. G-protein coupled reaction mediate calcium signaling by increase intracellular calcium level resulting in gallbladder contraction and secretes of bile and bile salts into the intestine. CCK binding to CCK-A receptor on pancreatic acinar cells initiates the release pancreatic enzymes through G-protein coupled receptor and calcium mediates pathway; both bile and pancreatic enzymes help to digest fatty acids nutrients in the small intestine[38]. CCK continues being secreted until all the absorption is complete and nutrients stimulation of I-cells has ceased.

CCK secretion is initiated by fatty acids and protein intake. Nonetheless, small amounts of CCK are released during the cephalic phase by the sight, smell or taste of food. A study found a CCK peak around 45-90 minutes after food intake for dogs[39]. Other studies reported that in healthy dogs, CCK levels peak 1 hour following

administration of corn oil[40]. This later study utilized long-chain fatty acids as a potent stimulator of canine CCK and pancreatic enzyme release.

CCK has four receptors: CCK-A receptor (CCK_AR), CCK-B receptor (CCK_BR), gastrin, and CG-4 receptors. CCK_AR and CCK_BR are the major receptors, in a human research, both receptors located in the upper gastrointestinal tracts has separate regulatory role of gastrin and CCK in terms of morphological and biochemical[41]. CCK_AR has high affinity for sulfated CCK only[42]. There are two CCK_AR types: one located in the pancreas, and the other one at gallbladder[38]. CCK_BR are found on cells throughout the central nervous system; some are located at parietal cells in the gastrointestinal system as well. CCK_BR is a receptor not only for CCK but also for gastrin binding[38,43].

There are several forms of CCK, and the most abundant forms of CCK found in dogs are CCK-58, K-39, CCK-33, and CCK-8[44,45]. These forms are classified based on the number of amino acids within the molecule. CCK-8 has 8 amino acids, the 8 amino acids are structural conserved binding site to the CCK receptor; whereas CCK-58 has a longer sequence, and the extra 50 amino acids may hinder its binding sterically[38]. CCK is primarily secreted from I-cells on the small intestine, but it has been found that some CCK is expressed in the brain and neurons[36]. Aside from food stimulation causing the release of CCK from duodenum, CCK is also released from the dorsal vagal complex within the brainstem during the cephalic phase of the food digestion process. Both the brain and digestive organs have CCK receptors. The forms of CCK that exist in the brain and gut are different: CCK-8 is the primary form found in brains of dogs. CCK is found in highest concentration within the intestine. CCK-58 is the most abundant form, other abundant forms are CCK-39, CCK-33, and CCK-8. [44]. CCK-58 is the major form of CCK, with 80% of immunoactivity in some species[38]. CCK-58 is the most abundant form of CCK in dog blood[46] but is degraded rapidly in blood plasma with a half-life of 2.6 minutes[47].

Because CCK is secreted within the brain and small intestine, to measure total CCK release, require sampling of the cerebrospinal fluid and jejunoileal mucosa[48]. In reality, measuring blood CCK is the most practical and adequately represents the food-stimulated CCL release into the blood following secretion. A challenge in accurately

measuring is that CCK is a very small amount peptide hormone and highly sensitive to the plasma protease degradation after release. The half-life time of CCK is around 2.6 minutes in dogs[39,47]. The average basal concentration is around 2.7±0.2 pM and reaches a maximum of 5.0±0.7 pM after a normal meal[39].

CCK is an important hormone in the gastrointestinal digestive process. Several methods have been used to measure circulating levels of the hormone, each with their advantages and limitations. Liddle (2011) describes a reliable CCK assay antibody as being able to distinguish at least three amino acid between the CCK and gastrin sequence[49]. Radioimmunoassay (RIA) is sensitive immunoassay technique that uses radiolabeled molecules for measuring small amounts of peptide; the level can be as low as picograms in units. The principle of RIA is that of competitive binding, which involves using a fixed number of antibodies to bind with labeled antigens called "tracer" and unlabeled antigens from the sample or standards. Once antigens and antibodies bind, the labeled tracer is detectable by the radioactive counter. Higher rates of the tracer activity indicate lower concentration of the sample antigen, and vice versa. With RIA testing, high affinity antibodies are required for testing a low concentration of CCK in some serum samples. Even though RIA has the advantage of high sensitivity, it requires specialized equipment and the use of radioactive materials.

Enzyme-linked immunoassay (ELISA) is a commonly used assay technique available for measuring CCK that is also based on antigen-antibody reaction. Rather than using radioactive reagents, ELISA relies on the activation of a chromophore or fluorescent compound to create a colorimetric signal. The two most commonly used types of ELISA are competitive ELISA and sandwich ELISA. The competitive ELISA combines an incubated mixture of sample antigen and test antibody to an antigen coated substrate. Samples with a high antigen concentration will bind more test antibodies, causing less binding of the reference antigen. Any unbound antibody is removed through plate washing. Depending upon the antigen concentration in the sample, A second enzyme-linked antibody that has the same binding affinity as the sample antibodies is applied to a pre-coated antigen well. A chromophore or fluorescent substrate is then added such that antibody-bound enzyme activates a substrate to a detectable color signal,

when the secondary antibody successfully binds to the test antibody. More color on the well or solid substrate indicates lower levels of sample antigen were present to bind the test antibody during incubation.

Sandwich ELISA's utilize pre-coated capture antibodies on the well surface. Sample antigens are added and bind the capture antibodies. After washing the plate to remove unbound antigen, detecting antibodies are applied that also bind the sample antigen. Following a second wash to remove unbound detection antibody, a colorimetric chemical such as tetramethylbenzidine (TMB) is added and a blue color develops proportionately to amount of sample analyte present. While RIA is generally more sensitive than ELISA, the simplicity of running ELISA assays and avoiding radiation hazards makes it a more practical approach in many laboratories.

Gastrin

Gastrin is a linear peptide hormone that is mainly secreted from the G-cells in the gastric mucosa on the stomach pyloric antrum after food intake and stimulates the secretion of hydrochloric acid (HCl) from the stomach to initiate digestion. The major gastrin forms found in dogs are gastrin-34, gastrin-17, and gastrin-14, with the concentration found in gastric fluid decreasing, respectively[50]. Gastrin is produced by the G cell located in the pyloric antrum, and the secretion is primarily stimulated by dietary protein[43]. Some G cells are also located within the duodenum and pancreas[51]. It is possible that gastrin stimulates pancreatic acinar cells to increase enzyme secretion to aid digestion.

The structure of gastrin is similar to CCK with both having common binding motifs. In mammals, only gastrin and CCK have the same carboxyl terminal[42] (Figure 1). CCK binding to the CCK-A receptor primarily stimulates gallbladder contraction and pancreatic enzyme secretion. The CCK-B receptor is predominantly located at the brain, is less selective, and binds to gastrin[42], gastrin and CCK can both bind to the CCK-B receptors.[43]. A clone experiment from a parietal cell shows that the gastrin receptor and the CCK-B receptor are identical[52]. Due to the similarity between gastrin and CCK, it is important to consider that CCK may have cross-reactivity with gastrin since they have

very similar molecular structure and binding receptors. Therefore, cross-reactivity with gastrin is an important feature is to consider in validating CCK assays[53]. Serum gastrin measurement can be done using radioimmunoassay or chemiluminometric assays which appear less sensitive than the radioimmunoassay[54]. The normal dog gastrin level is around 10-40ng/L[55], which is 3.46-13.85 pM.

Canine specific Pancreatic lipase (cPLI)

Lipases are a type of enzyme that help break down dietary fats into smaller fatty acids during digestion. Lipases are mainly produced from the stomach and pancreas, and to a lesser amount are secreted by lingual serous glands[56] to break down dietary fats into fatty acids[57]. Pancreatic lipase (PLI) is an enzyme secreted by the pancreas that is mainly responsible for aiding the digestion of triglycerides[58]. Pancreatic lipase can also stimulate the release of CCK[40], further promoting it's excretion into the duodenum. When dietary fatty acids arrive at the duodenum, they are broken down into fatty acid droplets by a combination of pancreatic lipase anchored by colipase to bile salts. This allows further emulsification of the lipid droplets into monoacylglycerides and free fatty acids for absorption[59].

Because serum lipases can be elevated for a variety of reasons and released from different tissues in the GI tract[60], measuring the total serum lipase yields poor specificity for diagnosing pancreatitis. The lipase secreted from the pancreas has a unique amino sequence that allows differentiation from other lipases in the body.[61] Canine pancreatic lipase (cPLI) is an immunoreactivity assay that provides a more specific measure of pancreatitis in dogs since it only measures the lipase secreted from pancreas[62]. In the case of active pancreatitis, high concentrations of pancreatic lipase are released into the circulation[23]. In the immunologic cPLI assay, antibodies are used to test the lipase concentration by binding with antigens using ELISA[23]. The validity of the cPLI assay to detect pancreatitis was compared the gold standard of pancreatic histology. To ensure the specificity, results were also validated using dogs with exocrine pancreatic insufficiency (EPI) to test pancreatic lipase levels. In a study comparing 25 dogs with EPI to controls, investigators found a median concentration about 0.1 μg/L in the dogs with EPI,

compared to 16.3 μg/L in the control group. This study demonstrated that cPLI has a high specificity for pancreatic lipase compared to lipase released from other tissues[23]. Radioimmunoassay and ELISA are both methods of measuring cPLI. Both analytical methods are sensitive, accurate and clinically applicatable[61]. A normal dog has serum cPLI levels that range between 0 to 200μg/L[63]; serum levels higher than 200μg/L is suggests pancreatitis. Normal dogs reach approximately 75.2μg/L after a standard meal intake[64].

DGGR lipase

In addition to the immunologic lipase assay, another common method for measuring lipases is by catalytic assay[23]. This method measures the hydrolysis of substrate by potential lipases, and uses colorimetric reaction to detect enzyme activity[65]. Using 1,2-diglyceride(1,2DiG) as the substrate of an analytical assay, hydrolysis by serum lipase can be determined serum lipase levels.[66] The assay requires complex and multistep colorimetric reaction, and previous tests indicate that the sensitivity of serum lipase is less than 60%[67] because 1,2DiG hydrolysis is not-specific to pancreatic lipase[68]. A better substrate was developed in 2001[68] which is 1,2-*o*-dilauryl-*rac*-glycero-3-glutaric acid-(69-methylresorufin) ester (DGGR). This substrate has higher sensitivity, higher pancreatic-enzyme-specificity and is more selective compared to 1,2DiG[65]. DGGR assay works as a substrate when cleaved by lipase to become a reliable assay for acute pancreatitis testing in dogs .[65] After DGGR became widely used in human pancreatitis diagnosis, interest in its usage within veterinary medicine grew. The between assay reliability experiment found that DGGR assay measured the within-run and within-day coefficient of variation (CV) is less than 3% and the day-to-day CV is 14%[65,69], this provides assurance of the DGGR assay reliability in measuring lipase activity in the serum of dogs. There was an "statistical agreement" established between cPLI and DGGR lipase assay, where the calculated Cohan's kappa coefficient value (κ value) fell into the 95% confident intervals, indicating that the amount of agreement between DGGR lipase and cPLI is modest[70,71]. Therefore, DGGR lipase assay does not have as strong predictive value for pancreatitis compared to cPLI, but it can still be considered a

reasonable screening test, especially for the rapid diagnosis of acute canine pancreatitis[72]. When using DGGR lipase clinically, it is better to measure cPLI level and compare the results of the two tests simultaneously.

Amylase

Amylase is a cytoplasmic enzyme that is made and secreted by the intestines and pancreas of dogs. Amylase is primarily released from the pancreas and hydrolyses the starch in the intestine during digestion. Amylase was initially used as a biomarker of acute pancreatitis, but it has been found suboptimal in 1970[23,73]. Amylase is not only secreted from the pancreas, in dogs, amylase is secreted from the intestine. The study shows that serum amylase is increased both during and following damage to the pancreas in dogs. However, serum amylase does not remain in serum or plasma as long as pancreatic lipase following injury.[74] Amylase is no longer considered a valid marker for pancreatitis testing due to variable levels in disease and lack of organ specificity[23]. A study by Strombeck et al. surveyed 713 cases of dogs, which included healthy dogs and dogs with suspected pancreatitis[75]. They measured serum lipase and serum amylase and found serum amylase concentrations are essentially the same between normal and dogs with suspected pancreatitis[75]. This finding led them to conclude that serum amylase is only valuable when compared with serum lipase concentrations when diagnosing pancreatitis[75]. Even though amylase is no longer a reliable marker in the diagnosis for pancreatitis, it is secreted from the pancreas in response to carbohydrate intake and may be used to detect some degree of exocrine pancreatic function. When considering changes in serum amylase levels, it needs to be considered that increased amylase can be caused by renal, hepatic, intestinal, and neoplastic diseases, as well as other non-pancreatic conditions[14].

Dietary fatty acids

General information

Dietary fatty acids are fatty acids sourced from fruits, vegetables, animal tissues, seeds, nuts, etcetera. The majority of fats are composed of long-chain fatty acids; the

main categories of dietary fats include saturated, mono-unsaturated, and polyunsaturated[76]. Dietary triglycerides are the lipid molecules that have three fatty acids chain attached to the glycerol backbone; in this case, the number of carbons on the backbone are determinants of fatty acid classification. These classifications include short chain (<6 carbons) triglycerides (SCTs), medium chain (6-12 carbons) triglycerides (Figure 2), and long chain (>12 carbons) triglycerides[77] (Figure 3). The fatty acid component of triglycerides may or may not contain double bonds. Saturated fatty acids are defined as "saturated" because they do not contain carbon-carbon double bonds as bonds are saturated with electrons. Unsaturated fatty acids contain at least one double bond (monounsaturated fatty acids) or multiple carbon-carbon double bonds (polyunsaturated fatty acids or PUFA)[77].

Triglycerides (TAG) are usually a typed of long-chain fat that have three fatty acids attached on a backbone with three carbons. Dietary triglycerides are hydrolyzed by pancreatic lipase to glycerol, free fatty acids, mono and diglyceries[78]. In human patients, triglyceride-induced pancreatitis is one form of the disease and is associated with triglyceride levels over 1000mg/dL[79]. In dogs, the association between chronic pancreatitis and hyperlipidemia is still unclear, but it has been suggested that they are bidirectional[80]. Testing serum triglycerides levels provides a reference of dietary intake, hormonal disorders, and the effectiveness of pancreatic lipase secretion and digestion of TAG. A normal dog's triglycerides reach peak level around 2 hours to 4 hours postprandially[81].

Cholesterol is an unsaturated alcohol compound as the precursor of various steroid hormones[82]. Cholesterol also provides fundamental structural functions in cell membranes and associated with lipoprotein composition. Depending on different types of lipoproteins, high density lipoprotein (HDL) contains higher protein and lower cholesterol composition, whereas very low-density lipoprotein (VLDL) contains much higher cholesterol composition. It has been demonstrated in people with acute pancreatitis that cholesterol levels reveal a U-shape curve in response to treatment. In the 648 cases of acute pancreatitis in the study, those with low fasting blood cholesterol and

high fasting blood cholesterol level had higher incidence of protracted hospital stay than patients with normal cholesterol level patients[83].

Following a meal, fats containing triglycerides and cholesterol are digested and absorbed into the bloodstream. They are then packed and transported in plasma by specific lipoprotein rich in triglycerides such as VLDLs[84].

Another type of fatty acid is medium-chain fatty acids, or medium-chain triglycerides (MCT). Historically, MCT has been used to treat some gastrointestinal disorders. Compared to long-chain triglyceride (LCT), MCT have a lower molecular weight and higher water solubility. MCT only contains saturated fatty acids; therefore, it has no carbon-carbon double bonds in the molecule. MCT are rapidly digested and absorbed. Thus, it has been used for certain gastrointestinal disorder patients with impaired fat digestion and absorption[85]. For instance, MCT has been used to manage fat intake in people with pancreatic insufficiency. In a study, 8 enzyme-insufficient patients with EPI consumed 69% MCT as fat sources for 10 weeks. The results of this diet showed minimal CCK released and a decrease of post-prandial abdominal pain[77,86]. Similarly, past studies have been conducted in people consuming either MCT or normal LCT. These studies found that when administrated isocalorically, MCT resulted in little CCK release compared to LCT administration. These researchers concluded from that MCT can help reduce exocrine pancreatic secretion compared to LCT[87,88].

MCT Digestion

Usually during the digestion of LCTs, when fatty acids reach the stomach and duodenum, the I-cell of the small intestine secretes cholecystokinin (CCK) to stimulate pancreatic enzyme release and help digestion. CCK promotes bile secretion from the gallbladder to help emulsify LCTs to smaller droplets for digestion[77,89]. Pancreatic lipase are the fat-splitting enzymes that are secreted by the pancreas. With the aid of co-lipase secreted from the pancreas, lipase cleaves triglycerides at the surface of emulsified fat droplets, helping fatty acids, cholesterol and monoglycerides form into micelles. Micelles diffuse through the unstirred water later of the intestine and are absorbed by the small intestine later to be packed into chylomicrons for future fatty acids transport,

storage, or energy use. However, MCT digestion is simpler and more rapid due to its features. MCT does not stimulate CCK[87,89]; instead, MCT are passively transported and absorbed directly from the small intestine lumen into the portal vein without emulsification or cleavage[77,85]. They are then rapidly oxidized in the liver without secretion of pancreatic enzymes[90], or being stored in the form of LCT in the liver[90]. MCT has been used for patients who suffer with fat digestion problems[85]. In this study, the role of MCT oil in CCK secretion, pancreatic exocrine stimulation, and lipid digestion is being considered for dietary management of pancreatitis in dogs.

Pancreatic stimulation by different fatty acids

When animals eat dietary fatty acid, lingual lipase is secreted from the tongue while gastric lipase is secreted from the stomach for early digestion of fat; however, the major digestive process occurs in the small intestine[91]. After partially digested fat reaches the small intestine, the stimulation of the I-cell in the small intestine is initiated to secrete CCK. CCK binds to receptors on the gallbladder and pancreas to secrete bile acids and pancreatic lipase, respectively, and to aid in TAG emulsification and hydrolysis. With the pancreas also secreting bicarbonate, TAGs are broken down into free fatty acids and monoglycerides. Then, they are packed by bile salts and become into micelles[91]. Because TAG is hydrophobic, in order to be absorbed across the hydrophilic unstirred water layer of the intestine, the fatty acids from TAG hydrolysis are arranged into water soluble micelles. The micelles usually contain free fatty acids, 2-monoacylglycerol, cholesterol and cholesterol ester, which are released into enterocytes of distal small intestine once micelles interact with the brush border of the small intestine. Next, re-esterification of fatty acids to TAG takes place within the enterocyte followed by repackaging into chylomicrons which transport, TAGs, cholesterol esters, cholesterol, and other nonpolar lipids through the circulation.

The most important difference between MCT and LCT is that LCT as typical dietary fatty acids require emulsification, hydrolysis, micelle transport and repackaging prior to release into the portal system while fatty acids shorter than 1-12 carbons, once in the mucosal cell may be directly absorbed into the portal circulation. Before using LCFs

as an energy source, they must be repackaged into chylomicrons in the form of triglycerides. MCTs are rapidly absorbed into the enterocytes and bypass the need to be packed into chylomicrons. A previous experiment shows that humans who took MCT as their fatty acids source had lower levels of triglycerides and cholesterols in their blood compared to those consuming LCT[92], demonstrating the effect on lipid metabolism. This provides a mechanism of why MCT intake can decrease serum triglycerides compared to an isocaloric LCT diet.

After consuming a meal, fatty acids reach the small intestine waiting to be emulsified by bile acids and hydrolyzed by pancreas secreted lipase. Pancreatic lipase plays an important role in this process, predominantly in the hydrolysis of triglycerides. It is required for dietary triglyceride digestion[93,94].

Through both direct and indirect action, dietary fats stimulate the release of pancreatic enzymes to promote their digestion. However, the role of fatty acid length and composition on pancreatic stimulation in dogs is unclear. A previous study in rats suggested that feeding a high MCT diet resulted in lower pancreatic lipase activity compared to feeding low and medium MCT levels[58]. It was also studied in infants that MCT can decrease pancreatic lipase secretion compared to LCT[58]. These findings are promising when considering the impact of fatty acid length on the release of CCK and markers of pancreatic lipase secretion (e.g., cPLI) in the dog. Therefore, we propose that a high MCT diet will have decreased cPLI serum levels as a marker for decreased pancreatic enzyme release.

CHAPTER TWO

Impact of fatty acid composition on markers of exocrine pancreatic stimulation

Introduction

Pancreatitis is a common disease in dogs and is characterized by the aberrant release of pancreatic lipase and protease enzymes. In normal pancreatic function, several intestinal hormones stimulate the release of pancreatic enzymes in response to dietary protein and fat. Typical nutrition plans for chronic pancreatitis in dogs involve balancing protein and limiting fat level in the diet to reduce pancreatic enzyme secretion[32]. However, there are some practical drawbacks to using low-fat diets for long-term pancreatitis management. Because fat is more calorically dense than protein and carbohydrates, low fat diets typically have a lower caloric density than traditional diets. In addition, dietary fat and protein are highly palatable to dogs and formulating diets that are moderate in protein and low in fat can reduce voluntary food intake. Consequently, it can be difficult to maintain ideal body weight if patients consume the diet for a long time. Understanding the effect of various fat sources on pancreatic stimulation could provide better dietary management of canine pancreatitis by increasing the caloric density of foods for dogs with chronic pancreatitis.

Most dietary fats are in the form of long-chain fatty acids (LCFA) and are potent stimulators of pancreatic enzyme release[88]. Long-chain fatty acids (LCFA) are the primary dietary form of lipids and contain at least 14 carbons. The fatty acid component of triglycerides may or may not contain double bonds. Saturated fatty acids do not contain carbon-carbon double bonds whereas unsaturated fatty acids contain at least one double bond (monounsaturated fatty acids) or multiple carbon-carbon double bonds (polyunsaturated fatty acids or PUFA). Medium-chain triglycerides (MCT) contain shorter chain fatty acids with 6 to 12 carbon atoms. Due to their smaller size and lipophilic properties, MCT can be rapidly absorbed into enterocytes and the portal circulation. In addition, research in humans suggests MCT do not stimulate the exocrine

pancreas or cholecystokinin (CCK) release to the same degree as LCFA[87]. CCK is a peptide hormone that is mainly produced by I-cells in the lining of the duodenum to stimulate exocrine pancreatic enzymes release. In a study of eight healthy adult humans, infusion of MCT into the stomach did not significantly increase amylase, lipase, or bilirubin within the duodenum. In contrast, LCFA significantly increased the release of all three. In the same study, plasma CCK was significantly higher than the baseline with the LCFA administration with MCT having no effect on CCK concentrations[87]. The impact of MCT on the stimulation of the exocrine pancreas has not been studied in dogs.

Canine-specific pancreatic lipase (cPLI) is a serum marker that reflects the amount of pancreatic lipases released into circulation. cPLI is often used to diagnose active pancreatitis in dogs[95]. Cholecystokinin (CCK) is an intestinal hormone that primarily stimulates pancreatic enzyme release and gallbladder contraction. Gastrin is a similar molecule to CCK; however, gastrin is released by the stomach in response to gastric distension. Both gastrin and CCK stimulate acinar cells of the exocrine pancreas to release digestive enzymes[43]. Measuring CCK, gastrin, and cPLI in healthy dogs following a meal may provide insight into the nutrient components that trigger pancreatitis.

The goal of this study was to evaluate markers of exocrine pancreatic stimulation in dogs eating a high MCT diet compared to diets high in saturated or unsaturated LCFA and a low-fat control diet. Additionally, this study assesses the role of fatty acid saturation in pancreatic stimulation. Determining whether differences exist between saturated and unsaturated LCFA is valuable since these are the main fats found in a typical diet. A human study conducted by Beardshall (1989) suggested that consumption of mono and polyunsaturated LCFA resulted in greater secretion of CCK compared to saturated fatty acids[96]. High levels of unsaturated fatty acids, but not saturated fatty acids, also induced intra-cellular trypsin activation and cell damage of pancreatic acinar cells in vitro.[97] Thus, this study considers that consumption of unsaturated fatty acids may result in more pancreatic stimulation compared to saturated fats in dogs. Finally, post-prandial concentration of cPLI in healthy dogs has not been evaluated and is another aim of this study.

Material and Methods

Experimental design

Utilizing a four-period by four-treatment crossover design, this study compared the concentrations of serum CCK, gastrin, and cPLI in twelve healthy, adult research dogs consuming meals with varying fatty acid content and composition. Four dietary treatment groups were studied, consisting of a control diet with minimal fat, a high long chain saturated fatty acid (LCSFA) diet with approximately 50% of metabolizable energy (ME) from butter, a high long chain unsaturated fatty acid (LCUFA) diet with approximately 50% ME from canola oil, and a high MCT diet containing approximately 25% ME from MCT oil and 25% ME from butter. Following a 24 hour fast, baseline blood samples were collected and each dog was fed a test diet. Blood sampling was repeated at 30, 120 and 180 minutes post-prandial. Following a 2-3-day wash-out period on the regular maintenance diet (Purina ONE® Lamb & Rice Formula Dry Dog Food, Nestlé Purina PetCare Company, St. Louis, MO) (Table 2), each dog was assigned a new treatment diet and the process was repeated until all dogs received all treatments (Figure 4).

Experimental subjects

Following approval by the University of Tennessee's Institutional Animal Care and Use Committee (IACUC 2862), twelve healthy, adult purpose-bred research beagles were enrolled in the study. All dogs were determined to be healthy upon physical examination and had received a complete blood count, chemistry panel, and urinalysis within the previous 12 months. Each dog was randomly assigned to one of 4 dietary treatment groups by blindly drawing each dog's name from a bowl.

Diet design

All treatment diets were made with a base of Pacific cod skinless filets (Great Value, Bentonville, AR), Mahatma extra-long enriched rice (Riviana, Houston TX), and fat free Swanson® chicken broth (Campbell Soup Company, Camden, NJ). The cod and rice were prepared based on the manufacturer's directions then combined and frozen. Fat

treatments and broth were added prior to feeding. The LCSFA group contained 50% ME from butter (Land O'Lakes, Saint Paul, MN). The LCUFA group contained 50% ME from canola oil (Great Value, Bentonville, AR). The high MCT group contained 25% ME from MCT oil (NOW®, Bloomingdale, IL) and 25% ME from butter. The MCT treatment group contained both MCT and butter due to palatability issues when feeding 50% ME from MCT oil. Dietary nutrient profiles of each group are listed in table 1.

Test meals provided 50% of a dog's daily energy requirements (DER) to mimic a twice daily feeding schedule. The control diet provided approximately 25% of DER since it contained no added fat source. The DER of each dog was calculated based on the formula:130*[BW(kg)^0.75][98].

Sample collection and analysis

Jugular blood samples were collected in a 2mL vacutainer serum clot activator tube (Greiner Bio-One North America Inc. Monroe, NC). Samples were immediately placed on ice and centrifuged in a refrigerated centrifuge at 3000x for 10 minutes to separate the serum. The serum was stored in cryogenic vials (Premium Vials, Tullytown, PA) and frozen at -80°C before being shipped on dry ice to the Texas A&M University (TAMU) Gastrointestinal Laboratory for sample analysis. The cPLI was measured using Idexx Spec PL ELISA kit (IDEXX Laboratories, Inc., Westbrook, ME). Gastrin measurement was conducted using Siemens Immulite XPi (Siemens Medical Solutions USA Inc. Malvern, PA). DGGR lipase was tested using Sentinel Lipase NG assay (Via Robert Koch, 2 - 20152 Milano, Italy). Amylase, triglycerides and cholesterol were measured using Beckman Coulter reagents (Beckman Coulter Inc., 250 South Kraemer Boulevard, Brea, CA). DGGR lipase, amylase, triglycerides, and cholesterol were run on a Beckman AU480 (Beckman Coulter Inc., 250 South Kraemer Boulevard, Brea, CA).

CCK measurements occurred at the primary institution using a canine-specific ELISA (ELISA kit 2885300, MyBioSource, San Diego, CA). The manufacturer-reported sensitivity was <4.0pg/mL, intra-assay variation ≤5.6%, inter-assay variation ≤7.9%. Gastrin cross-reactivity of the CCK ELISA kit was also tested through spike and

recovery with Biomatik dog recombinant gastrin #RPU54348 (Biomatik USA LLC, Wilmington, DE). Recombinant gastrin was tested in the same concentrations as the CCK standard curve and didn't show cross-reactivity; the CCK ELISA kit has not been tested with natural gastrin.

Statistical analysis

A power analysis was performed using PASS 2021 (Power Analysis and Sample Size Software (2021). NCSS, LLC. Kaysville, Utah, USA, ncss.com/software/pass). Based on detecting two-fold difference in cPLI, a 4-treatment cross-over design with a sample of 12 subjects measured at 4 time points achieves 80% power to detect treatment differences among the means at a 0.05 significance level. The standard deviation across subjects for cPLI (primary aim) at the same time point is assumed to be 75 ng/mL. The pattern of the covariance matrix is to have all correlations equal with a correlation of 0.50 between the first and second time point measurements.

Repeated measures mixed model ANOVA as a crossover design, using a Kronecker product unstructured covariance matrix, was applied to test the within subject effects of treatment and time on cPLI, gastrin, amylase, CCK, cholesterol, triglycerides and DGGR lipase. Ranked transformation was applied to cPLI and DGGR lipase to address non-normal distribution of the residuals. Post hoc pairwise comparisons were run using Tukey-Kramer adjusted p-values. Shapiro-Wilk tests for normality were used to evaluate normality of the residuals. All statistical assumptions were sufficiently met. Statistical analysis was performed using SAS (version 9.4, Cary, North Carolina 27 513, USA, Release TS1M7). Statistical significance was defined as $p<.05$.

Results

A total of 14 dogs were enrolled in the study and were determined healthy based on physical examination. One dog was removed due to diarrhea that occurred while eating the regular maintenance diet. Another dog was removed for refusing to eat the MCT treatment diet. No data from these dogs was included in the final analysis. The mean body weight, body condition score (BCS), and age of the remaining 12 dogs were

12.14 kg ± 7.5 and 6/9 ± 3, and 5 years ± 2 respectively. There were 8 neutered males and 4 spayed females.

There were no treatment effects on cPLI (Figure 6), gastrin, amylase, DGGR lipase or cholesterol. However, triglyceride concentrations differed significantly by each treatment at both timepoint 120 minutes and 180 minutes ($p<.0001$). Triglyceride concentrations peaked at 120 minutes post-meal for LCSFA (154.65 mg/dL ± 11.37) and LCUFA (114.22 mg/dL ± 10.09), with the largest difference noted at this point, but then declined toward baseline at 180 minutes after feeding. The high MCT group had significantly lower triglyceride levels than the long-chain fatty acid groups at 120 minutes (58.9 mg/dL ± 4.92), but failed to reach its peak by the 180 minute timepoint (76.76 mg/dL ± 18.52). As expected, the low-fat control diet had the lowest 120-minute triglyceride concentration at 43.18 mg/dL ± 3.13. (Figure 5). CCK also showed no treatment or time effect in the study. This finding combined with the unexpected secretion pattern relative to mealtime or composition questions the validity of the assay and the CCK results are therefore considered unreliable without additional validation of the assay kit.

Discussion

In human studies, medium chain triglycerides (MCT) are more rapidly absorbed and result in less pancreatic enzyme release compared to long chain fatty acids. The goal of this study was to determine if this same effect occurs in dogs by measuring markers of exocrine pancreatic stimulation[87]. Based on our results, the replacement of LCFA with MCT has little effect on pancreatic lipase release in healthy dogs.

There are several explanations for this observation. Although MCT is expected to be broken down and absorbed in the small intestine without stimulating pancreatic lipase, the present experiment had to use a combination of LCSFA and MCT in the high MCT group due to the poor palatability of MCT alone. The addition of LCFA may have produced enough gastrin and pancreatic lipase release to confound the effects of MCT. Unfortunately, the CCK ELISA assay in this study could not be validated with recombinant canine CCK. Thus, the effects of MCT on this important hormone can not

be confidently assessed. In addition, this study used healthy dogs with presumably normal pancreatic function. The influence of dietary fat may be less pronounced in this population. A previous study has fed healthy dogs a diet with less percentage by weight of crude fat with extra pancreatic enzymes and MCTs. It concluded that the diet with MCT and supplemental pancreatic enzymes improved the pancreatic response from healthy dogs in terms of a noticeably lower cPLI marker, but the difference was not statistically significant enough (P= 0.2)[99]. In both the present study and the previously mentioned study, healthy dogs were the subjects tested. Despite high fatty acid intake, the healthy pancreas can release appropriate amounts of enzymes. Alterations in the dietary fatty acid composition in dogs with chronic pancreatitis may yield different results.

cPLI was the main marker used to measure pancreatic enzyme stimulation activity, and has been reported as a reliable marker when samples were collected at 2, 3, and 4 hours with 88% to 100% sensitivity[100] for both cPLI and triglycerides, respectively. In a prior study, gastrin concentrations peaked at 30 minutes post-prandial and remained elevated for at least 2 hours following a meal[101]. Those findings were used to determine optimal blood sampling times in this study.

Considering cPLI had no significant changes, it is unsurprising that amylase and gastrin also demonstrated minimal change. Changes in amylase concentration tend to parallel the pancreatic lipase level in dogs with pancreatitis[102]. In addition, gastrin and amylase secretion is not stimulated by fatty acids alone and may have been impacted by the protein and carbohydrates within the test diets.

The anticipatory effect of the cephalic phase of digestion could also have influenced the time effect of dietary treatments, and was not considered in the experimental design. There are three main phases of gastrointestinal peptides secretion and activity in response to a meal: the cephalic phase, gastric phase and intestinal phase. The cephalic phase occurs when dogs smell, sight, or taste leads to anticipation of food intake; these sensations could also bring the first phase of pancreatic secretion[103] to enhance the efficiency of future digestion[104]. CCK, gastrin, lipase, and pancreatic polypeptides are all known to be secreted in response to the cephalic phase[104]. CCK is primarily secreted in the duodenum after food intake but is also released from the central

nervous system to act as neurotransmitter. During the present experiment, there was advanced preparation of food prior to it being offered to the dogs. Because all of the dogs were living in the same unit, once one dog had been fed and the blood drawn, other dogs in the same unit were able to smell the food and anticipate being fed. This could have created an anticipatory period of feeding for them. Since CCK is secreted during the cephalic phase of digestion, it makes sense that the baseline CCK could become elevated sooner and exceed the levels found at 120 minutes. Previous research has found that beagle dogs had normal serum CCK levels around 5-10pg/mL, with the peak at 45 minutes to 90 minutes, returning to baseline 4 hours post-prandial[39,105]. This study's blood sampling time points were 30 minutes, 120 minutes and 180 minutes; there is a possibility that CCK levels raised to detectable peak levels post-prandial and were missed at the blood collection time points.

The only marker found to have significantly changed between different treatments was the serum triglycerides level. Within the gastrointestinal tract, MCT and LCT are digested to their respective fatty acids; however, LCFA are repackaged as LCT into chylomicrons for transport via the lymphatic system via the peripheral circulation. Medium-chain fatty acids (MCFA), because of their shorter chain lengths, do not require chylomicron formation for their absorption and transport[106]. Therefore, MCFA travel directly to the liver via the portal circulation, bypassing peripheral tissues such as adipose tissue. The different mode of transport for MCT compared to LCT allows for quicker absorption and utilization of MCT. MCFA are mostly oxidized by the liver for use as energy sources and therefore have been reported to act more like glucose than fats[107]. The findings of this study are similar to previous research in humans which demonstrated that even though MCT produced less triglycerides levels in serum, the chylomicron triglycerides levels still contained a large amount of medium-chain fatty acids[92]. Thus, there was little effect of MCT on total cholesterol, but significantly lower triglyceride concentrations. Since there is a significant decrease of serum triglycerides levels in the high MCT group compared to the LCSFA and LCUFA groups, this diet might be applicable to helping with hyperlipidemia and hypertriglyceridemia in dogs and warrants further research.

In the current study, all treatments demonstrated peak triglyceride levels within 180 minutes except the high MCT group. The reason for this peak delay could be because MCT are directly absorbed into the blood stream then move to the liver to be packed into long chain-fatty acid pool after a series of transformations, and then packed into chylomicrons and released out of the liver for usage[90]. Even though MCT is absorbed faster than LCT, the process of MCT being packed into chylomicron is longer than LCT, therefore, it takes longer for MCT to reach the peak level.

Conclusion

Using MCT compared to long chain saturated or unsaturated fatty acids as the fatty acid source reduced post-prandial triglyceride levels in dogs, which could prove helpful in managing hyperlipidemia and hypertriglyceridemia. In terms of decreasing pancreatic enzymes release, MCT supplementation does not seem to have a significant effect in pancreatic enzyme secretion in healthy dogs. In order to help dogs with pancreatitis, future research should focus on MCT as the dietary fatty acids source in dogs with clinical pancreatitis to better evaluate the potential impacts.

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APPENDIX

Table 1 Nutrient profile of diets (grams/1000Kcal)

Nutrient	Control	LCSFA	LCUFA	High MCT
Protein	116	60	60	60
Total fat	4	57	57	56
Carbohydrate	115	59	59	60
PUFA	1.4	2.7	16.1	1.7
MUFA	0.8	16.2	35.1	8.1
MCT	0	5.6	0	31.5
Saturated FA	0.8	35.2	4.5	44.5

LCSFA: long chain saturated fatty acids group; LCUFA: long chain unsaturated fatty acids group; High MCT: high medium chain triglycerides; PUFA: polyunsaturated fatty acids; MUFA: monounsaturated fatty acids; MCT: medium chain triglycerides.

Table 2 Treatment distribution and timetable

Week 1

Group	Monday	Tuesday	Wednesday	Thursday	Friday
1	High MCT			LCSFA	
2	LCSFA			Control	
3		Control			LCUFA
4		LCUFA			High MCT

Week 2

Group	Monday	Tuesday	Wednesday	Thursday	Friday
1	Control			LCUFA	
2	LCUFA			High MCT	
3		High MCT			LCSFA
4		LCSFA			Control

Table 3 cPLI mean data (ng/mL)

Analysis Variable: cPLI							
treatment	**time**	**N Obs**	**N**	**Mean**	**Std Dev**	**Minimum**	**Maximum**
LCSFA	**0**	**12**	12	46.67	26.84	30	128
	30	**12**	12	47.42	26.81	29	121
	120	**12**	12	49.50	29.71	30	138
	180	**12**	12	52.42	34.16	29	152
LCUFA	**0**	**12**	12	44.33	22.90	29	111
	30	**12**	12	44.67	25.21	29	115
	120	**12**	12	52.08	26.57	29	119
	180	**12**	12	47.92	21.86	31	103
Control	**0**	**12**	12	44.33	20.56	29	101
	30	**12**	12	46.67	32.91	29	147
	120	**12**	12	56.00	47.38	29	199
	180	**12**	12	54.58	47.90	29	196
High MCT	**0**	**12**	12	42.58	13.14	29	70
	30	**12**	12	43.33	12.77	29	70
	120	**12**	12	45.75	13.87	29	73
	180	**12**	12	44.67	12.32	31	70

Table 4 Gastrin mean data (ng/L)

Analysis Variable: Gastrin							
treatment	**time**	**N Obs**	**N**	**Mean**	**Std Dev**	**Minimum**	**Maximum**
LCSFA	**0**	**12**	12	13.59	3.85	7.07	19.7
	30	**12**	12	14.30	4.43	7.07	20.9
	120	**12**	12	14.62	3.84	7.07	20.7
	180	**12**	12	14.21	4.91	7.07	23.3
LCUFA	**0**	**12**	12	12.69	3.80	7.07	19.7
	30	**12**	12	13.36	4.71	7.07	24.4
	120	**12**	12	13.89	4.72	7.07	23.9
	180	**12**	12	13.47	5.00	7.07	21.7
Control	**0**	**12**	12	14.08	5.76	7.07	26.4
	30	**12**	12	15.71	8.45	7.07	39.4
	120	**12**	12	13.32	3.70	7.07	20.3
	180	**12**	12	12.91	4.51	7.07	20.9
High MCT	**0**	**12**	12	13.15	3.93	7.07	17.4
	30	**12**	12	14.43	4.41	7.07	20.1
	120	**12**	12	13.50	4.62	7.07	23.7
	180	**12**	12	12.25	4.16	7.07	17.2

Table 5 CCK mean data (pg/mL)

Analysis Variable: CCK							
treatment	**time**	**N Obs**	**N**	**Mean**	**Std Dev**	**Minimum**	**Maximum**
LCSFA	**0**	**12**	12	14.02	6.34	7.65	26.58
	30	**12**	12	14.31	7.08	7.53	26.11
	120	**12**	12	12.74	5.99	6.91	26.58
	180	**12**	12	13.06	7.35	6.58	30.64
LCUFA	**0**	**12**	12	13.14	5.84	7.12	26.49
	30	**12**	12	14.79	7.28	7.78	25.74
	120	**12**	12	12.36	5.90	5.51	26.11
	180	**12**	12	13.04	5.84	6.92	22.34
Control	**0**	**12**	12	13.69	6.29	7.12	26.58
	30	**12**	12	14.10	7.16	7.71	29.03
	120	**12**	12	12.87	5.71	7.10	22.81
	180	**12**	12	14.19	7.83	6.89	30.36
High MCT	**0**	**12**	12	13.12	6.25	7.65	29.04
	30	**12**	12	13.84	7.14	7.72	29.89
	120	**12**	12	12.09	5.71	7.10	25.64
	180	**12**	12	12.66	7.70	5.89	32.53

Table 6 DGGR lipase mean data (IU/L)

Analysis Variable: DGGR lipase							
treatment	**time**	**N Obs**	**N**	**Mean**	**Std Dev**	**Minimum**	**Maximum**
LCSFA	**0**	**12**	11	57.86	47.35	22.03	195.49
	30	**12**	11	57.96	48.26	22.94	197.94
	120	**12**	12	60.04	48.81	23.4	204.52
	180	**12**	12	60.64	49.66	22.64	204.67
LCUFA	**0**	**12**	12	51.21	36.75	27.84	161.23
	30	**12**	12	53.53	41.78	26.31	179.89
	120	**12**	12	56.60	40.05	29.22	174.99
	180	**12**	12	54.99	36.60	26.62	161.84
Control	**0**	**12**	12	54.48	38.99	23.86	169.33
	30	**12**	12	57.92	52.20	24.78	217.82
	120	**12**	12	66.27	64.38	24.93	257.6
	180	**12**	12	63.48	59.75	22.64	237.4
High MCT	**0**	**12**	12	52.07	26.54	22.49	110.14
	30	**12**	12	52.99	28.76	22.18	118.55
	120	**12**	12	54.14	30.10	22.18	118.7
	180	**12**	12	53.30	28.06	23.56	108.91

Table 7 Amylase mean data (U/dL)

Analysis Variable: Amylase							
treatment	**time**	**N Obs**	**N**	**Mean**	**Std Dev**	**Minimum**	**Maximum**
LCSFA	**0**	**12**	12	522.83	123.02	377	789
	30	**12**	12	507.50	117.90	373	759
	120	**12**	12	503.58	117.68	354	713
	180	**12**	12	500.42	111.21	368	689
LCUFA	**0**	**12**	12	500.33	106.23	362	693
	30	**12**	12	499.00	109.81	343	704
	120	**12**	12	498.42	106.33	349	679
	180	**12**	12	490.17	96.14	359	684
Control	**0**	**12**	12	509.17	106.45	329	725
	30	**12**	12	498.67	112.39	323	702
	120	**12**	12	505.25	114.17	358	723
	180	**12**	12	505.75	113.44	331	704
High MCT	**0**	**12**	12	525.75	126.02	363	778
	30	**12**	12	518.25	126.75	357	783
	120	**12**	12	502.08	113.99	351	722
	180	**12**	12	516.08	124.52	363	767

Table 8 Cholesterol mean data (mg/dL)

Analysis Variable: Cholesterol							
treatment	**time**	**N Obs**	**N**	**Mean**	**Std Dev**	**Minimum**	**Maximum**
LCSFA	**0**	**12**	12	212.06	36.53	143.03	283.9
	30	**12**	12	206.16	41.07	140.9	275.91
	120	**12**	12	199.36	38.13	140.5	278.44
	180	**12**	12	195.44	38.07	138.28	265.23
LCUFA	**0**	**12**	12	198.71	29.68	148.57	244.8
	30	**12**	12	199.11	33.57	148.42	246.95
	120	**12**	12	190.53	30.08	143.43	238.47
	180	**12**	12	186.48	30.20	143.99	235.39
Control	**0**	**12**	12	207.22	32.10	154.27	262.06
	30	**12**	12	198.49	33.94	145.96	247.9
	120	**12**	12	192.56	32.20	136.54	239.03
	180	**12**	12	191.41	32.77	141.13	244.25
High MCT	**0**	**12**	12	205.99	31.26	159.34	258.82
	30	**12**	12	201.33	33.04	150.55	248.76
	120	**12**	12	194.72	33.58	140.66	238.63
	180	**12**	12	196.92	32.36	137.65	246.71

Table 9 Triglycerides mean data (mg/dL)

Analysis Variable: Triglycerides							
treatment	**time**	**N Obs**	**N**	**Mean**	**Std Dev**	**Minimum**	**Maximum**
LCSFA	**0**	**12**	11	52.74	19.56	31.3	87.9
	30	**12**	11	47.94	12.60	33.15	68.51
	120	**12**	12	154.65	39.37	98.96	226.64
	180	**12**	12	136.99	43.65	68.79	246.21
LCUFA	**0**	**12**	12	50.54	15.59	31.92	88.85
	30	**12**	12	50.33	17.06	29.98	82.97
	120	**12**	12	114.22	34.94	63.85	175.09
	180	**12**	12	108.47	36.07	56.18	155.55
Control	**0**	**12**	12	55.30	16.67	34.66	89.06
	30	**12**	12	46.69	18.12	28.1	84.67
	120	**12**	12	43.18	10.84	32.23	65.02
	180	**12**	12	42.20	10.49	27.66	67.59
High MCT	**0**	**12**	12	50.33	14.94	31.23	87
	30	**12**	12	40.61	11.39	30.03	71.6
	120	**12**	12	58.90	17.04	40.9	90.33
	180	**12**	12	76.76	18.52	46	112.83

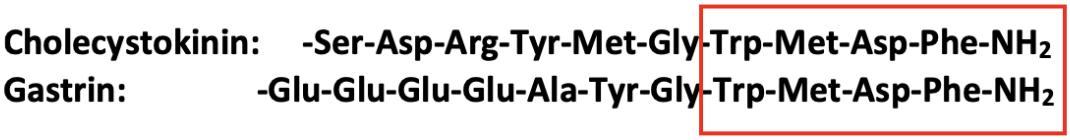

Figure 1 The homologous bioactive sequence of cholecystokinin and gastrin, modified from Jens FR et al (2007) [42]

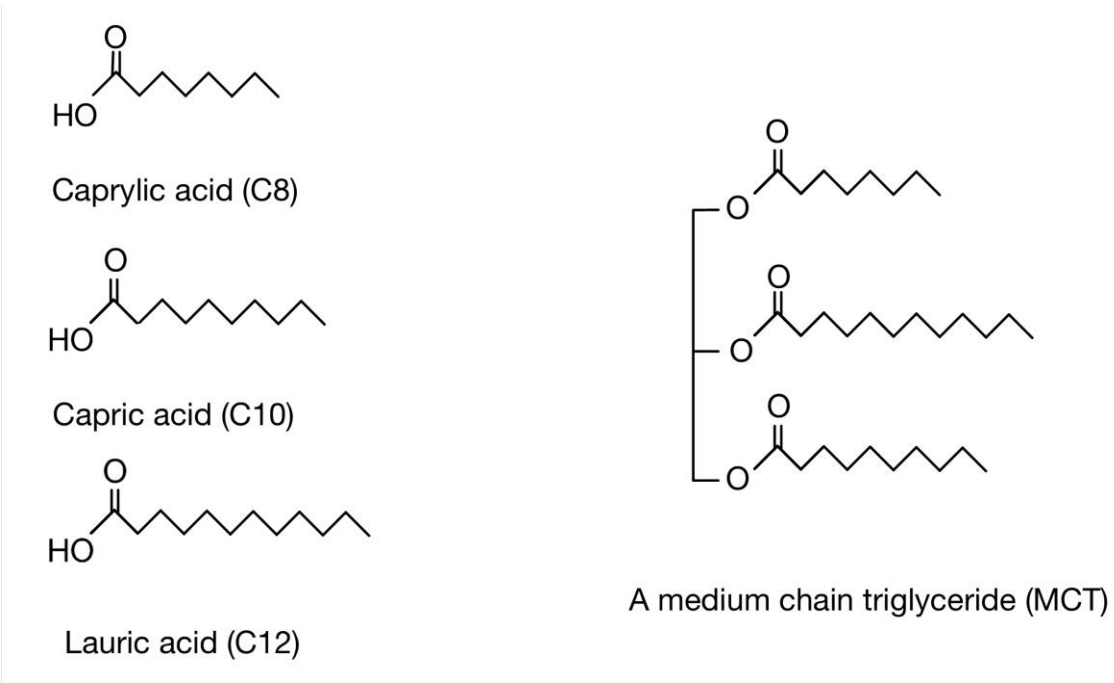

Figure 2 Structure examples of a medium chain fatty acid and triglycerides, modified from Cassiday L (2016) [108]

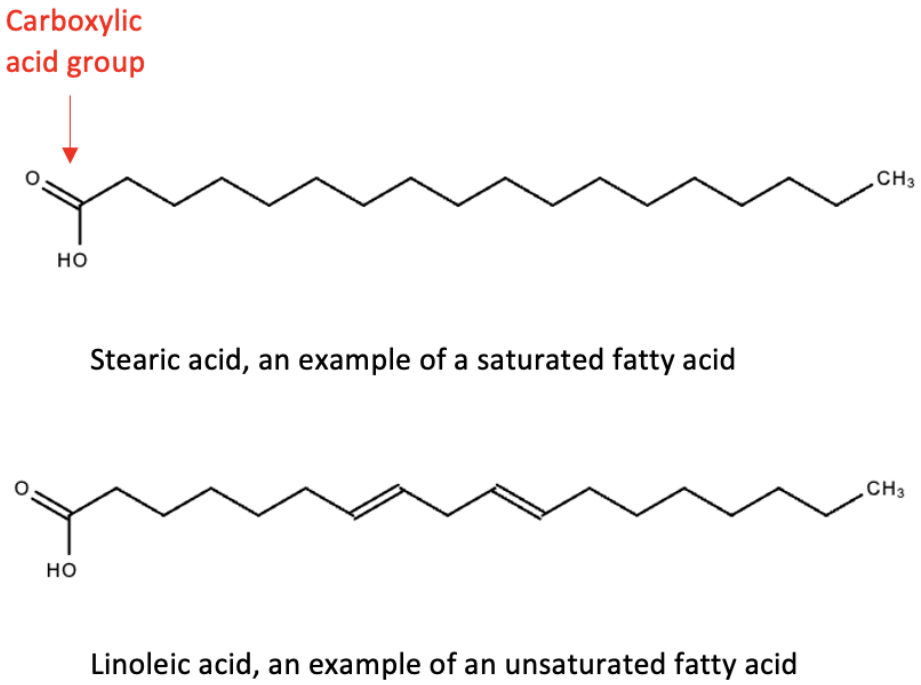

Figure 3 Structure example of long chain fatty acid, modified from Epomedicine (2022) [109]

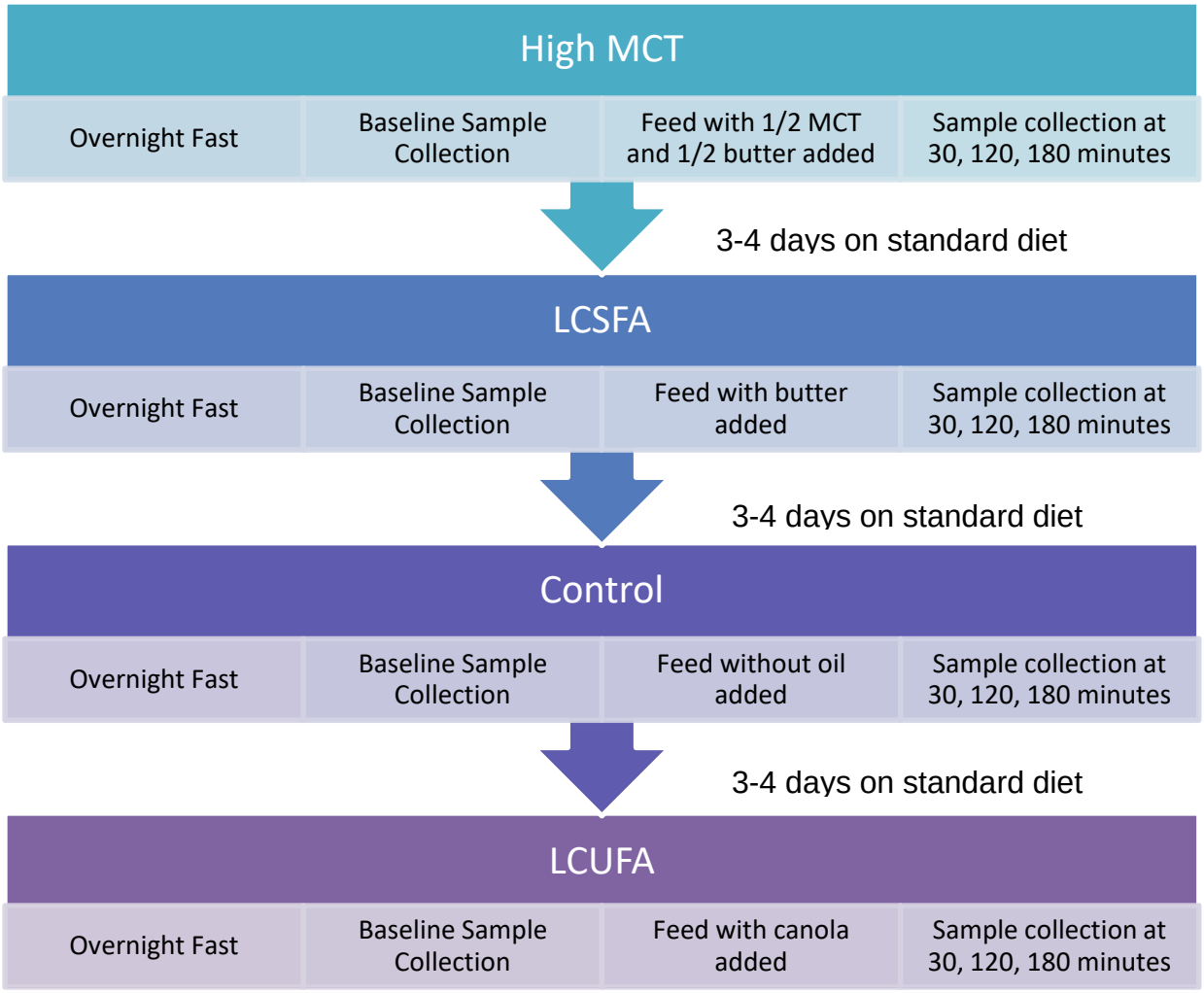

Figure 4 Flow chart process of treatments (example as Group 1)

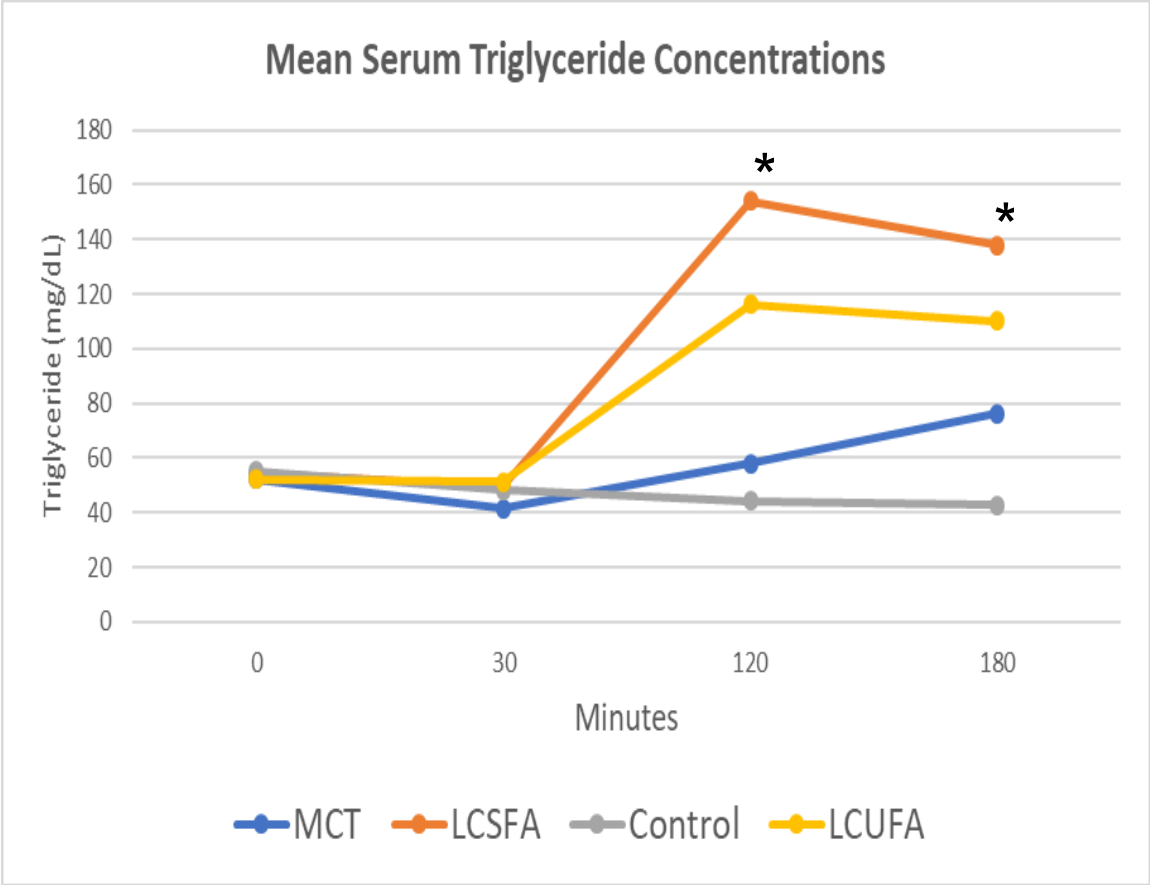

Figure 5 Mean triglycerides level of treatment groups over time: *The high MCT group had lower serum triglyceride levels than the LCSFA and LCUFA at times 120 and 180 minutes (p<.001).*

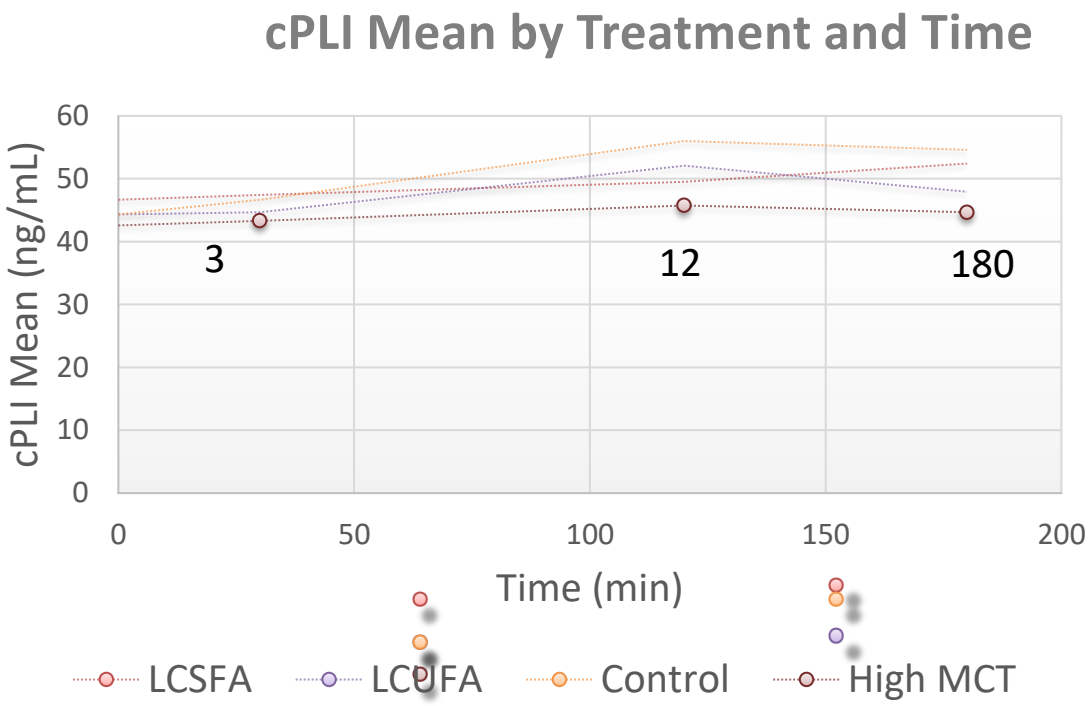

Figure 6 cPLI mean by treatment and time: There were no statistical differences in serum cPLI between treatment groups at any timepoint. However, overall cPLI concentrations demonstrated a mild increase over time with 30 minutes being lower than 120 minutes ($p<.004$).

VITA

Originally from China, Yunyi Zhang grew up in Beijing. After high school, she attended the University of Connecticut and received a Bachelor of Science degree in Pathobiology and Veterinary Science. She then attended the University of Tennessee, Knoxville to pursue her Master of Science in Comparative and Experimental Medicine with a concentration in small animal nutrition. Her research interest is focused on whether medium-chain triglycerides can decrease pancreatic enzyme release in dogs to help future pancreatitis patients with improved dietary management. She is grateful for all support from her family, professors, and friends.