University of Alberta

Starch: An Alternative Energy Source for Cats

by

Kimberley Berendt

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Abstract

Two studies examined the effects of 3 commercial cat diets varying in glycemic index (GI) based on ingredient composition and starch content (34.1, 29.5, and 23.6% NFE for high, medium, and low GI, respectively) on metabolizable energy (ME), indirect calorimetry and blood measurements. In Study 1, the modified Atwater equation underestimated diet ME by 12%. In Study 2, the RQ decreased with GI ($P<0.001$) and the REE did not change with diet. Postprandial fat oxidation was inversely related to GI ($P<0.050$). Postprandial carbohydrate oxidation decreased with GI ($P<0.001$). Interstitial glucose decreased with GI ($P<0.014$). Postprandial serum glucose did not change with diet. Postprandial serum insulin was highest for the high GI diet and lowest for the medium GI diet ($P<0.016$). The responses of cats to dietary starch levels are unique and more prolonged and less pronounced than other species such as humans or dogs.

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List of Abbreviations

°C	degree Celcius	**AGT1**	alanine:glyoxylate aminotransferase 1
cal	calorie	**Arg**	arginine
kcal	kilocalorie	**BCS**	body condition score
g	gram	**BW**	body weight
mg	milligram	**CFU**	colony-forming units
kg	kilogram	**CCK**	cholecystokinin
m	meter	**CP**	crude protein
cm	centimeter	**CSS**	complete sum of squares
L	liter	**Cys**	cysteine
dL	deciliter	**DE**	digestible energy
s	second(s)	**DM**	dry matter
min	minute(s)	**DMI**	dry matter intake
h	hour(s)	**e.g.**	example given
d	day(s)	**Eq.**	equation(s)
wk	week(s)	**EUN**	endogenous urinary nitrogen
yr	year(s)	**FE**	fecal energy
n	sample size	**FEO**	fecal energy out
μIU	micro International Units	**FOS**	fructooligosaccharide(s)
AA	amino acid(s)		
ADFI	average daily feed intake		

GaE gaseous energy

GE gross energy

G:F gain-to-feed ratio

GI glycemic index

GL glycemic load

Gln glutamine

Glu glutamic acid

Gly glycine

IL interleukin

LPL lipoprotein lipase

ME metabolizable energy

MED metabolizable energy density

Met methionine

mRNA messenger ribonucleic acid

n-3 FA omega 3 fatty acid(s)

n-6 FA omega 6 fatty acid(s)

NE net energy

NFE nitrogen-free extract

NPN non-protein nitrogen

NRC National Research Council

NSP non-starch polysaccharide(s)

OM organic matter

P phosphorous

RQ respiratory quotient

rRNA ribosomal ribonucleic acid

SAS Statistical Analysis System

SCFA short-chain fatty acid(s)

Ser serine

TDF total dietary fiber

U enzyme unit

UE urinary energy

vs. versus

Chapter 1: Literature Review

1.1 Introduction

"Raw" and "natural" are terms that are often used in pet food commercials, and a consumer-driven trend towards pet foods that are organic and less processed is currently growing in the pet food industry. However, in cats, a grain-free diet might not be as beneficial as advertized when compared to a commercial diet that includes carbohydrate sources. Although the current paradigm indicates that the domestic cat is a carnivorous species (Verbrugghe et al., 2011), recent research has demonstrated the physiological ability of felines to successfully digest and metabolize starch (de-Oliveira et al., 2008). Addition of carbohydrate to the diet of a carnivorous species, however, has not been thoroughly studied. In cats, dietary starch has been blamed for obesity and increased fecal output, yet little data is available to support this proposed cause-and-effect relationship (Vester, 2010). Recent results indicate that addition of dietary starch may complicate glucose control in diabetic cats (de-Oliveira et al., 2008), but starch inclusion also has some notable positive effects on healthy cats. These effects are mainly seen in the examination of gastrointestinal microflora populations (Hooda et al., 2013), which in turn benefit a multitude of other bodily systems (Reddy, 1999; Roberfroid, 2000; Swanson et al., 2002). High carbohydrate diets have even been prescribed for feline patients with severe pancreatitis (Kirk, 2006). In total, it is unclear what the optimum

amount and type of carbohydrate are for the domestic cat to optimize health and wellbeing.

A certain level of carbohydrate inclusion is required for a dry, extruded diet in the form of kibble, which is highly convenient for the consumer. The starch content allows the kibble to keep its shape and texture (Forrester and Kirk, 2009). The process of extrusion, drying and enrobing is the most cost effective way presently available to produce a microbially-stable product that can handle storage and transport for an extended period of time. Adding to the cost effectiveness is that carbohydrate ingredients are cheaper than protein ingredients. These effects are then passed down to the consumer, who benefits not only from the money saved but also from the convenience of storage and shelf life (Forrester and Kirk, 2009) and the confidence of a more microbially-stable product vs. feeding raw or homemade diets (Weese et al., 2005).

Despite strong arguments within the industry regarding the negative impact of addition of carbohydrates to feline diets, and the absence of a tolerable upper limit of dietary carbohydrates, new research is ongoing to establish the mechanisms of carbohydrate metabolism in cats. As we build on our scientific understanding, functional uses of carbohydrates in cats are being discovered. This literature review will explore general carbohydrate composition, digestion and metabolism of carbohydrates by cats, carbohydrate requirements of cats, and effects of carbohydrate addition to grain-free diets.

1.2 Carbohydrate composition

Carbohydrates are molecules containing the chemical structure $C_x(H_2O)_Y$ (BeMiller and Huber, 2008). The main carbohydrates in the diet of humans are polysaccharides and oligosaccharides, which are broken down to form monosaccharides. Members of the carbohydrate family include starch, fiber, non-starch polysaccharides (cellulose, hemicellulose, pectin), and other unique categories of carbohydrates. These categories do not mutually exclude each other, and will be described in detail in the following sections.

1.2.1 Sugars

Sugars are the end products of carbohydrate digestion, or may alternatively be ingested directly. Sugars include the small molecule monosaccharides, e.g., glucose, galactose, and fructose, and disaccharides, e.g. lactose and sucrose (Slade and Levine, 1989; Biliaderis, 1990).

1.2.2 Starch

Starch is a category of carbohydrate unto itself, due to its unique chemical and physical structure. Starch is made up of granules, which only partially hydrate in water (Slade and Levine, 1989; Biliaderis, 1990), making them useful as adhesives, to add structure to foods, retain moisture, or thicken solutions. Starch may be gelatinized by cooking or heating in water, which makes it more digestible (Slade and Levine, 1989; Biliaderis, 1990). Gelatinization includes granule swelling and loss of structural order that, if

exaggerated, is irreversible and creates complete disruption of order within the starch matrix (Biliaderis, 1990; Slade and Levine, 1989). Starch is the primary energy reserve in plants, and is comprised of 2 main components: amylose and amylopectin.

Amylose is a linear chain of 1-4 linked α-D-glucopyranosyl units that may contain some branches connected to the linear chain via α-D 1-6 linkages (Whistler et al., 1984; Wurzburg, 1986). This creates a helical shape in the physical structure of starch, with a hydrophobic inner core (Figure 1.1). Other molecules may associate with this core, forming complexes. Most starch sources contain 20 to 30% amylose (Cummings and Englyst, 1995), with some starches, such as high-amylose corn starch, having greater amounts, from 50 to 70% (Whistler et al., 1984; Wurzburg, 1986).

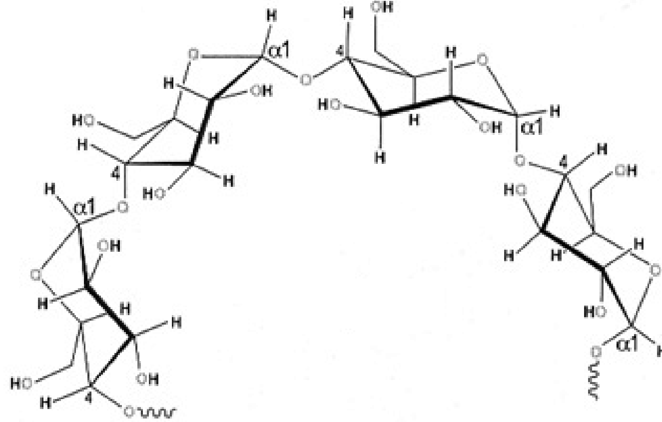

Figure 1.1. Amylose: A molecular representation of the two components of the starch molecule (Adapted from Helmberger, 2009).

Amylopectin is a very large, multi-branched compound that forms a double helical structure (Figure 1.2). Amylopectin might be the largest naturally-occurring molecule (Whistler et al., 1984; Wurzburg, 1986) and compromises up to 70 to 80% of total starch (Cummings and Englyst, 1995). Certain compounds, such as waxy starches, have much higher amounts by definition, due to their very low amylose content of less than 2% (Whistler et al., 1984; Wurzburg, 1986).

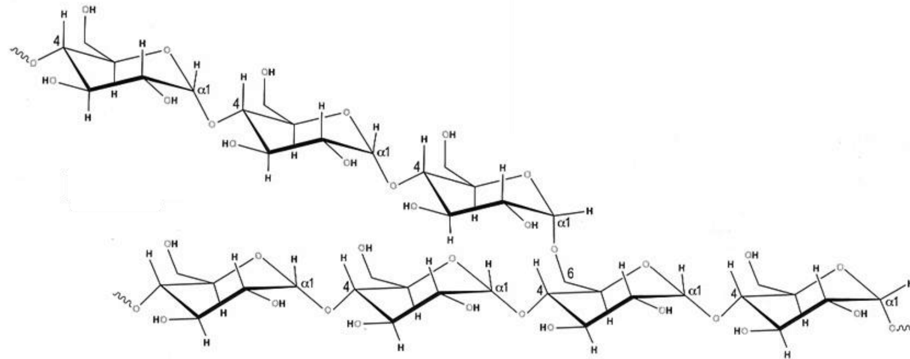

Figure 1.2. Amylopectin: A molecular representation of the two components of the starch molecule. (Adapted from Helmberger, 2009).

In starch, amylopectin double helices pack together to create a crystalline structure, while the amylose-rich amorphous regions are less ordered (Zobel, 1988). These alternating layers of crystalline (amylopectin) and non-crystalline (amylose) regions arrange in a radial formation, forming a starch granule structure (Zobel, 1988). Granules vary in shape depending on grain source, which creates variation in nutrient composition and digestibility (Zobel, 1988). Besides grain source, starch digestibility depends on amylose to amylopectin ratio, interaction of the starch granule with protein, cellular

structure and strength of the grain, enzyme inhibitors, and a variety of other factors (Rooney and Pflugfelder, 1986). High amylopectin starches are generally more digestible than high amylose starches (Rooney and Pflugfelder, 1986). All starches will include trace amounts of protein, ash and lipid.

Starches can be modified chemically and physically to improve processing characteristics such as gelatinization. Esterification and etherification, and combinations of these chemical reactions, can modify the functionality of food starches. This can include cross-linking starches to increase gelatinization temperature, and create a more pH-stable starch that can be more easily used for pastes (BeMiller, 1993; Whistler et al., 1984; Wurzburg, 1986). Starches may also be stabilized to become less likely to retrograde and to improve freeze-thaw stability (BeMiller, 1993; Whistler et al., 1984; Wurzburg, 1986). Manipulation of these properties is useful to reduce cooking time, increase solubility, and change viscosity.

Starch is classified as rapidly digestible, slowly digestible, or resistant, depending on a variety of factors such as grain size, cooking, and chemical modification (Spears and Fahey, 2004). Rapidly and slowly digestible starch are almost completely digested in the small intestine at different rates (quickly vs. slowly, respectively), while resistant starch, predictably, is resistant to small intestinal digestion (Cummings and Englyst, 1995). The extrusion process used in most pet foods increases starch digestibility, providing additional nutritional components to the animal; however, a small

portion of resistant starch remains, which can increase if retrogradation occurs (Spears and Fahey, 2004). Resistant starch may have similar health benefits to fiber, including increased bacterial fermentation and production of short-chain fatty acids (SCFA), which reduce intestinal pH, prevent colonization by some pathogenic microorganisms, and promote gut motility and mucus production (Spears and Fahey, 2004). In canines, in vitro ileal fermentation of legumes containing high resistant starch (24.7%) increased SCFA concentration (7.8 mmol/g OM fermented) compared to cereal grains (5.9 mmol/g OM fermented), flours (5.0 mmol/g OM fermented), grain-based food products such as pasta and rolled oats (3.9 mmol/g OM fermented) and reference substrates corn starch, potato starch, and amylomaize (3.6, 0.64, and 0.8 mmol/g OM fermented, respectively) (Bednar et al., 2001). Studies on resistant starch have not yet been completed using cats, but are likely to be different given the gross morphological differences in the colon.

Notably, specific effects vary between starch sources. Similar to fiber, increased bacterial mass will increase fecal bulk, which may be undesirable to some pet owners (Spears and Fahey, 2004). Fecal bulk increased by 90 g in dogs fed a diet containing resistant starch compared to slowly digestible starch (42.6 vs. 76.1% total tract digestibility, respectively) (Murray et al., 1998). While the specific health-promoting effects of resistant starch have mainly been demonstrated in humans, similar effects could be hypothesized in dogs. However, knowledge on effects of starch in cats is limited and requires further investigation.

1.2.3 Fiber

Fiber is defined as any plant substance that goes through the small intestine mostly undigested (Cho and Dreher, 2001), and includes resistant starch, prebiotics, and non-starch polysaccharides (NSP). The term non-starch polysaccharide (NSP) is sometimes used interchangeably with the term fiber (Englyst, 1989); however, fiber would more closely be defined as the sum of lignin and NSP (Montagne et al., 2003; Rodriguez et al., 2006). NSP are substances such as cellulose, hemicellulose and pectin that form gels or gums, which can be used to improve food functionality (Cho and Dreher, 2001; FAO, 2002). Dietary fiber is a common ingredient used to add bulk to feed and serves to improve intestinal health by maintaining rate of motility within the gut, preventing constipation and increasing satiety. Soluble fiber is also useful in preventing loose stools (Cho and Dreher, 2001). Fiber can be classified as soluble and insoluble.

Soluble fiber can be fermented in the intestinal tract, producing gas and SCFA while lowering pH and preventing the growth of some harmful microorganisms (Cho and Dreher, 2001). β-Glucans, a type of soluble fiber mostly found in barley and oat bran, lower serum cholesterol in humans through a combination of decreased absorption of bile acids, interference with fat and cholesterol absorption, and delayed gastric emptying (Queenan et al., 2007). β-Glucans also lower serum blood glucose postprandially, which is useful for individuals with diabetes (Rahar et al., 2011).

Insoluble fiber is unfermented in the digestive tract, thus promoting gut motility, an effect that decreases the risk of colon cancer in humans (Kritchevsky, 1986). Insoluble fiber may also decrease blood cholesterol levels in humans through their bile acid reducing action, lowering the risk for cardiovascular disease (Kritchevsky, 1986). In cats, the increased excretion of bile acids would increase the excretion of taurine, an amino acid conjugated to bile acids. This effect of certain levels of fiber may be detrimental for cats, as taurine is crucial to prevent dilated cardiomyopathy and retinal degeneration (Stratton-Phelps et al., 2002).

Cellulose is a very high molecular weight compound containing linear repeating β-D-glucopyranosyl units and is classified as a non-starch polysaccharide (Young and Rowell, 1986). This change in base structure from starch makes cellulose insoluble and unable to be digested in non-ruminant species and is thus categorized as fiber. Cellulose molecules can combine to create polycrystalline, fibrous bundles and, like the granule structure of starch, will form crystalline and amorphous regions (Young and Rowell, 1986). Cellulose is often used to add bulk to low-calorie baked goods.

A unique category of fiber are prebiotics, which are specific fermentable components of fiber that selectively promote the growth of beneficial microorganisms in the gut (Biradar et al., 2005). Examples of commonly used prebiotics include inulin and fructooligosaccharides (FOS). Prebiotics have beneficial health effects for the host, with potential anticarcinogenic properties (Reddy, 1999), blood glucose regulation (Roberfroid, 2000), and

antibiotic effects by supporting a healthy microbial flora (Swanson et al., 2002). Both inulin and FOS have a glycemic index of zero, and can improve the texture in food products (Flamm et al., 2001).

1.3 Carbohydrate metabolism in cats

Limited information exists on the mechanics of carbohydrate digestion and metabolism in cats. This is likely because cats are considered obligate carnivores due to the identification of unique nutrient requirements such as taurine and arachidonic acid, both of which are only found in animal-based proteins. Much more information is available regarding carbohydrate metabolism of omnivorous species, which can be used to tentatively hypothesize effects on carnivorous species.

1.3.1 Starch

1.3.1.1 Taste receptors

Cats and other obligate carnivores do not respond to foods that have a sweet taste, a trait that differentiates them from herbivores and omnivores (Li et al., 2005); thus, the cat is less inclined to eat foods containing high amounts of sugars. The basis for this trait may lie at the molecular level, specifically 2 G-protein coupled receptors, T1R2 and T1R3, which form a dimer to create the sweet taste receptor (Li et al., 2005). While cats have a functional T1R3 used to recognize umami taste associated with meat, the T1R2 receptor is considered an unexpressed pseudogene (Li et al., 2005).

The cat is thus unable to taste sweetness, a trait that was not important in evolution, because the domestic feline still remains mostly a carnivore today.

1.3.1.2 Transporters

Cats absorb glucose similarly to other species, mainly through active Na^+-dependent transport but also through passive Na^+-independent transport in the small intestine (Washabu et al., 1986). In adult cats, maximal lactase activity occurs in the jejunum, followed by the duodenum and ileum, but the activities of sucrase, maltase and isomaltase increase from duodenum to jejunum and ileum (Figure 1.3; Kienzle, 1993b).

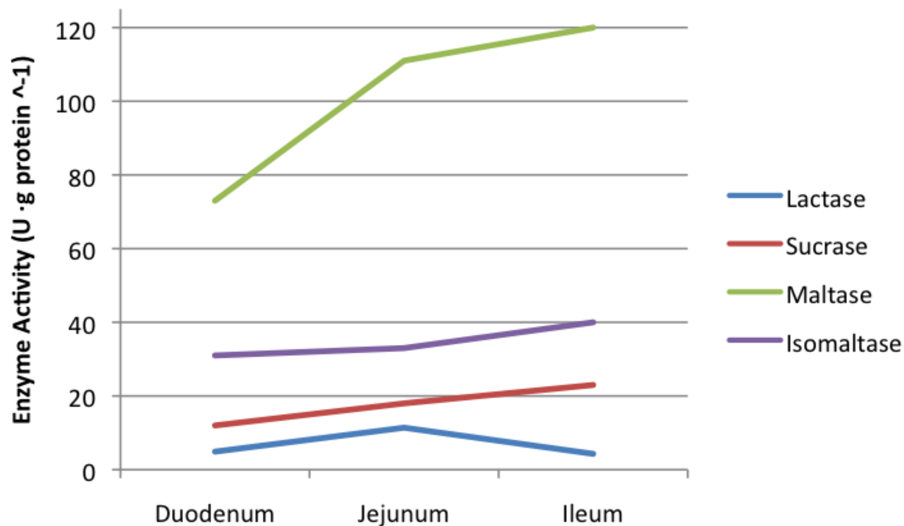

Figure 1.3. Adult feline changes in enzyme levels through the small intestine.

Conflicting information exists on whether or not glucose tolerance in dogs decreases with age due to changes in enzymatic activity. Older dogs have increased plasma glucose concentrations (97.0 ± 2.3 mg/dL) and decreased

glucose clearance rates (1.9 ± 0.5 mg/L min) compared to younger dogs (91.1 ± 2.4 mg/dL and 2.9 ± 0.4 mg/L min, respectively), leading to decreased glucose tolerance in aged canines (Strasser et al., 1993). In contrast, plasma glucose concentrations of older vs. younger dogs did not differ in similar experiments (Lowseth et al., 1990; Sheffy et al., 1985; Fukuda et al., 1989). The best way to determine whether glucose tolerance in dogs does in fact decrease with age may be to test glycemic response with an oral or intravenous glucose tolerance test, rather than monitoring fasting glucose levels over time.

1.3.1.3 Enzymes

In both kittens and puppies, lactase activity is high while nursing and decreases at weaning (Kienzle, 1988). In felines, lactase activity decreases from 96 ± 66 U g protein^{-1} at less than 1 week of age to 7 U g protein^{-1} in adult cats (Kienzle, 1993b), while adult levels of lactase are seen in as few as 29 days in puppies (Welsh and Walker, 1965). Maltase activity in kittens is inversely related to lactase, increasing with age (from 66 ± 77 U g protein^{-1} at less than 1 week of age to 102 ± 58 U g protein^{-1} in adult cats) (Kienzle, 1993b).

In dogs, intestinal activities of lactase, sucrase and maltase are 1.5 to 4.5 times those of cats (Batchelor et al., 2011). Sucrase activity is low to nonexistent in the feline intestine compared to other species, resulting in inability to digest moderate amounts (36% of diet) of sucrose, leading to

diarrhea (Meyer and Kienzle, 1991). Cats are also unable to digest galactose sufficiently. When fed moderate amounts of galactose (5.6 g/kg BW per d, 39% of diet), toxicity effects were evident at much lower levels than those shown by other laboratory animals (Kienzle, 1994). This is due to the inhibition of carbohydrate metabolism, particularly glucose-6-phosphatase, through accumulation of galactose-1-phosphate (Kienzle, 1994). For these reasons, it is best to avoid ingredients containing high levels of glucose and galactose, such as gravies, in cat diets.

One major difference between dogs and cats is that cats lack significant glucokinase, the enzyme that allows glucose to enter a cell and be phosphorylated (Tanaka, 2005). Compared to dogs, cats use less efficient enzymes, including hexokinase, to phosphorylate glucose; however, increased hexokinase in cats allows the process to proceed sufficiently (Tanaka, 2005). Other enzymes also influence the metabolism of starch in mammals; however, there is a dearth of information on cats and more study is required. For example, the slow metabolism of fructose in cats may indicate insufficient fructokinase in addition to glucokinase (Kienzle, 1995).

In cats, the highest pancreatic amylase activity is found in the jejunum (26.4 ± 29.4 U g protein^{-1}), followed by the ileum (16.2 ± 14.0 U g protein^{-1}) and colon (13.0 ± 12.2 U g protein^{-1}), with lowest activity in the duodenum (8.7 ± 6.8 U g protein^{-1}) (Kienzle, 1993a).

In the duodenum, pancreatic α-amylase will cleave the starch molecule into disaccharides, trisaccharides and branched α-dextrins (Gray, 1992).

Dogs have increased amylase activity compared to their feline counterparts, and their pancreatic amylase seems to be more sensitive to starch as well (Kienzle, 1988, 1993a). Cats do not have salivary amylase, contrary to other species (Kienzle, 1993a).

1.3.1.4 Starch Source

Starch source has a large effect on digestibility, as some plant species, such as legumes, have very low starch digestibility due to their granule structure and the difficulty with which amylase can penetrate (Englyst et al., 1992). Physical and chemical processing affects starch digestibility in cats differently. Processing increases starch surface area, allowing increased access for enzymes such as amylase. However, extreme processing, such as heating with an excess of water, may cause gelatinization, resulting in retrogradation and poor digestibility (Berry, 1986). In cats, apparent digestibility of starch increased from 92-97% through grinding of wheat starch, and cooking corn starch increased digestibility from 79-88% (Morris, 1977a). Grinding extent is also critical to starch digestibility. Cats had a greater apparent starch digestibility coefficient (0.794 vs. 0.937) for coarsely ground maize compared to finely ground maize; coarsely ground maize coefficient increased to 0.881 when cook was added (Morris et al., 1977b). This is comparable to the dog's ability to digest starch at close to 100% (NRC, 2006).

High temperature extrusion decreases resistant starch (1 to 21% of total starch content of extruded corn, potato, rice and sorghum vs. 21 to 69% in native substrates) and increases rapidly digestible starch (69 to 92% of total starch content of extruded corn, potato, rice and sorghum vs. 25 to 48% in native substrates) content of cereal grains, thereby increasing digestibility in dogs (Murray et al., 2001).

1.3.1.5 Effect on other nutrients

Addition of carbohydrate in feline diets may also affect the digestibility of other nutrients. Kienzle (1994) added 36% sucrose to a feline diet and showed an increase in apparent digestion of both magnesium (52.6 ± 21.2% vs. 17.8 ± 7.1%) and phosphorus (61.2 ± 23.2% vs. 23.1 ± 9.2%) compared to cats fed carbohydrate-free diets consisting mainly of meat meal and animal fat. Further, apparent protein digestibility was decreased (77.1 ± 3.2% vs. 86.9 ± 1.9%, respectively) when cats were fed raw corn starch compared to a carbohydrate-free diet (Kienzle, 1994).

An optimal starch intake level has not yet been established for the cat, however ongoing research will yield results that will optimize the benefits of starch addition to the feline diet without causing symptoms of excess.

1.3.2 Fiber

In monogastric species, fiber is ingested and passes through the stomach and small intestine largely intact until it reaches the distal intestine where it

is fermented by microbes, causing the release of gasses and SCFA (Roberfroid, 1993). Most SCFA are metabolized by the liver, while some may be converted to other molecules; for example, propionate's conversion to succinyl coenzyme A (Roberfroid, 1993). Lactate serves as a precursor for gluconeogenesis (Delzenne and Roberfroid, 1994), the process primarily used by cats to generate energy. Very high SCFA concentrations in the feline gut may result in watery feces and increased output, indicating that the cat has a limit to the amount of fiber that can be fermented; however, moderate SCFA concentrations decrease fecal moisture content (Sunvold et al., 1995a) and are therefore beneficial to health.

Cats do not possess a functional cecum and have a very small colon compared to other monogastric species, resulting in a limited capacity to digest fiber (Sunvold et al., 1995a). Even so, multiple studies using both *in vivo* and *in vitro* techniques suggest that cats ferment fiber to a similar extent as other monogastric species (Sunvold et al., 1995b). As such, cats may be able to utilize non-starch polysaccharides just as readily as dogs (Sunvold et al., 1995b). In fact, cats supplemented with fructooligosaccharides had decreased populations of the pathogenic bacteria *Escherichia coli* (75% mean reduction) and *Clostridium perfringens* (98% mean reduction) in their feces compared to dogs fed the same diet (Sparkes et al., 1998). Thus, fermentation of resistant starch is likely similar in cats as other monogastric species.

Similar to humans and dogs, fiber may be used in cats for weight control. The increased bulk in the diet has a satiating effect while decreasing the

caloric intake (Cho and Dreher, 2001). Similarly to starch, however, the source of fiber strongly affects the extent of breakdown. Combinations of rapidly fermentable fiber sources have shown adverse effects in cats attributed to bacterial overgrowth in the small intestine, decreased pancreatic enzyme activity due to a decrease in intestinal pH, and decreased nutrient absorption due to the bulky nature of the fiber (Sunvold, 1995b). The majority of fiber and prebiotic research within the companion animal industry has been completed on canines, and there is still a wealth of information to be gathered regarding fiber fermentation in cats.

1.4 Carbohydrate incorporation in feline diets

Recently, researchers hypothesized that increased carbohydrates in cat food would increase adipose tissue and body mass of feline offspring, and performed one of the first *in utero* comparisons of cats fed high protein (HP) (52.9% CP, 23.5% fat, 10.8% digestible carbohydrate) and high carbohydrate (HC) (34.3% CP, 19.2% fat, 30.8% digestible carbohydrate) diets (Vester et al., 2009a). When 8 pregnant queens were divided into 2 groups and fed either the HP or HC diet, there was no difference in birth weight observed between kittens (Vester et al., 2009a; Lauten et al., 2000). There was also no difference in body condition scores in kittens at 8 months of age after they had been weaned onto the diet of their mother and fed ad libitum (23.50% fat HP vs. 19.23% fat HC) (Vester et al., 2009b; Lauten et al., 2000).

In another study, weight gain after spaying, often blamed on carbohydrate-containing pet foods, was challenged by researchers to be due to other underlying causes instead of carbohydrate content of diet (Vester et al., 2009a; Belsito et al., 2009). Adult cats (n=8) were spayed after a 4-week baseline period, and their food intake was subsequently altered to maintain body weight until week 12. After this period cats were allowed to *eat ad libitum* for 12 weeks. Instead of being a direct result of carbohydrate intake, weight gain after spaying appeared to be due to an increase in food intake and decrease in activity level (30 ± 1.8 mean activity counts per 0.25 min during 0 to 12 weeks vs. 14.2 ± 1.8 during 12 to 24 weeks). This study found that a 30% caloric restriction was necessary to prevent weight gain after ovariohysterectomy (Belsito et al., 2009). These findings indicate that other factors may be involved in feline weight gain, including an alteration of hormone secretions and hormonal reactions after spaying (Vester et al., 2009a). These include a decrease in leptin, the hormone responsible for satiation, and adipose mRNA abundance of lipoprotein lipase (LPL), a regulator of lipid storage and blood lipid concentrations. A subsequent increase in adipose mRNA interleukin-6 (IL-6) expression (which is inhibited by LPL) is indicative of obesity, however this study suggests that it may indicate an increase in proinflammatory cytokine production that is not linked to weight gain (Figure 1.4;Belsito et al., 2009).

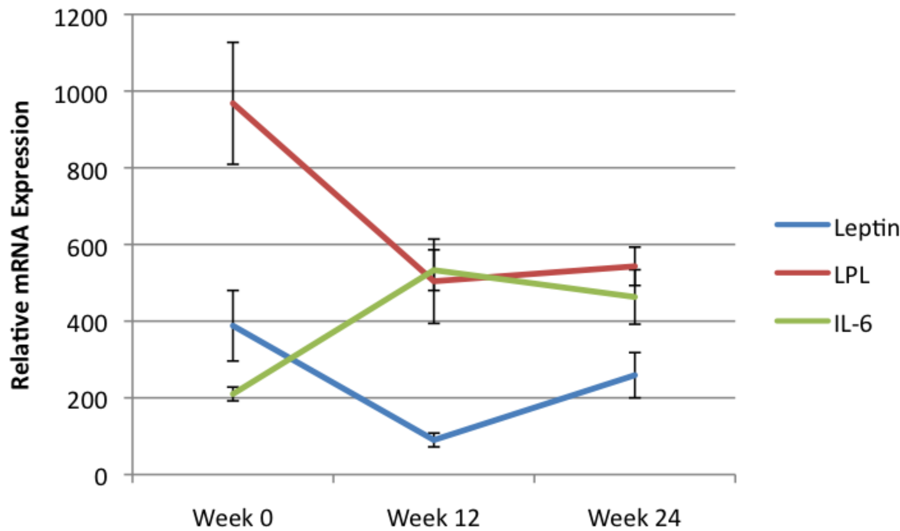

Figure 1.4. Relative mRNA expression at week 0, 12, and 24 of leptin, lipoprotein lipase (LPL), and interleukin-6 (IL-6). Food was restricted from weeks 0-12 and fed ad libitum from weeks 12-24.

Inclusion of carbohydrates in diets may also benefit the health of some cats. A high carbohydrate, moderate protein, low fat diet is currently the prescribed intervention for cats with severe pancreatitis (Kirk, 2006). Cats with pancreatitis have impaired pancreatic enzyme secretion, releasing active enzymes that begin to digest the organ instead of their inactive forms that will be activated in the small intestine (Friess et al., 1998). High dietary protein levels stimulate cholecystokinin (CCK), the peptide hormone responsible for initiating pancreatic enzyme secretion for the digestion of protein, amino acids, and fat. CCK is a key factor in the control of enzyme release from the pancreas (Ma and Szurszewski, 1995) and increased

pancreatic enzyme release may increase inflammation of this organ. This condition can also proceed to pancreatic necrosis in cats (Zhao et al., 1998).

Raw, grain-free pet food can increase risk of illness due to pathogens, which could also harm the pet owners if they become infected by handling the food, bowl, or feces (Weese et al., 2005). The lack of heat processing of these raw meat diets may allow potential pathogens to remain in pet food. In 25 commercially available raw diets (13 for dogs, 8 for cats, 4 unspecified), coliforms were present in 100% of samples (used as an indication of sanitation; counts ranging from $3.5 \cdot 10^3$ to $9.4 \cdot 10^6$ CFU/g), *Escherichia coli* was present in 64% of samples (16 diets), monophasic *Salmonella Typhimurium* was detected in 20% of samples (5 diets), *Clostridium perfringens* was found in 20% of samples (5 diets), and *Staphylococcus aureus* was found in 4% of samples (1 diet) (Weese et al., 2005). These pathogens may harm both humans and pets at small dosages, and given the mostly unsubstantiated nature of health claims of raw pet diets, this risk should be taken strongly into consideration (Weese et al., 2005).

In addition to the harmful bacteria found in raw pet diets, high protein/low carbohydrate diets may alter the natural intestinal microflora. Hooda et al. (2013) demonstrated the microbiological changes that occur in cats' feces due to dietary carbohydrate and protein fraction alterations. This study used kittens, since gut colonization occurs very soon after birth and in humans is considered stable and identical to adults at 2 years of age, predicting lifelong gut health. The study presumed this process to be similar

in the feline species. Kittens were fed 1 of 2 diets immediately after weaning: high protein, low carbohydrate (HPLC) (52.9% CP, 2.0% TDF) or moderate protein, moderate carbohydrate (MPMC) (34.3% CP, 6.9% TDF). The kittens were assigned identical diet as their mothers; feces were sampled at 8, 12, and 16 weeks of age for analysis via 16S rRNA gene pyrosequencing. The kittens fed HPLC had lower levels of the health promoting bacteria *Bifidobacteria* (0.06, 0.04, 0.10% of sequences), *Megasphaera elsdenii* (0.01, 0.10, 1.80%), a butyrate producing bacteria that has been used as a probiotic in other species, and *Lactobacilli* (1.49, 0.08, 0.05%) in their feces. These results indicate that a diet high in protein may reduce host intestinal health, and future health of the cat, since a high protein diet fed at an early age would set the stage for lifelong intestinal microflora. The kittens fed MPMC had greater levels of *Megasphaera* (17.9, 33.0, 23.9% of sequences), and higher levels of *Lactobacilli* (7.0, 0.1, 0.8%) and *Bifidobacteria* (12.0, 17.6, 20.8%) that had positive effects on intestinal structure and integrity and immune system development with early colonization. This study indicates that at least in kittens, a high protein diet is not optimal, and a moderate protein diet is better for healthy intestinal bacterial colonization.

Obesity and potentially resultant type II diabetes affect an increasing proportion of domestic cats in North America. Research is ongoing to define the causes and management options for these conditions. In the United States, 35% of adult cats are overweight, with 6.4% classified as obese (Lund et al., 2005). Type II diabetes mellitus is most common in cats, with an

incidence of 0.25 to 2% in Australian cats (Rand et al., 2004). The cause of diabetes development includes a variety of factors, including environmental effects, age, activity, and genetic predisposition, as evidenced in the high incidence in the Burmese cat (Rand et al., 2004). As with diabetic humans, blood glucose and insulin response must be controlled, limiting high glucose spikes postprandially. Thus, high glycemic index grains such as rice should be limited as they cause spikes in blood glucose levels postprandially, are not satiating and thus lead to hunger and weight gain, and may cause pancreatic β-cell exhaustion (Rand et al., 2004). Cats at risk for diabetes development may benefit from a diet high in protein, low in starch and moderate in fat (Rand et al., 2004), while diabetic cats should be fed very low starch diets to reduce hyperglycemia and the subsequent insulin dose (Rand et al., 2004). Besides limited starch, diets with a moderate level of soluble fiber may benefit diabetic cats, as fiber addition may help delay gastric emptying and promote satiety. Dietary prebiotics may also benefit overweight cats. Dietary prebiotics (inulin and oligofructose) may control glycemic response in obese domestic cats through reducing hepatic gluconeogenesis and enhancing glycolysis (Verbrugghe et al., 2009).

1.5 Impact of dietary carbohydrates in cats

Effects of 6 carbohydrate sources on postprandial glucose and insulin responses were recently studied in dogs and cats, providing a direct comparison on starch digestion between the 2 species (de-Oliveira et al.,

2008; Carciofi et al., 2008). Carbohydrate sources included cassava flour, brewers rice, corn, sorghum, peas, and lentils; all diets were formulated to contain 35% starch through the addition of soybean protein, poultry by-product and poultry fat (de-Oliveira et al., 2008). Fiber content varied among the six diets, but salt, vitamins and minerals were constant.

Cats were able to digest finely ground, cooked starch in all diets at a rate of 93%, only slightly lower than dogs at 98% (de-Oliveira et al., 2008; Carciofi et al., 2008). Interestingly, corn caused the greatest increase in postprandial glucose response in cats (mean 10.2 mg/dL, maximum incremental concentration 24.8 mg/dL) and was also the only carbohydrate source that stimulated a postprandial insulin response (mean 18.4 μIU/mL, maximum incremental concentration 32.2 mg/dL) (de-Oliveira et al., 2008). Results were similar for dogs, with corn, brewers rice, and cassava flour having the most increased postprandial glucose and insulin responses (Carciofi et al., 2008).

Differences in digestibility and postprandial glucose/insulin responses among the 6 carbohydrate sources were attributed to differences in composition among sources, including grain structure and fiber content, because increased fiber decreased starch content (de-Oliveira et al., 2008). Brewers rice and cassava flour are hulled and polished, resulting in lower total dietary fiber. Legume starches generally contain more amylose than cereals (de-Oliveira et al., 2008), which was inversely correlated to glucose response in both cats and dogs (Carciofi et al., 2008; de-Oliveira et al., 2008).

In felines, postprandial glucose and insulin response was affected less by starch compared to canines or humans (de-Oliveira et al., 2008). De-Oliveira et al. (2008) proposed that the cat's metabolism slows and prolongs starch digestion due to the lack of glucokinase (Kienzle, 1994) and preferential use of AA for energy. This combination delays glucose and insulin rate of appearance in the bloodstream of cats compared with dogs or humans (de-Oliveira et al., 2008). The prolonged glycemic response benefits cats with diabetes, as elevated blood glucose should be avoided, and the effects may also be beneficial for managing pregnancy, lactation, stress, infection, cancer, or aging (Carciofi et al., 2008).

Figure 1.5 depicts the cat's typical response to an intravenous glucose tolerance test (Hoenig et al., 2002). Cats were given 5 different doses of 50% dextrose: 0.3, 0.5, 0.8, 1.0 and 1.3 g/kg BW. Blood sampling for glucose determination occurred before sampling, and 5, 10, 15, 30, 45, 60, 90 and 120 min after dextrose injection.

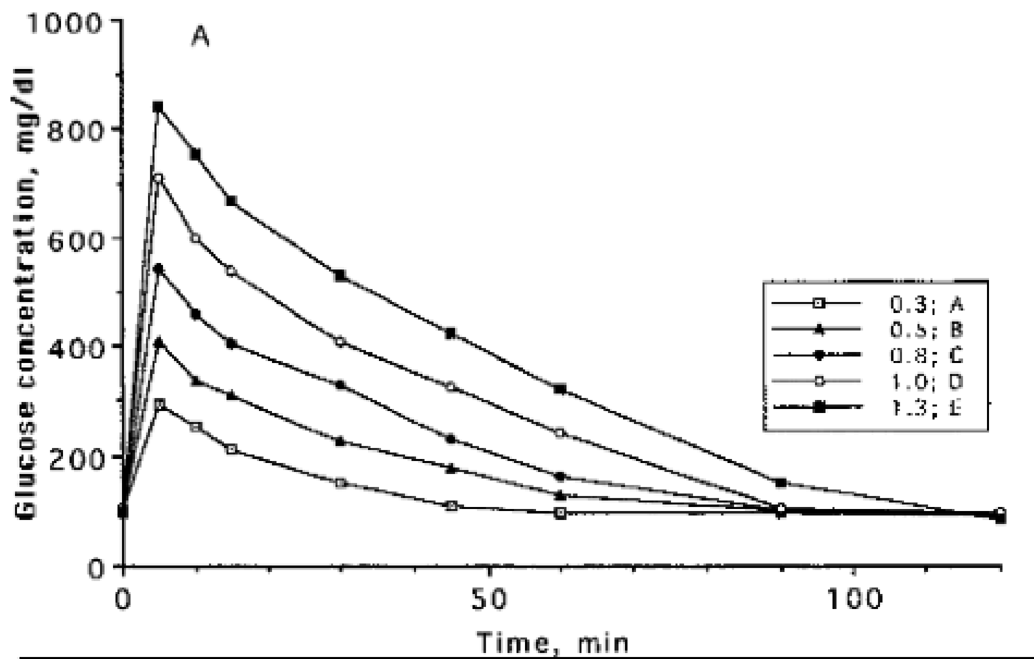

Figure 1.5. Glucose responses of cats injected with 5 concentrations of 50% dextrose: 0.3 (A), 0.5 (B), 0.8 (C), 1.0 (D) and 1.3 (E) g/kg BW. Blood sampling occurred once before sampling, as well as 5, 10, 15, 30, 45, 60, 90 and 120 min after dextrose injection (Hoenig et al., 2002).

Healthy adult cats have fasting blood glucose concentrations of 60-120 mg/dL (Hoenig et al., 2002). As seen in Figure 1.4, cats reach peak blood glucose within 10 min of glucose injection, followed by a gradual decline and return to baseline levels approximately 100 min post-injection. It is important to note that the cat's blood glucose response to a meal would be lower in magnitude and more prolonged than this test, depending on the macronutrient composition of the diet.

1.6 Energy requirements of cats

To maximize health and longevity of cats, nutritional status must be carefully monitored, because aging in pets is related to nutritional demand. While human energy requirements decrease with age (Harper, 1998), the same relation may not exist for cats. Human and canine maintenance requirements decrease approximately 20% with age due to lower basal metabolic rate and reduced physical activity (Harper, 1998). The resulting decreased energy requirements should be compensated with a decrease in food intake. In cats, however, it is not necessary to alter food intake with age, as maintenance energy requirements are unchanged due to the relative inactivity displayed throughout life (Harper, 1998); thus, the lack of decrease in activity leads to an unchanged basal metabolic rate. Although a relatively simple explanation, more research is required to associate physiological processes with aging and their affects on the metabolic rate so that the mechanisms behind maintenance energy requirements are understood. NRC (2006) describes a decrease in maintenance energy requirements of 20% for cats along with dogs, but seems to misattribute this conclusion to Harper.

Energy can be obtained from any of the dietary macronutrients. Carbohydrates and protein have energy contents of 4 kcal/g, while fat is more energy dense at 9 kcal/g (Rolls et al., 2005). Carbohydrates are generally the largest component of pet diets, including some cat diets (Forrester and Kirk, 2009), making them the largest contributor to dietary

energy. Due to time constraints and practicality this study will be looking solely at energy from carbohydrates.

1.7 Summary

It is unclear whether cats are best fed low or moderate carbohydrate diets and whether the type of carbohydrate plays a significant role in the dietary content. This puts the rationale used by the consumer-driven aspects of the cat food industry in question, where raw and grain-free diets are thought to be the best option for a healthy cat. The extent of dietary carbohydrate digestion in cats is currently unknown; thus, future research in feline nutrition can determine optimal starch content of diets. New information will lead to advances in feline health, and this new frontier in feline nutrition may include diets with starch added to improve the overall health and longevity of cats.

1.8 Thesis hypotheses

Three diets were compared: Innova Adult Cat, Purina Chicken and Rice and Iams ProActive Kitten. We hypothesized that:

1. **Glycemic index comparison:** Innova Adult Dry Cat Food would produce the lowest glycemic index (GI) due to its high level of protein and low level of nitrogen-free extract (NFE), an approximation of starch content. Also, its main ingredients for carbohydrates are brown rice and

peas, which are considered to be low GI. Purina ONE Chicken and Rice would have the highest GI due to its high NFE content and inclusion of rice, a higher GI grain. Iams Kitten Proactive Health would have a moderate GI.

2. **Digestibility:** No diet would be significantly more digestible than another, and all would be highly digestible.

3. **Respiratory quotient and energy expenditure:** The low GI diet would have a lower respiratory quotient (RQ) than the high GI diet. A lower RQ indicates less glucose oxidation. We did not expect large variability in energy expenditure because the experimental diets are not exaggerated in macronutrient composition.

4. **Glucose and insulin response:** We hypothesized that if cats can digest and utilize different amount of dietary carbohydrates with a predicted range in GI, cats fed the high GI diet would have the fastest and highest glucose and insulin response peak postprandially, while cats fed the low GI would have a lower and more sustained response. Low GI diets were also expected to lower interstitial glucose.

1.9 Research objectives

To determine the cat's whole body energetic response, we compared 3 diets of differing expected glycemic response using indirect calorimetry. The diets were also compared to determine differences in the cat's

concentrations of interstitial glucose, plasma blood glucose and insulin before and after feeding.

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Chapter 2. A study of starch digestion and metabolism in domestic cats using metabolizable energy determination to discover effects of low, medium and high glycemic index cat foods[1]

2.1 Introduction

The energy value of feline diets is expressed as metabolizable energy (ME), which is measured as gross energy (GE) minus energy lost in feces and urine (Livesy, 2001). For cats fed at their maintenance requirement, ME reflects energy used for total body heat production (Livesy, 2001). In feline research, diet ME is commonly predicted using generally accepted algorithms, because routine ME measurements are not practical, ethical, or financially feasible. The ME value can be predicted by the Atwater (1902) equation with assigned coefficients for the 3 macronutrients: protein, carbohydrate measured as N-free extract (NFE), and fat. Two equations exist (Atwater, 1902; AAFCO, 1997), traditional and modified:

Traditional: $$\mathrm{ME} = 4 \times \mathrm{CP}\ (\%) + 4 \times \mathrm{NFE}\ (\%) + 9 \times \text{crude fat}\ (\%) \qquad \text{[Eq. 1]}$$

Modified: $$\mathrm{ME} = 3.5 \times \mathrm{CP}\ (\%) + 3.5 \times \mathrm{NFE}\ (\%) + 8.5 \times \text{crude fat}\ (\%) \qquad \text{[Eq. 2]}$$

The assigned coefficients are based on the nutrient-specific heat of combustion adjusted for energy losses in feces and urine (Atwater, 1902).

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[1]A version of this manuscript will be submitted to a scientific journal (Authors K. Berendt, A. K. Shoveller, M. Guevera, and R. T. Zijlstra).

Since the traditional Atwater equation overestimates ME (Kendall, 1982), a modified Atwater equation was developed (AAFCO, 1997) and is recommended to estimate diet ME for dogs and cats (AAFCO, 2008). A diet ME value of 3.5 kcal/g is assumed, and diets with an ME value greater than 4.0 kcal/g should be corrected for energy density by multiplying the amount/kg DM for each ingredient by the energy density of the diet and dividing by 4,000 (AAFCO. 2008). Neither Atwater equation accurately predicts the ME value of pet foods, because the coefficients are unreliable (NRC, 2006). Thus, coefficients of 4 for both protein and carbohydrates and of 8.5 for fat are recommended for cats (NRC, 2006); however, these have not been validated. Finally, the relation between measured ME and glycemic index (GI) of cat diets is unknown and may provide information that would aid in dietary carbohydrate selection.

We hypothesized that measured diet ME values are inversely related to the predicted GI. Objectives were to 1) measure the ME value of 3 diets differing in predicted glycemic response and 2) compare measured to predicted diet ME values.

2.2 Materials and Methods

All procedures were reviewed and approved by Procter & Gamble Pet Care's Institutional Animal Care and Use Committee and were in accordance with USDA and AAALAC guidelines.

2.2.1 Experimental Diets and Design

Three diets were studied (Table 2.1): Purina ONE Chicken and Rice (Nestlé, St. Louis, MO), Iams Kitten Proactive Health (Procter & Gamble, Cincinatti, OH), and Innova Dry Adult Cat Food (Procter & Gamble, Cincinatti, OH). These diets were selected to have a high, medium, and low GI, respectively, based on their guaranteed nutrient analysis profiles (Table 2.1). Innova was predicted to have the lowest GI because carbohydrates were included as whole grain barley and brown rice, 2 low GI ingredients. Iams Kitten was predicted to be medium GI, because of its corn meal and sorghum inclusion, considered to be moderate GI ingredients in pet foods. Purina ONE was predicted to have the highest GI due to its inclusion of brewers rice that is considered a high GI grain (Rand et al., 2003).

Twelve cats were provided with each test diet in a quadruple 3 × 3 Latin square. Access to diet was restricted and diet allowance was based on calculated K values for each cat to maintain initial body weight (BW). As per NRC (2006), 50 was used for the constant K and multiplied by the cat's BW to calculate the energy requirement for maintenance per cat. This was then used to calculate diet allowance based on the energy density of each diet. The amount fed averaged 44 ± 8 g/d and ranged from 32 to 55 g/d. Each period lasted 10 d, with 5 d of acclimation to diet followed by 5 d of collections.

2.2.2 Experimental Procedures

Domestic shorthair cats of similar age (4.9 ± 1.2 yr) were used. Both neutered males and spayed females were included (6 males, 6 females). Initial BW average 4.4 ± 0.8 kg and ranged from 3.0 to 5.7 kg.

Cats were previously acclimated to housing facilities and cages. Cats were provided by the Pet Health and Nutrition Centre (PHNC) at Procter & Gamble Pet Care (Lewisburg, OH) and received veterinary exams to ensure health prior to and during the study.

Cats were fasted overnight and weighed prior to feeding the morning of d 1 and 6. Cats were fed at 07:00 daily. During the acclimation (d 1 to 5), cats were housed in a one-room free-living group environment (13.94 m^2) with room enrichment including perches, toys, beds, scratching posts, and climbing apparatus. Human socialization was provided daily for a minimum of 20 min. The light schedule followed a pattern of 12 h light beginning at 06:30 and 12 h darkness beginning at 18:30. Room temperature was maintained at 22°C and relative humidity was 50 to 60%. Surfaces were cleaned daily and disinfected weekly with Nolvasan disinfectant (Allivet, St. Hialeah, FL). Cats were placed in individual metabolism pens for feeding each day from 06:30 until 13:00, and were then moved back into their free-living environment.

During the collections (d 6 to 10), cats were housed in individual stainless steel metabolism cages (0.61 m length × 0.61 m width x 0.62 m height; Suburban Surgical Company, Wheeling, IL). The cages were equipped with

water bottles that cats have been acclimated to using readily. Water was given freely. Two labeled urine collection bottles per cat fitted with screened funnels and containing 10 mL HCl as preservative were placed under each cage. Each cat had its own labeled urine composite bottle, with empty weight recorded and filled with 50 mL HCl. These bottles were refrigerated at 3°C. Feces and urine collections started at 08:00 on d 6. Feces were collected, weighed, and scored using the Procter & Gamble standard operating procedure for collections and scoring. This included prompt collection of fresh feces into individual bags and scoring of feces on a scale of 0-5 with 0 as no stool and 5 as extremely dry. After weighing, feces were frozen in individual composite bags at –16°C. Urine bottles were emptied daily into composite bottles and refrigerated at 3°C. Daily, orts were weighed at 13:00. Clinical observations were recorded, but none were thought to be significant enough to alter results.

On d 11, cats' fasted weights were recorded and final feces and urine samples were collected at 08:00. Orts were recorded. Collected urine was weighed and transferred into composite bottles, which were weighed at the end of the period. Urine composites were mixed thoroughly and two 50 mL urine sub-samples were prepared for subsequent analyses. Collected feces were transferred to the individual cat's fecal composite bag, which was weighed. An aliquoted sample of each cat's fecal composite was weighed. Feces were then frozen for 24 h and freeze-dried for 3 d. Once completed,

feces were weighed again. Diet and freeze-dried feces were ground to fine particle matter using a hand grinder and analyzed.

2.2.3 Chemical Analyses and Calculations

Proximate analyses were completed in triplicate for each of the 3 experimental diets and fecal samples using AOAC procedures (1997; Table 2.2, Table 2.3). Ether extract was analyzed following acid hydrolysis (954.02), and DM was determined by vacuum drying at 100°C for 24 h (934.01). The CP was determined by oxidation using a CP/N analyzer (990.03; Leco Corp., St Joseph, MI). Crude fiber was analyzed through a ceramic fiber filtration method (962.09). Starch content was approximated as the value for N-free extract (NFE), calculated as 100 - protein (%) – fat (%) – fiber (%) –ash (%) – moisture (%) (AAFCO, 2008) (979.10) Ash was found after exposure at 550°C for 4 h (942.05), P through spectrophotometry (964.06), and Ca by atomic absorption spectrometry with electrothermal furnace (968.08). The GE was determined by bomb calorimetry (C-2000; IKA Staufen, Germany).

Total energy intake of each cat was determined using the formula:

$$GE(cal/g) = \frac{\text{Feed intake}(g) \times \text{energy}(cal)}{1000} \quad \text{[Eq. 3]}$$

Fecal energy out (FEO) was calculated as:

$$FEO = \text{Fecal output corrected for DM\%} \times \frac{\text{Corrected fecal proximate energy DM}}{1000} \quad \text{[Eq. 4]}$$

The DE of each diet was calculated by subtracting energy lost in feces from the determined GE:

$$\mathrm{DE} = \mathrm{GE} - \mathrm{FEO} \qquad \text{[Eq. 5]}$$

$$\mathrm{DE\%} = \left(\frac{\mathrm{DE}}{\mathrm{GE}}\right) \times 100 \qquad \text{[Eq. 6]}$$

Diet ME was calculated by subtracting energy in feces and urine from GE:

$$\mathrm{ME} = \mathrm{GE} - (\mathrm{FEO} + \text{urine energy out}) \qquad \text{[Eq. 7]}$$

$$\mathrm{ME\%} = \left(\frac{\mathrm{ME}}{\mathrm{GE}}\right) \times 100 \qquad \text{[Eq. 8]}$$

The constant K represents the amount of energy spent per kg BW, and is used to determine food allowance per day to maintain BW. It is calculated using the following equation:

$$\text{Calculated K} = \frac{\text{Total daily intake (as fed, g/d)} \times \text{Energy density (cal/g)}}{\text{Body weight (g)}} \qquad \text{[Eq. 9]}$$

2.2.4 Statistical Analyses

Measured and calculated variables were analyzed using the GLIMMIX procedure of SAS (version 9.3) with cat as the experimental unit, cat and period as the random effects, and diet as the fixed effect. In case diet effect was significant, means were separated using the least significant difference. An alpha of 0.05 was used. Data were reported as least-squares means.

2.3 Results

The proximate analyses of diets corresponded closely to the labels on each package (Table 2.2). The nutrient composition thus complied with the minimum and maximum composition indicated for each nutrient, except the crude fiber content in the Innova diet, which when analyzed in triplicate was 0.08% higher than the guaranteed maximum of 2.5%. No values for TDF were given in the guaranteed nutrient analyses for comparison with laboratory values.

Daily food intake (g) was lower ($P < 0.05$; Table 2.4) for the high GI diet than the low and medium GI diets. Calculated K, projected daily intake, and resulting GE intake per day differed among the 3 diets. The K value was greatest for the low GI diet, intermediate for medium, and lowest for the high GI diet (P=<0.001). The trend (P=<0.001) for projected daily intake was opposite, with the greatest values for the high GI diet, intermediate for medium, and lowest for the low GI diet. The GE intake per day as calculated per individual cat was greatest for the low GI diet, intermediate for the medium GI diet, and was lowest for the high GI diet.

Cats fed the low GI had the greatest fecal output (wet, g/d), which was greater than the medium GI treatment, and lowest for the high GI treatment ($P < 0.05$; Table 2.4). Fecal output (100% DM) was greatest for the medium GI, which was not different than the low GI group, but significantly greater than the high GI group ($P < 0.05$). Urinary N was greater ($P < 0.05$) for the

low GI than the medium and high GI diets. However, when corrected for intake, these differences no longer exist.

The OM digestibility was greater ($P < 0.05$; Table 2.6) for the high GI than the medium GI diet, and neither treatment differed from the low GI group. Protein digestibility was greatest ($P < 0.05$) for the low GI diet, followed by the high GI diet and was lowest for the medium GI diet. Fat digestibility was greater ($P < 0.05$) for the low and medium GI than the high GI diets. Ash digestibility did not differ among diets. Protein, fat, fiber and ash intake differed accordingly.

On a daily basis, GE and actual ME intake were greatest ($P < 0.05$; Table 2.6) for the low GI diet, intermediate for the medium GI diet and lowest for the high GI diet. Per unit of feed, GE, urinary energy, and actual ME were the greatest ($P < 0.001$; Table 2.7) for the low GI diet. GE and actual ME were lowest for the high GI diet ($P<0.001$), with intermediate values for the medium ME diet. UE was lowest for the medium GI diet ($P<0.001$), with intermediate values for the high GI diet.

Measured ME is compared with ME calculated using both the traditional and modified Atwater equations in Table 2.9. The measured ME value was greatest ($P < 0.05$; Table 2.8) for the low GI diet, intermediate for the medium GI diet, and lowest for the high GI diet. The ME value calculated using both ME equations followed the same ranking as for the measured ME value and because only one number per diet can be calculated we cannot assess this numerical ranking statistically. The measured ME values were greater than

calculated ME values for all 3 diets (Figure 2.1). Therefore, both Atwater equations underestimate ME. The modified Atwater equation consistently underestimated measured ME values by approximately 12% for all diets (11.9, 10.8, and 13.6% for high, medium, and low GI diets, respectively). The traditional Atwater equation comes closest to the measured ME values (2.0, 1.5, and 4.6% for high, medium, and low GI diets, respectively). The inaccurate ME values obtained from the modified Atwater ME equation were used to calculate daily feeding amounts and resulted in a daily caloric surplus (Table 2.9).

2.4 Discussion

Pet foods can be certified as low GI based on human *in vitro* and *in vivo* trials and a potential limitation of this study is that we predicted GI based on diet ingredient and nutrient composition on commercially available pet foods and did not quantify GI. The ME value was greatest for the low GI diet and lowest for the high GI diet. For all 3 diets, ME determined in cats exceeded the ME calculated based on the Atwater equation. The discrepancy calls into question the appropriateness of these equations for calculating diet ME value to determine the daily food allowance for cats. This study used a very homogenous group of cats of medium age and body condition score, and did not look at how the Atwater equation would fare at predicting ME for cats of different body weights, ages, or physiological stages.

The 3 experimental diets varied in macronutrient composition, especially for NFE, causing macronutrient intake to differ among diets. The low GI diet

contained mostly animal-based protein and fat ingredients, followed by whole grains. These ingredients contributed to its high protein and fat content, with a low NFE content due to fewer grain products. The high GI diet contained brewers rice and wheat flour as main ingredients, thereby increasing NFE content. Because NFE is crudely correlated with starch content, the NFE content indicate a greater starch load in the high GI treatment because of the high level of starch combined with high GI carbohydrate sources (Jenkins et al., 2002).

Fecal and urine output characteristics differed minimally among diets. Daily urinary energy excretion did not differ, but cats fed the low GI diet had the greatest urinary N excretion, likely due to its greater content of dietary protein, causing the greatest urinary energy loss per kg of diet. These data may also suggest that 39% protein with the relative contributions of the indispensable amino acids exceed the protein requirement for cats (NRC, 2006). Fecal characteristics did not differ among the 3 diets, except fecal output (wet) and fecal output (100% DM) due to diet intake. The lower intake of the high GI diet with equal digestibility equates to less fecal output. Fecal output could be normalized using the following ratio, as calculated in Table 2.4:

Fecal output (wet) (g) : 100 g DM intake [Eq. 10]

This measure provides similar values for the medium and low GI diets. However, the high GI diet has less fecal output based on intake that might have resulted from the higher moisture percentage of this diet.

We observed an inverse relation between predicted GI and the ME value of the 3 diets. As a result, actual ME intake was greatest for cats fed the low GI diet. The difference in ME intake was not caused by changes in digestibility of energy, but by a greater GE content of the low GI diet that consequently increased diet GE and ME values compared to high GI diets, with medium GI being intermediate. Our measured ME values were overall higher than ME values found in dogs (Yamka et al., 2007) due to the higher energy density observed in cat diets because of a higher fat content. In addition, fat has a higher digestibility than other macronutrients, thus the effect is compounded. The main reason for the greater ME content of the low GI diet is likely its greater fat content compared to the high and medium GI diets. Fat digestibility was also greater for the low and medium GI diets causing a further increase in ME value. Structural limitations of plant-based fat sources such as corn may reduce enzyme access, and thereby lower fat digestibility for the high GI diet (Singh et al., 2013). Macronutrient digestibility differed among the 3 diets that may have further contributed to changes in ME values. As described previously, in the present study, we predicted estimated GI based on type of carbohydrates and total NFE content (de Oliveira et al., 2008). A high GI diet should perhaps indicate a high ME value if we predict using the human model; however, the opposite was observed in the present study. The ME value reflects total tract apparent digestibility of energy, which includes fermentation. We cannot isolate the small intestine in cats to determine starch disappearance while maintaining a minimally invasive

approach, although it would be a better indicator of GI. Apparent total tract digestibility also does not correct for endogenous losses of N originating from sloughed intestinal epithelial cells, un-recycled enzymes, and excreted bacterial protein. Therefore, total tract protein digestibility will not reflect amino acid digestibility. Protein digestibility differed among the 3 diets, however, the magnitude of change was small and reflects the inclusion of different protein sources in the diets.

A key finding of the present study was a consistent, 12% underestimation of the modified Atwater calculation of ME. When the ME value was calculated using the traditional Atwater equation, values were much closer to the measured ME values. The underestimation is likely caused by the high quality of these 3 diets. The medium and high GI diets are considered premium pet foods, whereas the low GI diet is considered super-premium. These labels are based on price of the diet that is driven by ingredient cost and marketing opportunity. Recently, the modified Atwater equations underestimated ME values by similar margins. Specifically, the modified Atwater equation underestimated the ME value by 15% by feeding low-ash poultry meal to female adult dogs (Yamka et al., 2007). However, the modified Atwater equation predicted the ME value accurately in some studies. In particular, 558 digestibility studies of commercial and non-commercial pet foods over 7 years predicted the ME value with an error of 0.16% for dogs and 1.57% for cats (Hall et al., 2013), likely due to the wide variety of pet food tested.

While the modified Atwater equation may yield reasonable estimations for average quality pet foods with traditional ingredients, use in premium or super-premium diets with increasingly novel ingredients to calculate feeding directions may cause overfeeding of energy and, subsequently, weight gain of pets due to a caloric surplus (Yamka et al., 2007). The opposite might also occur: use of the modified Atwater to determine ME of low quality diets high in poorly-digestible ingredients may overestimate the ME value and subsequently cause weight loss. Because cat foods are generally more energy dense than dog foods, we recommend that the traditional Atwater equation instead of the modified Atwater equation should be used to calculate ME for all cat diets. Alternative methods may also be considered to predict diet ME values more accurately. Fractioning CP and NFE into smaller categories and using regression coefficients could provide a more accurate prediction of the ME value (Yamka et al., 2007). Fractioning CP into total AA and non-AA portions could reduce the overestimation of CP. An equation that includes measured GE and specific coefficients for moisture, protein, fat, and fiber may increase the accuracy to predict the ME value further (Hall et al., 2013). The following equation is proposed:

$$\begin{aligned} ME = -541 + (0.923 \times GE(kcal/kg)) + (14.68 \times \%fat) - (44.31 \times \%crude\ fiber) - \\ (4.21 \times \%protein) + (4.80 \times \%moisture) \end{aligned} \quad \text{[Eq. 11]}$$

Similar methods using GE have been developed previously (Kendall et al., 1985; Kienzle et al., 1998; Kuhlmann et al., 1993). Various equations, models, and theories have been proposed as better predictors of the diet ME value

than the modified or traditional Atwater equations, but one has yet to become the new standard.

The current requirement for pet food labels in the United States includes the guaranteed nutrient analysis, but does not include the GE or ME value (FDA, 2010). Many companies include GE content, and some companies include ME density (MED), calculated using ME, in their pet food labels (Hill et al., 2009). Commonly, for practical and financial reasons the ME value is calculated based on proximate analyses and Atwater calculation.

In conclusion, the current study found measured diet ME values to be inversely related to our predicted GI of the diets, not necessarily due to GI but more likely due to the higher fat content and digestibility of the low GI diet. Due to the limitations incurred when using commercial diets, it was not possible to hold fat and protein constant, and therefore we cannot make a conclusion directly relating ME to GI. Furthermore, we confirmed that the modified Atwater equation does not give an accurate estimate of ME of high quality pet diets. Additionally, the equations might not be appropriate for any cat diet due to the overall higher energy density than dog foods. We recommended that the traditional Atwater coefficients are used to calculate diet ME values to avoid errors in feeding guidelines and subsequent weight gain in pets, until future research establishes a more accurate equation to calculate the ME value of cat food.

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Table 2.1. Guaranteed nutrient composition and predicted glycemic index of the 3 experimental diets.

	Predicted GI[1]		
Item (as fed, %)	High	Medium	Low
CP, min.	34.0	33.0	36.0
Crude fat, min.	13.0	21.0	20.0
Crude fiber, max.	2.0	3.0	2.5
Moisture, max.	12.0	10.0	10.0
Ash, max.	-	7.0	-
Mg, max.	-	0.1	-
Taurine	0.15	0.16	0.3
n-6 Fatty acid, min.	1.5	2.76	3.6
n-3 Fatty acid, min.	-	0.29	0.35

[1]GI = glycemic index.

[2]Purina ONE Chicken and Rice (Nestlé, St. Louis, MO) with main ingredients: Chicken, brewer's rice, corn gluten meal, poultry by-product meal, wheat flour, animal fat preserved with mixed-tocopherols, whole grain corn, soy protein isolate, fish meal, animal liver flavor, KCl, H_3PO_4, $CaCO_3$, caramel color, choline chloride, and salt.

[3]Iams Kitten Proactive Health (Procter & Gamble, Cincinatti, OH) with main ingredients: Chicken, chicken by-product meal, corn meal, chicken fat preserved with mixed tocopherols, dried beet pulp, ground whole grain sorghum, dried egg product, natural flavor, fish oil preserved with mixed

tocopherols, KCl, fructooligosaccharides, choline chloride, $CaCO_3$, brewer's dried yeast, DL-Met, and salt.

[4]Innova (Procter & Gamble, Cincinatti, OH) with main ingredients: Turkey, chicken, chicken meal, whole grain barley and whole grain brown rice, chicken fat preserved with mixed tocopherols, peas, natural flavors, apples, herring, flaxseed, eggs, blueberries, pumpkin, tomatoes, sunflower oil, KCl, DL-Met, carrots, pears, cranberries, menhaden oil, cottage cheese, taurine, green beans, alfalfa sprouts, parsnips, and salt.

Table 2.2. Analyzed nutrient composition of the 3 experimental diets[1,2,3].

Item	High GI	Medium GI	Low GI
CP[4], %	38.02	35.86	42.06
Moisture, %	7.16	6.76	5.31
Ash, %	6.36	6.31	6.38
NFE,[5] %	34.1	29.5	23.6
Starch, %	36.75	30.72	23.56
Ether extract, %	15.7	22.2	22.9
Crude Fat, %	10.83	20.02	20.42
Crude fiber, %	1.17	1.78	2.58
ADF[6], %	1.88	2.95	2.43
NDF[7], %	7.36	12.58	10.57
Available Lysine, %	1.62	1.91	2.80
GE, kcal/kg	4,916	5,253	5,462
Calculated ME[8], kcal/kg	3,752	4,081	4,137
DM digestibility[9], %	91.14	90.74	92.70

[1]Each diet was analyzed in triplicate.

[2]High GI diet was Purina ONE Chicken and Rice (Nestlé, St. Louis, MO), and the medium and low GI diets were Iams Kitten Proactive Health and Innova, respectively (Procter & Gamble, Cincinatti, OH).

[3]Results (except moisture) presented on a dry-matter basis.

[4]Percentage N X 6.25.

[5]NFE = N-free extract.

[6]ADF = Acid detergent fiber.

[7]NDF = Neutral detergent fiber.

[8]Calculated with modified Atwater equation (AAFCO, 1997):

$$\mathrm{ME}\,(\mathrm{kcal/kg}) = 3.5 \times \mathrm{CP}\ (\%) + 3.5 \times \mathrm{carbohydrate}\ (\%) + 3.5 \times \mathrm{crude\ fat}\ (\%).$$

[9]Determined using *in vitro* dry matter digestibility laboratory analysis.

Table 2.3. Analyzed amino acid composition of the 3 experimental diets[1, 2, 3].

Amino acid (%)	High GI	Medium GI	Low GI
Taurine	0.22	0.26	0.37
Hydroxyproline	0.50	0.73	0.69
Aspartic Acid	2.76	2.77	3.50
Threonine	1.29	1.32	1.61
Serine	1.50	1.28	1.38
Glutamic Acid	6.00	4.30	5.52
Proline	2.55	1.82	2.00
Lanthionine	0.00	0.00	0.00
Glycine	2.08	2.61	2.70
Alanine	2.48	2.15	2.40
Cysteine	0.48	0.42	0.42
Valine	1.66	1.61	1.94
Methionine	0.75	1.08	1.38
Isoleucine	1.40	1.32	1.70
Leucine	3.81	2.61	3.01
Tyrosine	1.38	1.08	1.30
Phenylalanine	1.78	1.37	1.64
Hydroxylysine	0.17	0.15	0.19
Ornithine	0.08	0.06	0.06
Lysine	1.74	2.11	2.94
Histidine	0.80	0.78	1.02
Arginine	1.92	2.16	2.70
Tryptophan	0.31	0.34	0.40
Total	35.65	32.33	38.87

[1]Each diet was analyzed in triplicate.

[2]High GI diet was Purina ONE Chicken and Rice (Nestlé, St. Louis, MO), and the medium and low GI diets were Iams Kitten Proactive Health and Innova, respectively (Procter & Gamble, Cincinatti, OH).

[3]Results presented on a dry-matter basis.

Table 2.4. Animal physical measurements and their relationship to diet intake.

Name	High GI	Medium GI	Low GI	SEM[1]	*P*-value
Initial BW, kg	4.5	4.4	4.4	0.2	0.649
Gain, kg	-0.04	0.03	0.01	0.01	0.516
ADFI, g/d	42.8[b]	45.0[a]	45.1[a]	1.2	<0.001
Gain:feed, kg/kg	-0.10	0.04	-0.01	0.01	0.480
Calculated K[2]	40.6[c]	46.3[b]	47.9[a]	1.0	<0.001
Projected intake[3] (K = 50), g/d	53.0[a]	49.0[b]	47.5[c]	1.5	<0.001

[a-c]Within a row, means without a common superscript differ ($P < 0.05$).

[1]Means were based on 12 observations per diet.

[2]K = a constant representing the amount of energy spent per kg BW to maintain BW, used to determine daily food intake using the equation:

$$\text{Calculated K} = \frac{\text{Total daily intake (as fed, g/d)} \times \text{Energy density (cal/g)}}{\text{Body weight (g)}}$$

[3]Projected intake was found using the equation:

$$\text{Projected Intake} = \frac{\text{Initial weight} \times 50}{(\text{Calculated ME}/1000)}.$$

Table 2.5. Feces and urine characteristics of cats fed 3 experimental diets.

Name	High GI	Medium GI	Low GI	SEM[1]	*P*-value
Primary stool score	4.0	4.0	3.7	0.06	0.229
Secondary stool score	3.0	3.5	3.4	0.10	0.160
Fecal output (wet), g/d	12.2[c]	15.5[b]	16.1[a]	3.95	0.050
Fecal DM, %	39.7	37.3	36.8	1.03	0.328
Fecal output (100% DM), g/d	4.8[b]	5.8[a]	5.5[ab]	0.20	0.022
Fecal output (wet), g/d : Energy intake, kcal EME[2]/d	0.07	0.08	0.08	0.01	0.369
Fecal output (wet) (g)/100 g DM intake	30.8	36.7	37.3	1.65	0.267
Fecal energy (cal/g d^{-1})	3576	3555	3478	24.74	0.243
Urine energy (cal/g d^{-1})	155	143	172	9.16	0.437
Urine N (mg/mL)	3.52[b]	3.36[b]	4.12[a]	0.10	0.003

[a-b]Within a row, means without a common superscript differ ($P < 0.05$).

[1]Means were based on 12 observations per diet.

[2]EME= Estimated ME, calculated using the modified Atwater equation (AAFCO, 1997):

$$\mathrm{ME}\left(\mathrm{kcal/kg}\right) = 3.5 \times \mathrm{CP}\ (\%) + 3.5 \times \mathrm{carbohydrate}\ (\%) + 3.5 \times \mathrm{crude\ fat}\ (\%).$$

Table 2.6. Intake and digestibility of individual ingredients, overall digestible and metabolizable energy of 3 experimental diets.

Name	High GI	Medium GI	Low GI	SEM	*P*-value
DM digestibility, %	87.6	86.2	87.0	0.41	0.128
OM digestibility, %	90.9[a]	89.5[b]	90.3[ab]	1.03	0.031
Protein digestibility, %	88.7[b]	87.3[c]	91.4[a]	0.49	<0.001
Fat digestibility, %	92.9[b]	95.4[a]	95.0[a]	0.25	<0.001
Ash digestibility, %	39.3	41.3	39.1	1.78	0.781
Total protein intake, g/d	14.9[b]	15.0[b]	17.6[a]	0.49	<0.001
Total fat intake, g/d	6.7[c]	10.0[b]	10.3[a]	0.37	<0.001
Total fiber intake, g/d	0.8[c]	1.1[b]	1.4[a]	0.05	<0.001
Total NFE[2] intake, g/d	14.6[a]	13.3[b]	10.6[c]	0.45	<0.001
Total ash intake, g/d	2.6[c]	2.9[a]	2.7[b]	0.08	<0.001

[a-c]Within a row, means without a common superscript differ ($P < 0.05$).

[1]Means were based on 12 observations per diet.

[2]NFE = N-free extract.

Table 2.7. Energy measurements of experimental diets on a daily basis (as fed).

Name	High GI	Medium GI	Low GI	SEM[1]	*P*-value
GE intake, kcal/d	210.16[c]	236.39[b]	246.15[a]	6.90	<0.001
Fecal energy, kcal/d	18.97	23.12	21.37	0.82	0.116
DE, %	90.80	90.30	91.30	0.29	0.185
Urinary energy, kcal/d	11.42[C]	11.25[BC]	13.86[A]	0.41	0.011
GE-FE-UE, kcal/d	182.71	206.22	215.78	6.42	0.093
Measured ME, kcal/d	182.10[c]	205.81[b]	215.46[a]	2.30	<0.001
ME, %	86.64	87.07	87.64	0.39	0.592
Calculated ME[2], kcal/d	160.40	174.50	186.60		

[a-c]Within a row, means without a common superscript differ ($P < 0.05$).

[1]Means were based on 12 observations per diet.

[2]Calculated with modified Atwater equation (AAFCO, 1997):

$$ME = 3.5 \times CP\ (\%) + 3.5 \times \text{carbohydrate}\ (\%) + 8.5 \times \text{crude fat}\ (\%).$$

Table 2.8. Energy measurements of experimental diets per 100 g diet (as fed).

Item, kcal/100 g diet	High GI	Medium GI	Low GI	SEM[1]	*P*-value
GE	491.60[c]	525.31[b]	546.88[a]	3.2	<0.001
Fecal energy	44.37	50.38	47.46	1.6	0.353
Urinary energy	19.84[b]	17.31[c]	20.02[a]	0.4	<0.001
GE-FE-UE	427.39[b]	457.62[a]	479.40[a]	3.2	<0.001
Actual ME	425.96[c]	457.35[b]	478.69[a]	3.6	<0.001
Calculated ME[2]	375.20	408.10	413.70		

[a-c]Within a row, means without a common superscript differ ($P < 0.05$).

[1]Means were based on 12 observations per diet.

[2]Calculated with modified Atwater equation (AAFCO, 1997):

$$ME = 3.5 \times CP\ (\%) + 3.5 \times \text{carbohydrate}\ (\%) + 8.5 \times \text{crude fat}\ (\%).$$

Table 2.9. Comparison of measured ME with ME calculated using Atwater and modified Atwater equations, and resulting caloric surplus per day.

	High GI	Medium GI	Low GI
Measured ME, kcal/kg as fed	4,259[c]	4,574[b]	4,787[a]
Calculated ME, kcal/kg as fed			
Modified Atwater[1]	3,752	4,081	4,137
Traditional Atwater[2]	4,176	4,505	4,565
Calorie surplus per day			
Modified Atwater	21.7	22.2	29.3
Traditional Atwater	3.6	3.1	10
Calorie surplus per day, %			
Modified Atwater	11.9	10.8	13.6
Traditional Atwater	2.0	1.5	4.6

[1]Calculated with modified Atwater equation (AAFCO, 1997):

$$ME = 3.5 \times CP\ (\%) + 3.5 \times carbohydrate\ (\%) + 8.5 \times crude\ fat\ (\%).$$

[2]Calculated with traditional Atwater equation (Atwater, 1902):

$$ME = 4 \times CP\ (\%) + 4 \times carbohydrate\ (\%) + 9 \times crude\ fat\ (\%).$$

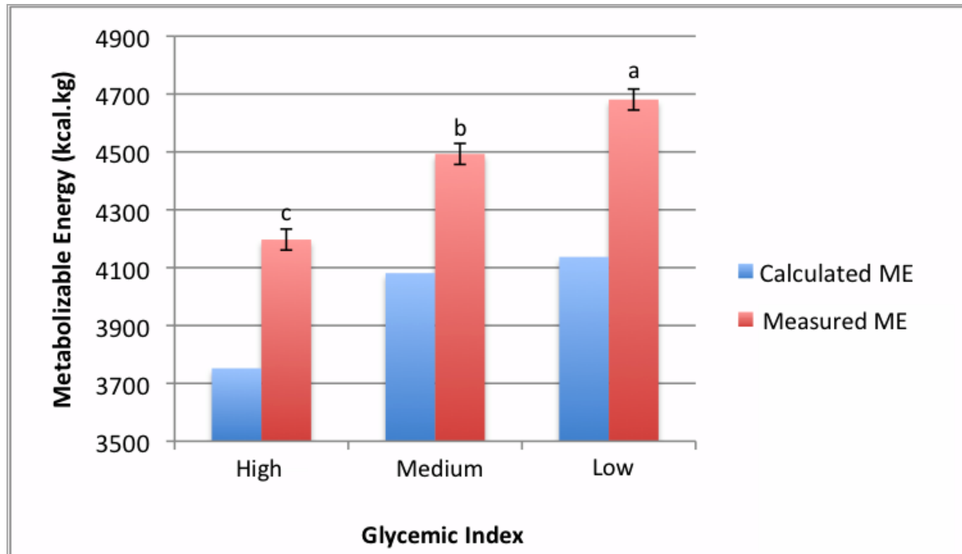

Figure 2.1. Measured ME and calculated ME using the modified Atwater equation compared for 3 diets differing in predicted glycemic index.

Chapter 3: Indirect calorimetry, real-time interstitial glucose monitoring and blood sampling to determine effects of low, medium and high glycemic index cat foods[1].

3.1 Introduction

The domestic cat is efficient at converting dietary protein into glucose for energy (LaFlamme, 2008). However, dry cat foods contain carbohydrates to enhance processing and product stability (Forrester and Kirk, 2009). The ability of cats to metabolize starch has been poorly defined. Glucose and insulin responses of cats have mainly been studied with regards to obesity and type II diabetes. These responses are lower in cats than dogs, likely due to low glucokinase activity in cats requiring compensation with hexokinase, slowing starch digestion and metabolism (de-Oliveira et al., 2008).

The glycemic response to an ingredient, commonly referred to as the glycemic index (GI), measures speed of dietary starch and sugar release as glucose into the bloodstream and can be a useful dietary classification tool in omnivorous monogastric nutrition (Giuberti et al., 2012). The GI has not been studied in carnivorous monogastrics such as the cat. The size of dietary starch fraction and starch source affects glucose and insulin responses (Carciofi et al., 2008).

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[1] A version of this manuscript will be submitted to a scientific journal (Authors K. Berendt, A. K. Shoveller, and R. T. Zijlstra).

Indirect calorimetry measures respiratory gases and can be used to calculate the metabolic rate of an animal (Ferrannini, 1988). Respiration calorimetry can be used to calculate respiratory quotient (RQ), daily resting energy expenditure (REE), carbohydrate and fat oxidation (Ferrannini, 1988). Real-time interstitial glucose monitoring is beginning to be practiced in animal research (Aussedat et al., 2000). Interstitial glucose monitors allow creation of a real-time curve of pre- and postprandial interstitial glucose. Blood sampling allows tracking of serum glucose and insulin responses to a diet, but with far fewer data points than real-time interstitial glucose monitoring.

We hypothesized that cats fed diets with greater inclusion of high GI ingredients will have greater serum glucose and insulin than cats fed medium and low GI diets. The objectives were to measure effects in cats of 3 diets differing in GI on RQ, REE, fat and carbohydrate oxidation using indirect calorimetry, interstitial glucose responses using real-time interstitial glucose sensors, and serum glucose and insulin using sequential blood sampling. The approach will define how cats digest and metabolize starch and indicate the usefulness of GI as a measure for cat foods.

3.2 Materials and Methods

All procedures were reviewed and approved by Procter & Gamble's Institutional Animal Care and Use Committee in accordance with IACUC guidelines and in compliance with USDA and AALAC.

3.2.1 Experimental Diets and Design

Three diets were studied: Innova Dry Adult Cat Food (Procter & Gamble, Cincinatti, OH), Iams Kitten Proactive Health (Procter & Gamble, Cincinatti, OH) and Purina ONE Chicken and Rice (Nestlé, St. Louis, MO). These diets were selected as hypothetically to be with a high, medium, and low GI, respectively, based on their guaranteed nutrient analyses and ingredient composition (Table 3.1). Proximate analyses completed on these diets are included in Table 3.2, with amino acid analysis included in Table 3.3. Cats were assigned to diets in a 3 × 3 Latin square, with cats cycling through all diets in 3 periods. In total, 19 cats were included in 6 complete and 1 incomplete 3 × 3 Latin squares. Cats were fed to 95% maintenance energy requirements to encourage consumption of all food provided. Female cats were fed 45 kcal ME/kg BW/d and male cats were fed 50 kcal ME/kg BW/d. A modified Atwater calculation using the coefficients 3.5, 3.5 and 8.5 for protein, carbohydrates, and fat, respectively, was used to determine the metabolizable energy (ME) content of each of the three diets (AAFCO, 1997). The resulting ME was used to determine their daily allotment amount of diet per cat. Cats were provided by and housed at the Pet Health and Nutrition Centre (PHNC) at Procter & Gamble Pet Care (Lewisburg, OH) and received veterinary exams to ensure health before and during the study.

Cats followed an 8 d prefeeding schedule followed by a 22 hr calorimetry chamber measurement on d 9. Also during d 9, interstitial blood glucose was

measured in real time via implanted interstitial glucose sensors. On d 10, saphenous vein blood was sampled sequentially before and after feeding for analysis of serum glucose and insulin. Food intake and orts were recorded daily, and BW was recorded on the last day of each period (d 10, 20, and 30 for each group).

3.2.2 Experimental Procedures

Domestic shorthair cats (*n* = 19) of similar age (4 to 5 yr) and body condition score (3-4.5 on a 9-point scale) were used. Both neutered males and spayed females were included (10 males, 9 females). Cats had been previously acclimated to calorimetry chambers and associated equipment so that stress during the trial would be minimized (Gooding et al., 2012). Cats were also trained to accept repeated (up to 8 per day) saphenous vein blood draws (Lockhart et al., 2011).

During the pre-feeding period, cats were housed in free-living group environments with room enrichment including perches, toys, beds, scratching posts, and climbing apparatus. Cats were able to choose to go outside through a swinging door during daylight hours; the swinging door was locked from 16:00 to 07:00. Lighting schedule followed a pattern of 12 h light beginning at 06:30 and 12 h darkness beginning at 18:30. Room temperature was kept at 22°C, and relative humidity was 50 to 60%. Water was provided freely via automatic waterers for the duration of the study.

Surfaces were cleaned weekly disinfecting with Nolvasan disinfectant (Allivet, St. Hialeah, FL) and daily cleaning was performed.

Cats were divided into 4 groups of 5 and 1 group of 4 to accommodate rotation through the 5 calorimetry chambers. Cats began the study with an 8 d pre-feeding period on 1 of the 3 diets. Day 1 for each group of 4 cats was staggered to ensure each group received exactly 8 d of pre-feeding before entering the calorimetry chamber on d 9. Cats were placed in individual cages for feeding at 07:00 each morning. Cats were given 60 min to eat the given food before orts (unconsumed food) were removed and weighed.

Early morning on d 9, the first group of cats was implanted with subcutaneous interstitial glucose sensors (Guardian Continuous Monitoring Devices, Medtronic Diabetes, Northridge, CA) placed between the 9th and last rib. Sensor cannulae were 14 mm, with an attached 4.17 × 3.56 × 0.94 cm transmitter that lies flat on top of the skin. Cats had a patch of fur shaved the day before to accommodate the sensor, which was secured with Polyskin II transparent dressing (Tyco Healthcare, Schaffhausen, Switzerland). Cats wore bodysuits to prevent scratching or licking the sensor or the dressing over the sensor, and harnesses over top to prevent escape from body suits.

After interstitial glucose sensor placement on d 9, the first group of cats was placed in the 5 calorimetry chambers for a 22 hr measurement. The calorimetry chambers (Qubit Systems, Kingston, ON, Canada) were composed of Plexiglas and measured 53.3 × 53.3 × 76.2 cm. Each contained a shelf, feeder, water bowl, hammock, litter box, toy, and free area with a fleece bed.

Water was given freely from water bowls. The chamber is large enough to provide enough separation between areas used for feeding, sleeping and elimination (Gooding et al., 2012). On a daily basis, the chambers and water bowls were disinfected and the litter, litter boxes, toys, hammock and bed were removed and cleaned. Calorimetry chambers were calibrated at 06:45 and 18:00.

On d 10, sequential blood sampling was completed. The first blood sample was taken 15 min before feeding (06:45, fasting), followed by samples at 30, 60, 120, 240, 360, 480, and 600 min after feeding. Per sample, 1 mL of blood was collected. Sampling was started on the right rear leg, moving upwards for each subsequent blood draw, and switched to the left rear leg on sample 4, or sooner if needed depending on the individual cat. Cats were kept in individual cages in the procedure room for the duration of the blood draws (05:45-16:15) for constant monitoring and convenience. Following the first period, cats were then switched to their next diet. The same schedule was used for all 3 periods of the study.

3.2.2.1 Indirect Calorimetry

To detect metabolic changes as a result of each of the 3 diets differing in hypothesized glycemic response, indirect calorimetry techniques were used to measure energy expenditure (kcal/kg $BW^{0.67}$/d) and rates of fat and carbohydrate oxidation. Breath samples were obtained in 30 min intervals where O_2 consumed (VO_2) and CO_2 produced (VCO_2) were measured. Levels

of O_2 and CO_2 in the respiratory chambers were measured with infrared O_2 and CO_2 analyzers (Qubit Systems, Kingston, ON, Canada). The calorimeter is open circuit and ventilated with room air being drawn through at a rate between 5 and 10 L/min, depending on the individual cat. Rate of airflow was measured using a mass flow meter to calculate total volume. Calibration of the analyzers and mass flow meters was performed prior to the study and continued every 12 h during the study or whenever a drift of >5% was observed on the half-hourly reference channel. Calibration was performed using standard gas mixtures of N and a span gas containing 1.012% CO_2 against known calibration standards. Measurements were recorded at t = –60 and –30 min, and measurements in the fed state were recorded from t = 0 onward.

3.2.2.2 Interstitial Measurements

On d 9, interstitial glucose measurements were automatically recorded from transmitters to a computer, recording glucose every 5 min. These real-time measurements were used to create a curve of interstitial glucose concentrations throughout the day for each of the cats. Calibration occurred 3 times per day at 08:00-08:30, 13:30-14:00, and 18:00-19:00. Calibration consisted of pricking each cat's paw pad and reading the blood glucose level using the Alphatrak 2 monitor (Abbot Animal Health, Abbott Park, IL). This reading was then programmed into the interstitial glucose monitor. All interstitial glucose measurements were in the fed state, due to the 2 h

calibration period required after sensor placement. The detection limit was 40 mg/dL and this value was used when the measurement was on or below this limit.

3.2.2.3 Blood Sampling

Fasting and multiple postprandial saphenous blood draws taken on d 10 were used to create a chart of both serum glucose and insulin response to the different diets. Blood was obtained using the BD Safety-Lok Vacutainer, 23 gauge with 1.91 cm needle and 30.5 cm tubing without anticoagulant. Samples were kept on ice for up to 1 h immediately after they were obtained, and were centrifuged using the Thermo Scientific IEC Centra GP8 centrifuge at 1862 *g* and -4°C for 15 min after clotting. Serum was then removed using a pipette and roughly divided in half into 2 tubes for glucose and insulin analyses. Samples were stored at -20°C until all samples for the study were obtained, at which point they were transported on dry ice to the laboratory for analysis. Serum glucose analysis was completed through colorimetric measurements (UV/vis spectrometry) with the Beckman Coulter AU480 automated chemistry analyzer (Indianapolis, IN). Serum insulin analysis was completed using a feline ELISA kit (Mercodia Inc., Winston Salem, NC). These measurements allow us to see the size of glucose and insulin spikes in relation to meal times and how long these concentrations were sustained before returning to fasting levels. Fasting serum glucose and insulin were obtained at t = -15 min. The remaining 7 time points were in the fed state.

Peak glucose and insulin were calculated by averaging serum concentrations among cats at each time period, with the greatest average indicating peak mean glucose or insulin.

3.2.3 Calculations

For study analysis, the following calculations were used.

Indirect calorimetry measures RQ, which is the ratio of CO_2 exhaled and O_2 used (Ferrannini, 1988):

$$RQ = \frac{\text{L } CO_2 \text{ produced}}{\text{L } O_2 \text{ consumed}} \quad \text{[Eq. 1]}$$

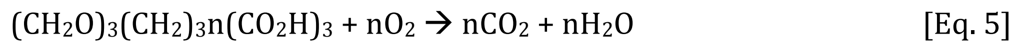
$$REE = \left[\frac{3.94 \times O_2 \text{ exchange } (\mu L/h)}{1000000} + \frac{1.11 \times CO_2 \text{ exchange } (\mu L/h)}{1000000}\right] \times 24h \quad \text{[Eq. 2]}$$

Carbohydrate oxidation (Ferrannini, 1988):

$$C_nH_{2n}O_n + nO_2 \rightarrow nCO_2 + nH_2O \quad \text{[Eq. 3]}$$

$$\frac{(4.585 \times (CO_2 \text{ exchange, } \mu L/h)) - (3.226 \times (O_2 \text{ exchange, } \mu L/h))}{1000000} \quad \text{[Eq. 4]}$$

Fat oxidation (Ferrannini, 1988):



$$(CH_2O)_3(CH_2)_3n(CO_2H)_3 + nO_2 \rightarrow nCO_2 + nH_2O \quad \text{[Eq. 5]}$$

$$\frac{(1.695 \times (O_2 \text{ exchange, } \mu L/h)) - (1.701 \times (CO_2 \text{ exchange, } \mu L/h))}{1000000} \quad \text{[Eq. 6]}$$

Equations 1 through 6 are measures of indirect calorimetry to determine the cat's energy reserves required for basal metabolism (Ferrannini, 1988).

$$\text{Glucose : Insulin ratio} = \frac{\text{Glucose (mg/dL)}}{\text{Insulin } (\mu IU/mL)} \quad \text{[Eq. 7]}$$

Equation 7 gives the glucose : insulin ratio of cats. This variable was not analyzed statistically.

3.2.4 Statistical Analyses

Statistical power was calculated prior to the study using SAS version 9.2 and for the population size of 19 cats was found to be 97.4% for energy expenditure, serum insulin and glucose concentrations (Appendix 1). Data used to calculate the statistical power came for energy expenditure from an abstract (Shoveller et al., 2010) and for blood glucose and insulin from a published paper (de-Oliveira et al., 2008).

Data was analyzed using the proc GLM, proc MIXED and proc GLIMMIX functions of SAS version 9.2 and 9.3 with cat as the experimental unit, cat and period as the random effects, diet as the fixed effect, and time as the repeated measure. Body weight was included as a covariate in the serum glucose and insulin analyses. Repeated measures analyses were performed for RQ, REE, serum glucose, serum insulin and interstitial glucose over time. The covariance structure used in the interstitial glucose analysis was Autoregressive Moving Average Model (ARMA 1,1) because it was found to have the lowest value for Akaike Information Criterion (AIC).

When the effect of diet was significant (alpha of 0.05), means were separated using the least significant difference. If $0.05 < P < 0.10$, a trend was described. Data were reported as least-squares means.

3.3 Results

On d 7 of period 3, blood was detected in the urine of one cat. The cat was given 150 mL of IV fluid and did not show other symptoms, and was allowed to continue the study after veterinary examination. On d 9 of period 2, one cat's sensor lost signal at around 21 h post meal feeding. For data analyses, this point became the end point of interstitial glucose measurement for all cats. The measurement period was thus shortened to approximately 20 h instead of the planned 22 h.

3.3.1 Indirect Calorimetry

Immediately following the meal, the RQ increased, reached plateau, and then declined (Figure 3.1). The RQ differed ($P<0.001$) among diets. The RQ over the 22 h was greatest ($P<0.05$) for the high GI diet, followed by the medium, and was lowest ($P<0.05$) for the low GI diet (Table 3.4; Figure 3.2). Fasted RQ did not differ among the 3 diets (Table 3.4). The RQ in the fed state was greatest ($P<0.05$) for the high GI diet, followed by the medium, and was lowest ($P<0.05$) for the low GI diet.

Following the meal, the REE increased gradually (Figure 3.3). For REE over 22 h, diets did not differ (Table 3.4; Figure 3.4). The REE in both fasting and fed state did not differ (Table 3.4).

Fat oxidation tended to differ ($P=0.057$; Table 3.4) among diets. Fat oxidation was greater ($P<0.05$) for the low than high GI diet, and was intermediate for the medium GI diet that did not differ from the high nor low

GI diets. In the fasted state, fat oxidation did not differ among the 3 diets. In the fed state, fat oxidation was greater (*P*<0.05) for the low than high GI diet, and was intermediate for the medium GI diet.

Carbohydrate oxidation differed (*P*<0.001; Table 3.4) among diets. Carbohydrate oxidation was greatest (*P*<0.05) for the high GI diet, followed by the medium, and was lowest (*P*<0.05) for the low GI diet. In the fasted state, carbohydrate oxidation did not differ among the 3 diets. In the fed state, carbohydrate oxidation was greatest (*P*<0.05) for the high GI diet, followed by the medium, and was lowest (*P*<0.05) for the low GI diet.

For the overall period, and for the fed and fasted states, an effect for time was observed (*P*<0.001) for RQ, REE, and fat and carbohydrate oxidation. Diet and time did not interact for any of the 4 variables.

3.3.2 Interstitial Glucose Monitoring

Interstitial glucose increased (Figure 3.5) over time for all 3 diets. Mean interstitial glucose concentration differed (*P*<0.001; Table 3.5) among the 3 diets. During the 20 h measurement, interstitial glucose was greater (*P*<0.05) for the high GI diet than the medium and low GI diets; medium and low GI diets did not differ. An effect of time points was observed (*P*<0.001), and there was a diet * time interaction (*P*<0.001).

The present study is one of the first to use continuous interstitial glucose sensors in cats, and some irregularities were observed. Occasionally, sensors recorded a reading of 40 mg/dL with little variation during the entire period.

This reading was due to interstitial glucose levels of the individual cat being below the threshold of 40 mg/dL. This occurred for 4 cats in period 1, 2 cats in period 2, and 1 cat in period 3. Of these, 4 instances were for cats fed the low GI diet, 2 for the medium GI diet, and 1 for the high GI diet.

3.3.3 Sequential Blood Sampling

Serum glucose peak was greatest preprandially or after an 18 h fast, followed by an immediate postprandial decline, and then reached plateau for the 3 diets (Figure 3.6). Serum glucose concentration did not differ overall (Table 3.5) or in the fasted or fed states. Serum glucose peaked at 15 min preprandial for the 3 diets. Body weight, included as a covariate, affected ($P<0.001$) serum glucose, as did time ($P<0.001$). Diet and time did not interact ($P<0.440$).

Serum insulin generally increased postprandially, followed by a plateau (Figure 3.6). Mean serum insulin differed ($P<0.001$; Table 3.5) among the 3 diets. Mean serum insulin was greater ($P<0.05$; Table 3.5) for the high than medium GI diet, and was intermediate for the low GI diet that did not differ from the high nor medium GI diets. In the fasted state, serum insulin did not differ among the 3 diets. In the fed state, serum insulin was greater ($P<0.05$) for the high than medium GI diet, and was intermediate for the low GI diet. Body weight, included as covariate, affected ($P<0.001$) serum insulin, as did time ($P<0.001$). Diet and time interacted ($P<0.003$). Average peak time for insulin was 60 min postprandial for the low GI diet, followed by the medium

(240 min) and high GI diet (360 min). Peak insulin did not differ among diets. Glucose : insulin can be seen in Table 3.5.

3.4 Discussion

3.4.1 Indirect Calorimetry

The RQ indicates the dietary macronutrient that is being used for energy. An RQ of 1.0 indicates sole carbohydrate oxidation and 0.7 indicates sole fat oxidation (Lusk, 1928). In the present study, RQ values were between 0.7 and 1.0, but closer to 0.7, indicating that cats fed the 3 diets tended toward oxidizing fat. The greater RQ for cats fed the high GI diet indicates that carbohydrates were used more than in cats fed medium or low GI diets. Cats fed the low GI diet that contained most fat content had the lowest RQ, similar to other feline studies that associated RQ to dietary macronutrient content. For example, increasing dietary fat content decreased RQ significantly (Gooding et al., 2013). Likewise, cats fed high carbohydrate diets had a greater RQ than cats fed high protein diets (Hoenig et al., 2007), similar to humans (Smith et al., 2000; Treuth et al., 2003). Dietary protein may complicate RQ analyses, because of possible protein catabolism in addition to fat and carbohydrate oxidation (Walsberg and Wolf, 1995). To eliminate confounding results, equations exist to calculate non-protein RQ using urinary N (Weir, 1949; Walsberg and Wolf, 1995); however, our cats were litterbox-trained, not allowing us to collect urine for N analysis.

Two peaks in RQ were visible and were consistent for all treatments (Figure 3.1). The first peak at t = 330 min is likely due to the effect of feeding, more delayed than expected in other species. Humans peak at 90 min (Akrabawi et al., 1996) and dogs at 75 min postprandially (Diamond and LeBlanc, 1987). The second peak at t = 630 min might be due to increased activity of cats during personnel changeover and subsequent calibration of calorimetry equipment in the afternoon, similar to increased RQ with increasing dog activity (Anderson and Lusk, 1917). The daily routine of staff is consistent and calibration occurred at the same time every day. Cats are kept very calm with little disturbance during the rest of the time. This second peak displays the sensitivity of the equipment used as well as the sensitivity of the cats to activity.

The lack of difference in REE among the 3 diets was expected. The REE increased over time, indicating that energy expenditure in cats changes during the day not based on specific diet fed, but instead likely due to daily schedule; for example, sleeping following mealtime. Previously, REE remained constant despite alterations in macronutrient fractions in cats fed isocaloric diets (Lester et al., 1999). In the present study with diets differing in starch content, cats were feed restricted at 95% of estimated ME requirement to encourage full meal consumption and ensure cats received identical calories across treatments, a factor that contributed to constant REE among diets. Changes in carbohydrate fraction did not change REE in humans (Thomas et al., 1992). The largest factors influencing REE are likely meal size

and frequency. For example, overfeeding decreased total energy expenditure of dogs (Pouteau et al., 2000). Thermogenesis doubled in dogs fed 4 small meals rather than 1 large meal, a difference attributed to repeated sensory stimulation (LeBlanc and Diamond, 1986). Thermogenesis is a component of daily resting energy expenditure, in addition to basal metabolic rate and physical energy expenditure (Westerterp, 2004).

Fat oxidation was greatest for the low GI diet, which is expected because this diet contained the most fat. Although increasing dietary fat content did not increase fat oxidation in humans (Schutz et al., 1989), this effect occurs in cats, underlining the unique energy metabolism in cats (Gooding et al., 2013; Lester et al., 1999). A greater fat : carbohydrate oxidation ratio reduces the RQ (Lester et al., 1999). That only the high and low GI diets differed in fat oxidation, with medium GI diet intermediate and the same as both high and low diets, fits with dietary nutrient profiles. Fat content was similar for low and medium GI diets, but was lower for the high GI diet. Because the medium GI diet did not differ from the high GI diet in fat oxidation, although their fat content differed, other factors not determined in the present study may have contributed such as differences in dietary omega fatty acid ratio, protein, or crude fiber content. Carbohydrate oxidation was greatest for the high GI diet, likely due to the greatest starch content, followed by the medium and low GI diets. Carbohydrate and fat oxidation were inversely related, similar to humans (Acheson et al., 1984).

3.4.2 Interstitial Glucose Monitoring

Interstitial glucose increased with diet GI, as hypothesized. Real-time interstitial glucose has not been studied thoroughly in cats. This minimally-invasive technique was used previously in diabetic humans, dogs, and horses (Wiedmeyer et al., 2003). In healthy animals, values ranged from 78 to 128 mg/dL for dogs, and from 72 to 114 mg/dL for horses (Wiedmeyer et al., 2003). In the present study using sensors developed for humans (FDA, 2007), some difficulties occurred. The lower limit of sensors was 40 mg/dL and was not low enough for some cats, causing inaccurate readings when interstitial glucose dropped below this limit. Sensor placement at the dorsal neck rather than in the thoracic region might improve readings (Hafner et al., 2013). For most cats, however, real-time interstitial glucose was successfully measured during the entire day. This technology can also monitor efficacy of dietary intervention in diabetic cats and dogs and surgery complications (Wiedmeyer and DeClue, 2008).

Interstitial glucose had 2 peaks for all 3 diets (Figure 3.5): one at 15:00 and one at 19:00 that coincided with 2 peaks in RQ. Likely, the first was due to effect of feeding (Stombaugh and Grifo, 1977) and the second due to personnel changeover. Stress and activity promote hyperglycemia in cats (Rand et al., 2002). Interstitial glucose may lag serum glucose (Rossetti, 2010). Serum glucose increased 300 min postprandial while interstitial glucose started to increase at 360 min postprandial, indicating a 60 min lag time in cats.

3.4.3 Sequential Blood Sampling

Blood sampling is also a minimally invasive technique that allows tracking of serum glucose over time, which may differ from interstitial glucose. Serum insulin can also be analyzed. Body weight was not expected to be significant as covariate, because it was corrected for by individualized diet allowance per cat (de-Oliveira et al., 2008) and each cat received each treatment. Cats in the present study were selected based on similar age and body condition score. Body condition score and accompanying body weight are well documented to play a role in glucose regulation and insulin sensitivity in cats (Rand et al., 2004). Although not the main focus, body condition may have influenced results and the association is a noteworthy byproduct of the present study.

In contrast to responses in humans and dogs, serum glucose peaked unexpectedly 15 min preprandially in cats in the present study, followed by a drop in serum glucose and a leveling out over time postprandially. In dogs, peak serum glucose occurred within 60 min of feeding and returned to baseline within 180 min (Carciofi et al., 2008). In humans, peak serum glucose occurred at t = 45 min postprandial and returned to baseline at t = 180 min postprandial (Johansson et al., 2013). Based on dog research (Carciofi et al., 2008) and other cat studies (de-Oliveira et al., 2008), we expected that serum glucose would peak postprandially. Previously, cats reached peak serum glucose at 8 h postprandial (de-Oliveira et al., 2008;

Farrow et al., 2013), which is much slower than omnivorous monogastric species; cats thus have a different glucose response. Although our sequential blood sampling was not of sufficient duration to measure time required to return to baseline serum insulin, a return to baseline levels between 8 to 24 h postprandially has been measured in cats (Farrow et al., 2013), which is much longer than in dogs or humans. Metabolism of dietary starch is thus different in cats than humans or dogs. One explanation for the elevated fasting serum glucose could be the morning activity level. Cats were moved to cages first thing in the morning and blood sampling commenced immediately. The same effect that was observed for interstitial glucose could have occurred here, with increased activity level promoting hyperglycemia (Rand et al., 2002). It may be advisable to ensure that cats are sufficiently relaxed prior to the initial blood sample.

The finding that hypothesized dietary GI did not affect serum glucose was unexpected. Previously, high GI ingredients marginally increased blood glucose postprandially in cats (de-Oliveira et al., 2008). High fat or high protein diets did not affect serum glucose in cats (Farrow et al., 2013), which might apply to the present study due to varying dietary fat content, but indicates that carbohydrate ingredients should not be investigated independently given that cats generally eat complete and balanced diets. The benefit of a low GI diet in humans or dogs is to avoid large glucose spikes postprandially and potential pancreatic β-cell exhaustion (Rand et al., 2004). However, if GI does not affect peak serum glucose in cats, other factors

should be considered for starch inclusion in cat diets. Feline β-cells are less sensitive to glucose than β-cells of omnivores (Curry et al., 1982) and might thus be less prone to exhaustion. Optimal dietary protein should be fed to cats due to their carnivorous nature. However, the cat might handle dietary starch to a certain level without detriment (Lauten et al., 2000; Vester et al., 2009; Hooda et al. 2013). Despite a lack of differences in serum glucose, interstitial glucose appears to be more sensitive than serum glucose to dietary hypothesized glycemic response. Based on the lack of difference in serum glucose in the present study, cats appeared to metabolize the carbohydrate intake of all three dietary treatments similarly. These particular cats may be well adapted to dietary changes due to their healthy weight and maintenance of insulin sensitivity. Moreover, the limited number of blood samples collected may have missed time points where important changes occurred. A real-time continuous monitoring system may be the most accurate method to detect changes in blood glucose in response to diet.

Interestingly, serum insulin, but not serum glucose, changed with diet. However, we did not observe increased serum insulin with increased dietary GI, as hypothesized. We expected serum glucose and insulin to be interdependent (de-Oliveira et al., 2008; Farrow et al., 2013). Because serum glucose did not follow our hypothesis, logically insulin also did not. Considering serum insulin in the fed state, only the high and medium GI diets differed, with low GI being intermediate and not different from either high or medium GI. Similarly, serum insulin increase postprandially only for the diet

with greatest GI in cats (de-Oliveira et al., 2008), and serum insulin did not differ between cats fed high fat and high starch diets (Gooding et al., 2013). In the present study, insulin peaked fastest for the low GI diet, followed by the medium and high GI diets, which could be associated with the unique nature of cats as obligate carnivores. Other factors than starch and GI must be examined with regards to insulin release. Cats meal-fed or with free access to high protein diets tended to reach peak insulin faster than cats fed high starch or high fat diets (Farrow et al., 2013). Amino acids stimulate insulin release in humans and dogs (Strack et al., 1994; van Loon et al., 2003), and certain amino acids act similarly in cats (Curry et al., 1982). In real-world application, this makes timing of insulin injection and dietary macronutrient profile very important for the owner of a type 2 diabetic cat to understand. However, low protein diets may also reach peak insulin faster than low starch diets in cats (Verbrugghe et al., 2010). Postprandial insulin patterns are thus not consistent among studies. More research is needed to develop a consensus. Until then, serum insulin may not be the best marker of a healthy metabolism in cats.

Cats apparently have a different insulin response than omnivorous monogastric species, and a response not expected based on their glucose response. Cats have a unique starch metabolism and starch has little effect on their postprandial blood glucose and insulin (Kienzle, 1994; Bouchard and Sunvold, 2000). This metabolic characteristic of cats has not been fully defined, but likely is associated with their enzymatic abilities and

preferential use of amino acids for energy. In the present study and other feline studies, blood glucose and insulin changed slightly with GI, whereas variations were larger in humans or dogs (Carciofi et al., 2008; Johansson et al., 2013). In the present study, glucose : insulin ratios were similar among diets, and although not analyzed statistically, these ratios indicate that insulin was released proportionally to glucose for all diets.

A mechanism for minimal insulin responses in cats was proposed (Hewson-Hughes et al., 2011). Briefly, the cat's inherent lack of glucokinase minimizes its ability to act as a glucose sensor in pancreatic β-cells, inhibiting β-cell metabolism of glucose and subsequent insulin secretion. Following this line of thinking, our results indicated that the medium GI diet elicited the lowest insulin response in young, healthy cats and provided evidence that a medium GI diet could be the best approach for long term metabolic health in cats as compared to the high and low GI diets.

In conclusion, while RQ, REE, and interstitial glucose were as expected, serum glucose and insulin showed some peculiarities that emphasize the unique metabolism of the cat. Overall, however, serum insulin and glucose indicate that cats have a prolonged and less pronounced response of glucose and insulin to dietary starch content. Cats responses to dietary starch are much lower in magnitude than for omnivorous monogastric species and in the present study did not differ based on dietary GI. Finally, GI was likely not as useful as other variables such as dietary macronutrient fractions.

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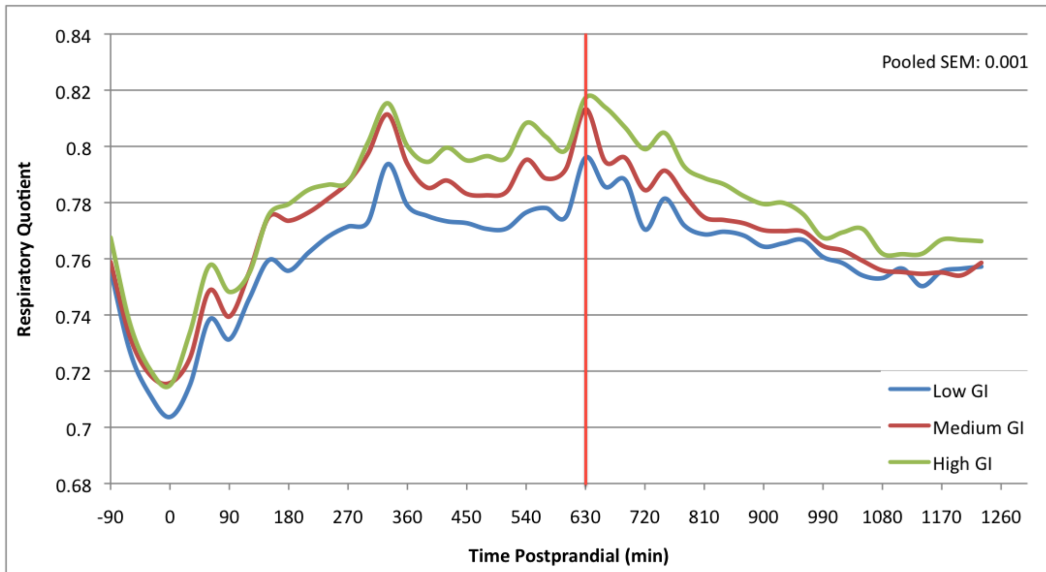

Figure 3.1. Respiratory quotient of cats fed diets with high, medium, and low predicted glycemic index (GI). Measurements were taken in 30 min intervals. Cats (n = 19) were fed at t = 0 min and equipment calibration occurred at t = 630 min.

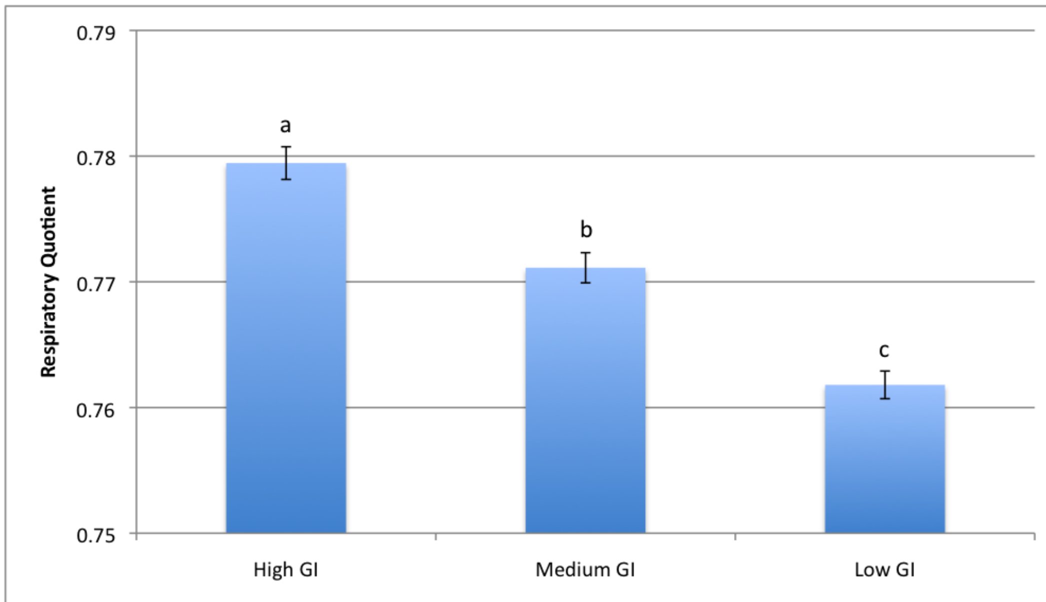

Figure 3.2. Respiratory quotient of cats (n=19) fed diets with high, medium, and low predicted glycemic index (GI) for the measurement period of 22 h.

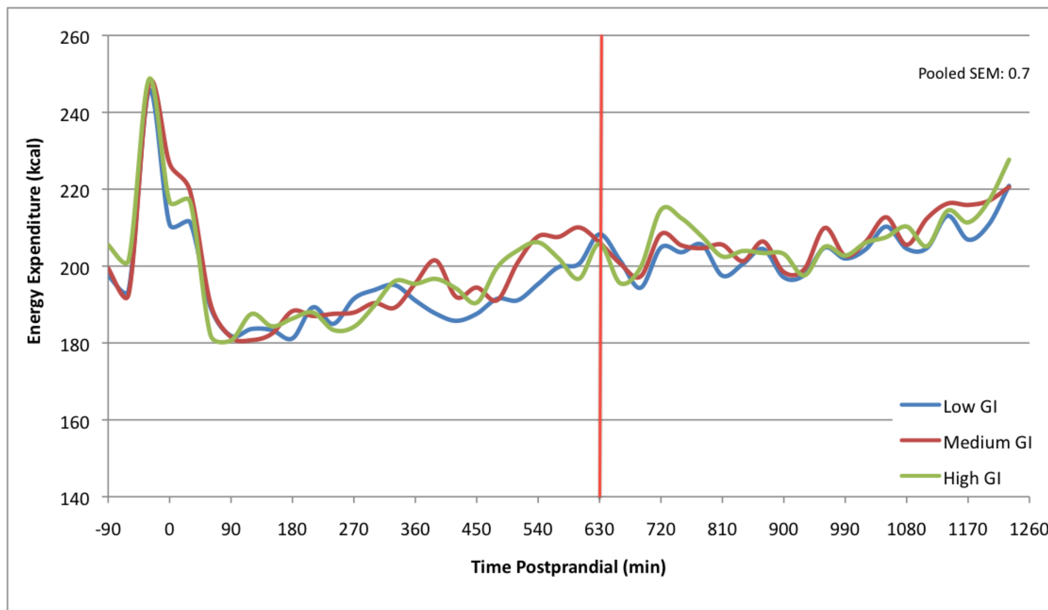

Figure 3.3. Total daily resting energy expenditure of cats fed diets with high, medium, and low predicted glycemic index (GI). Measurements were taken in 30 min intervals. Cats (n = 19) were fed at t = 0 min and equipment calibration occurred at t = 630 min.

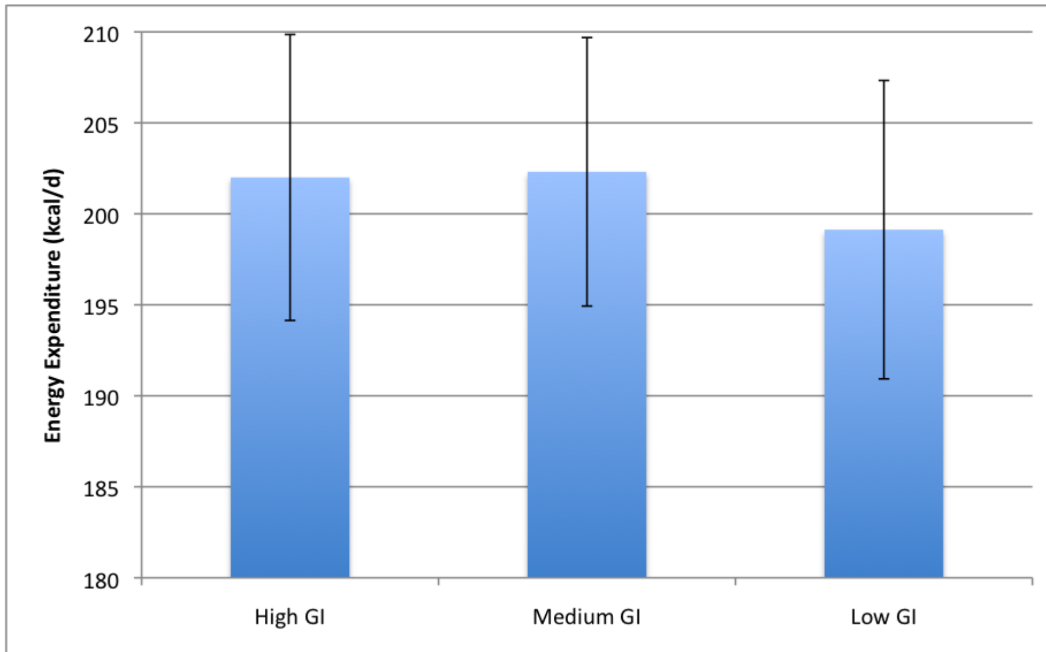

Figure 3.4. Total daily resting energy expenditure of cats (n=19) fed diets with high, medium, and low predicted glycemic index (GI) for the measurement period of 22 h.

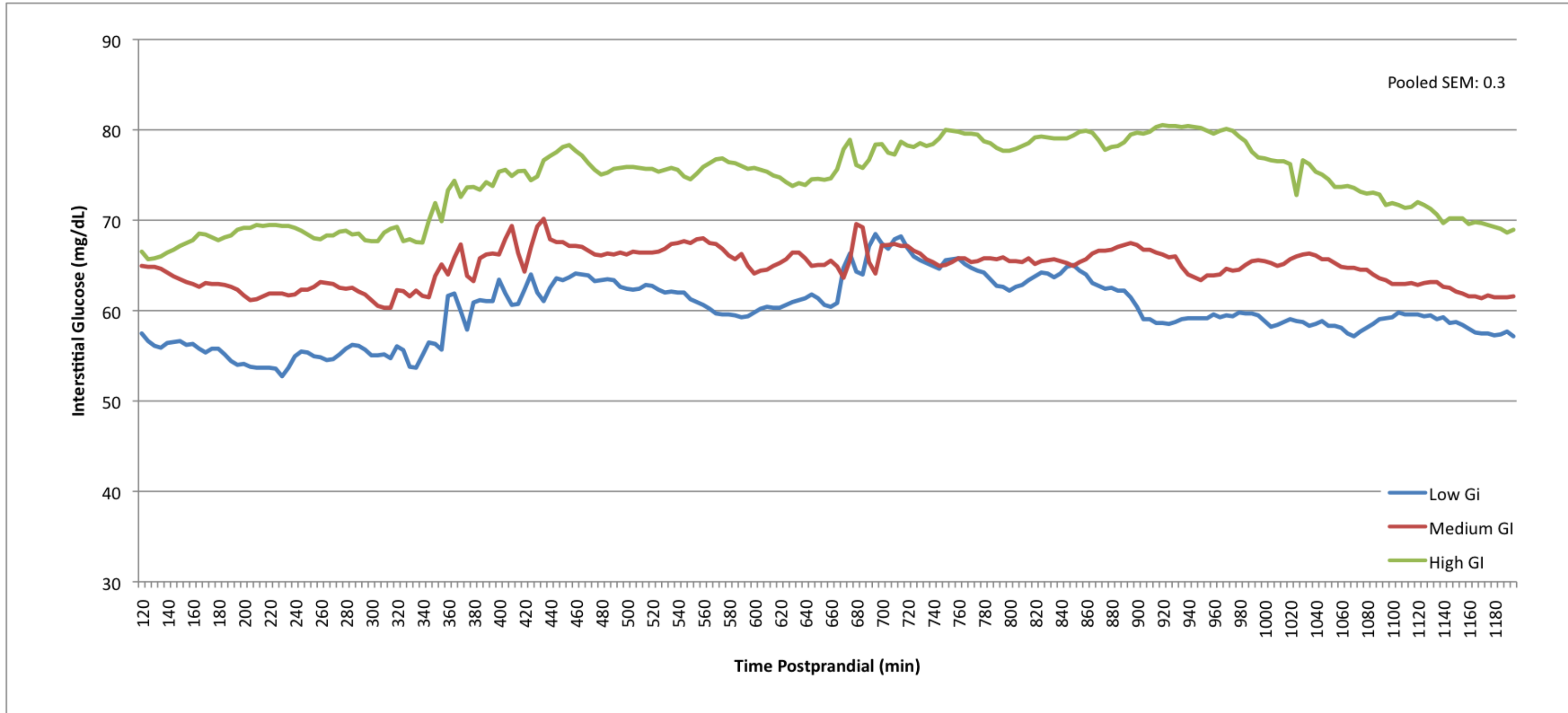

Figure 3.5. Interstitial glucose concentrations of cats (n=19) fed diets with high, medium, and low predicted glycemic index (GI) over a measurement period of approximately 20 h on d 9. Cats were fed at t = 0 min.

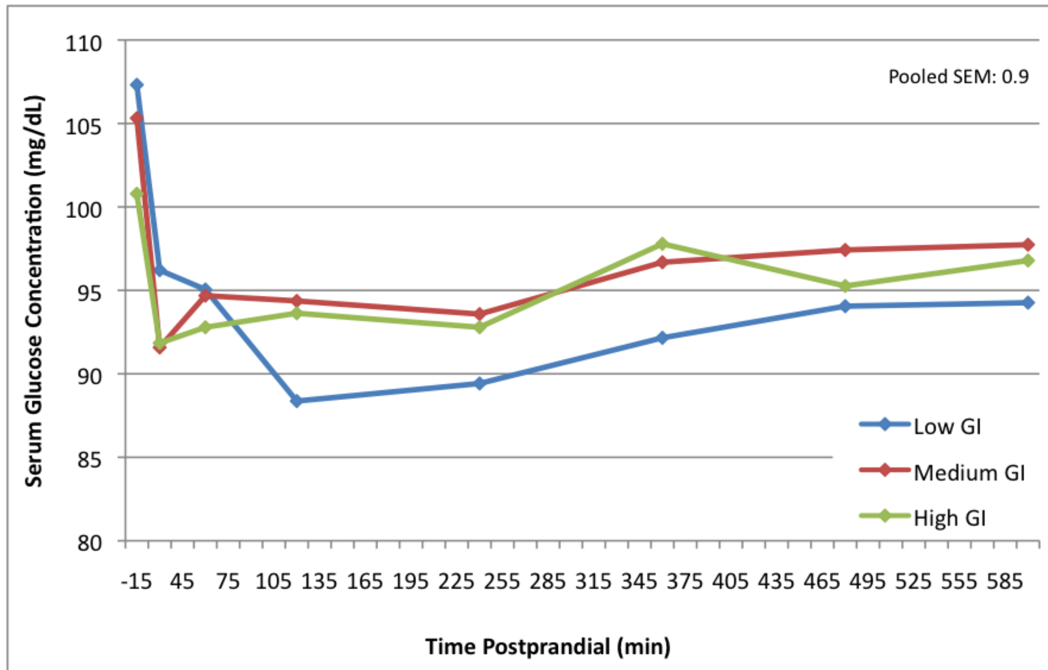

Figure 3.6. Serum glucose concentrations of cats (n=19) fed diets with high, medium, and low predicted glycemic index (GI) after 8 sequential blood draws on d 10 (*P*<0.591). Cats were fed at t = 0 min.

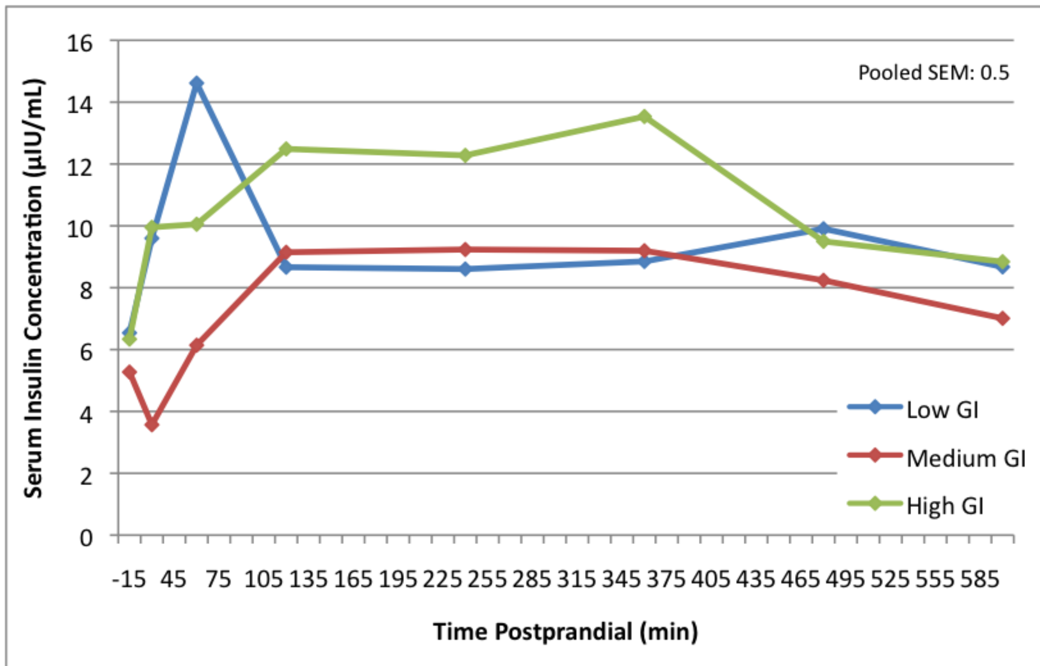

Figure 3.7. Serum insulin concentrations of cats (n=19) fed diets with high, medium, and low predicted glycemic index (GI) after 8 sequential blood draws on d 10 (*P*<0.591). Cats were fed at t = 0 min.

Table 3.1. Guaranteed nutrient composition and predicted glycemic index of the 3 experimental diets.

	Predicted GI[1]		
Item (as fed, %)	High	Medium	Low
CP, min.	34.0	33.0	36.0
Crude fat, min.	13.0	21.0	20.0
Crude fiber, max.	2.0	3.0	2.5
Moisture, max.	12.0	10.0	10.0
Ash, max.	-	7.0	-
Mg, max.	-	0.1	-
Taurine	0.15	0.16	0.3
n-6 Fatty acid, min.	1.5	2.76	3.6
n-3 Fatty acid, min.	-	0.29	0.35

[1]GI = glycemic index.

[2]Purina ONE Chicken and Rice (Nestlé, St. Louis, MO) with main ingredients: Chicken, brewer's rice, corn gluten meal, poultry by-product meal, wheat flour, animal fat preserved with mixed-tocopherols, whole grain corn, soy protein isolate, fish meal, animal liver flavor, KCl, H_3PO_4, $CaCO_3$, caramel color, choline chloride, and salt.

[3]Iams Kitten Proactive Health (Procter & Gamble, Cincinatti, OH) with main ingredients: Chicken, chicken by-product meal, corn meal, chicken fat preserved with mixed tocopherols, dried beet pulp, ground whole grain sorghum, dried egg product, natural flavor, fish oil preserved with mixed

tocopherols, KCl, fructooligosaccharides, choline chloride, $CaCO_3$, brewer's dried yeast, DL-Met, and salt.

[4]Innova (Procter & Gamble, Cincinatti, OH) with main ingredients: Turkey, chicken, chicken meal, whole grain barley and whole grain brown rice, chicken fat preserved with mixed tocopherols, peas, natural flavors, apples, herring, flaxseed, eggs, blueberries, pumpkin, tomatoes, sunflower oil, KCl, DL-Met, carrots, pears, cranberries, menhaden oil, cottage cheese, taurine, green beans, alfalfa sprouts, parsnips, and salt.

Table 3.2. Analyzed nutrient composition of the 3 experimental diets[1,2,3].

Item	High GI	Medium GI	Low GI
CP[4], %	38.02	35.86	42.06
Moisture, %	7.16	6.76	5.31
Ash, %	6.36	6.31	6.38
NFE,[5] %	34.1	29.5	23.6
Starch, %	36.75	30.72	23.56
Ether extract, %	15.7	22.2	22.9
Crude Fat, %	10.83	20.02	20.42
Crude fiber, %	1.17	1.78	2.58
ADF[6], %	1.88	2.95	2.43
NDF[7], %	7.36	12.58	10.57
Available Lysine, %	1.62	1.91	2.80
GE, kcal/kg	4,916	5,253	5,462
Calculated ME[8], kcal/kg	3,752	4,081	4,137
DM digestibility[9], %	91.14	90.74	92.70

[1]Each diet was analyzed in triplicate.

[2]High GI diet was Purina ONE Chicken and Rice (Nestlé, St. Louis, MO), and the medium and low GI diets were Iams Kitten Proactive Health and Innova, respectively (Procter & Gamble, Cincinatti, OH).

[3]Results (except moisture) presented on a dry-matter basis.

[4]Percentage N X 6.25.

[5]NFE = N-free extract.

[6]ADF = Acid detergent fiber.

[7]NDF = Neutral detergent fiber.

[8]Calculated with modified Atwater equation (AAFCO, 1997):

$$ME\left(\text{kcal/kg}\right) = 3.5 \times \text{CP } (\%) + 3.5 \times \text{carbohydrate } (\%) + 3.5 \times \text{crude fat } (\%).$$

[9]Determined using *in vitro* dry matter digestibility laboratory analysis.

Table 3.3. Analyzed amino acid composition of the 3 experimental diets[1, 2, 3].

Amino acid (%)	High GI	Medium GI	Low GI
Taurine	0.22	0.26	0.37
Hydroxyproline	0.50	0.73	0.69
Aspartic Acid	2.76	2.77	3.50
Threonine	1.29	1.32	1.61
Serine	1.50	1.28	1.38
Glutamic Acid	6.00	4.30	5.52
Proline	2.55	1.82	2.00
Lanthionine	0.00	0.00	0.00
Glycine	2.08	2.61	2.70
Alanine	2.48	2.15	2.40
Cysteine	0.48	0.42	0.42
Valine	1.66	1.61	1.94
Methionine	0.75	1.08	1.38
Isoleucine	1.40	1.32	1.70
Leucine	3.81	2.61	3.01
Tyrosine	1.38	1.08	1.30
Phenylalanine	1.78	1.37	1.64
Hydroxylysine	0.17	0.15	0.19
Ornithine	0.08	0.06	0.06
Lysine	1.74	2.11	2.94
Histidine	0.80	0.78	1.02
Arginine	1.92	2.16	2.70
Tryptophan	0.31	0.34	0.40
Total	35.65	32.33	38.87

[1]Each diet was analyzed in triplicate.

[2]High GI diet was Purina ONE Chicken and Rice (Nestlé, St. Louis, MO), and the medium and low GI diets were Iams Kitten Proactive Health and Innova, respectively (Procter & Gamble, Cincinatti, OH).

[3]Results presented on a dry-matter basis.

Table 3.4. Indirect calorimetry measurements of cats (n=19) fed diets with high, medium, and low predicted glycemic index (GI) for the measurement period of 22 h.

	GI				
Variable	High	Medium	Low	SEM	*P*-value
RQ					
Mean	0.78[a]	0.77[b]	0.76[c]	0.001	<0.001
Mean Fasting[1]	0.75	0.75	0.74	0.005	0.452
Mean Fed[2]	0.78[a]	0.77[b]	0.76[c]	0.001	<0.001
REE (kcal/d)					
Mean	202.0	202.3	199.1	0.72	0.767
Mean Fasting	203.6	196.1	195.7	6.814	0.765
Mean Fed	201.9	202.6	199.3	1.260	0.778
Fat oxidation (g/h)					
Mean	0.65[b]	0.68[ab]	0.69[a]	0.006	0.057
Mean Fasting	0.74	0.74	0.74	0.031	0.985
Mean Fed	0.64[b]	0.68[ab]	0.69[a]	0.006	0.050
Carbohydrate oxidation (g/h)					
Mean	0.61[a]	0.52[b]	0.46[c]	0.010	<0.001
Mean Fasting	0.39	0.32	0.29	0.041	0.358
Mean Fed	0.62[a]	0.53[b]	0.46[c]	0.010	<0.001

[1]Fasting measurements were taken at t = -60 and t = -30 min.

[2]Fed measurements were taken at t = 0 min onward.

Table 3.5. Interstitial glucose and sequential blood sampling measurements of cats (n=19) fed diets with high, medium, and low predicted glycemic index (GI) for the measurement period of 1 d.

	GI				
Variable	High	Medium	Low	SEM	*P*-value
Interstitial glucose					
Mean (mg/dL d^{-1})	73.4[a]	64.4[b]	59.7[b]	0.29	0.014
Serum glucose (mg/dL d^{-1})					
Mean	95.2	96.4	94.6	0.93	0.591
Mean Fasting[1]	100.8	105.3	107.3	2.47	0.169
Mean Fed[2]	94.4	95.2	92.8	0.55	0.680
Mean Peak	100.8	105.3	107.3	2.86	0.839
Serum insulin (μIU/mL d^{-1})					
Mean	10.4[a]	7.2[b]	9.4[ab]	0.48	<0.001
Mean Fasting	6.34	5.27	6.54	0.265	0.468
Mean Fed	10.95[a]	7.50[b]	9.84[ab]	0.31	0.016
Mean Peak	13.5	9.24	14.6	1.80	0.240
Glucose : Insulin					
Mean	9.18	13.3	10.0		
Mean Fasting	15.9	20.0	16.4		
Mean Fed	8.62	12.7	9.43		

[1]Fasting measurements were taken at t = -60 and t = -30 min.

[2]Fed measurements were taken at t = 0 min onward.

Chapter 4. Thesis summary and Implications

4.1 Project Summary

This thesis included 2 chapters to add to the body of research to understand the digestive peculiarities of domestic cats. Chapter 2 examined the effect of diet glycemic index (GI) on its metabolizable energy (ME) value. Determined ME values were then compared to ME values predicted by 2 common equations, the traditional and modified Atwater equations (Atwater, 1902; AAFCO, 1997). It was hypothesized that measured diet ME values would be inversely related to predicted GI of diets.

Chapter 3 analyzed the physiological effects of 3 commercial diets with high, medium, and low predicted GI using indirect calorimetry, sequential blood sampling, and real-time continuous interstitial glucose monitoring. This gave us information on changes in respiratory quotient (RQ), total energy expenditure (TEE), carbohydrate oxidation, fat oxidation, serum glucose and insulin, and interstitial glucose levels. It was hypothesized that greater dietary GI would decrease energy metabolism and increase serum glucose and insulin compared to medium and low GI diets.

4.2 Conclusions and Implications

In Chapter 2, the ME value was greatest for the low GI diet, and least for the high GI diet, in agreement with our hypothesis. However, instead being a direct effect of GI, the greater fat content and digestibility of the low GI diet might have increased ME. Because commercial diets were used,

macronutrient fractions were not kept constant; thus, a direct relation between GI and ME could not be made.

Inaccuracies in the modified Atwater equation became apparent, which was troubling, because it is the most prevalent predictive equation used in the pet food industry to calculate daily food allotment. For all 3 diets, ME determined in cats exceeded the ME predicted using the modified Atwater equation. Determined ME values were also greater than values predicted using the traditional Atwater equation, but by a smaller margin. These underestimated ME values might be due to the high quality via high energy digestibility of the 3 diets. The modified Atwater equation might be satisfactory for average quality pet foods with traditional ingredients, but the traditional Atwater equation is a better indicator for premium pet foods that incorporate ingredients that have greater digestibility than average ingredients. Cat diets are more energy dense in general than dog diets; therefore, the traditional Atwater equation should be used for all cat diets. In high quality diets, use of the modified Atwater may underestimate dietary ME values resulting in subsequent overfeeding and weight gain (Yamka et al., 2007).

In Chapter 3, cats fed the high GI diet had a greater RQ, indicative of increased carbohydrate oxidation at the expense of fat oxidation. The TEE did not differ among GI diets, because cats were fed identical calories. Fat oxidation was greatest for the low GI diet, which contained most fat. Although increasing fat intake does not increase fat oxidation in humans

(Schutz et al., 1989), this effect is seen in cats (Gooding et al., 2013; Lester et al., 1999). Carbohydrate oxidation was greatest for the high GI diet, which had the greatest starch content. Carbohydrate and fat oxidation were inversely related, similar to humans (Acheson et al., 1984).

Interstitial glucose data over time was in agreement with our hypothesis, with the greatest value for the high GI diet, followed by the medium and low GI diets. Interstitial glucose changes lagged 60 min behind serum glucose.

Serum glucose did not follow our hypothesis, and did not differ among diets. Serum glucose did not peak postprandially; rather, serum glucose peaked 15 min preprandially and leveled out over time. This finding contrasted cat studies with a postprandial glucose peak followed by return to baseline (de-Oliveira et al., 2008), but may have been due to increased activity level of cats following their transfer to cages resulting in hyperglycemia (Rand et al., 2002). Dietary fat and protein did not affect serum glucose in cats previously (Farrow et al., 2013), which could relate to this study as fat content differed between diets. Diet GI did not affect peak or overall serum glucose; thus, avoiding postprandial glucose spikes and potential β-cell exhaustion might not be a concern in cats (Rand et al., 2004). As indicated in Chapter 1, cats may metabolize a certain amount of dietary starch without detriment (Lauten et al., 2000; Vester et al., 2009; Hooda et al. 2013). In Chapter 3, cats managed up to 37% dietary starch without experiencing ill effects.

Interestingly, although diet GI did not change serum glucose, serum insulin was greater for the high GI diet than the medium GI diet in the fed state. Our hypothesis was that serum insulin and glucose would be interdependent, which was not the case, but the high GI diet provoking the greatest insulin response aligned with our hypothesis. Although it seems strange that serum insulin would be affected by dietary GI independent of glucose, the cat's inherent lack of glucokinase may minimize its ability to act as a glucose sensor in pancreatic β-cells, inhibiting β-cell metabolism of glucose and subsequent insulin secretion (Hewson-Hughes et al., 2011). Insulin responses of cats were inconsistent among studies. The overarching theme, however, is that feline insulin and glucose responses are less pronounced and more prolonged by starch than in omnivorous monogastric species (Kienzle, 1994; Bouchard and Sunvold, 2000; Carciofi et al., 2008; Johansson et al., 2013).

4.3 Limitations in the Research

In both chapters, commercial diets were used, and GI was predicted based on dietary ingredients and nutrient composition. The GI was not quantified, because the mixture of dietary ingredients was not included in human food GI databases. Instead, macronutrient fractions were more useful than GI to explain results. Moreover, since macronutrient fractions were not held constant, the greater fat content in the medium and low GI diets may be important. Another measurement such as glycemic load (GL), the weight of

dietary glucose required to raise blood glucose by the same amount as the amount of food (Wolever et al., 2004), may be more appropriate. GL can be calculated using the following equation (Wolever et al., 2004):

$$GL = \frac{GI \times \text{available carbohydrate (g)}}{100} \qquad \text{[Eq. 1]}$$

However, a numerical value for GI is still required for this measurement.

In Chapter 3, interstitial glucose sensor consistency was problematic. Since the interstitial glucose sensors were developed for human use (FDA, 2007), the lower limit of sensors of 40 mg/dL was not low enough for some cats, mainly those fed the low GI diet, and caused inaccurate readings when interstitial glucose dropped below this limit. Although the sensors had been used successfully in humans, horses, and dogs (Wiedmeyer et al., 2003), using more sensitive sensors with a lower threshold might be more suitable for future feline research. Sensor placement may also be reevaluated, because research might be more consistent with sensors placed at the dorsal neck rather than in the thoracic region (Hafner et al., 2013).

In Chapter 3, body weight was unexpectedly a significant covariate, because it had been corrected for by individualized diet allowance per cat (de-Oliveira et al., 2008). Body weight is known to influence glucose regulation and insulin sensitivity in cats (Rand et al., 2004), however this was not investigated in this study. We also could have examined female vs. male effects, because cat peak glucose and postprandial glycemia differ between sexes (Farrow et al., 2013).

4.4 Future Research

Although this study expands the knowledge about the domestic cat's ability to digest and metabolize starch, much is still unknown. Feline digestive physiology has many idiosyncrasies, and mechanisms cannot be effectively extrapolated from humans or dogs. In the growing pet food industry driven by consumer demand for high quality diets, a focus to develop diets optimal for cat health and longevity is essential so that feline research catches up to canine research.

The theory proposed by Hewson-Hughes et al. (2011) for the cat's minimal insulin response is compelling. Characterizing the cat's pancreatic β-cell activity in relation to enzyme levels of glucokinase may explain the minimal metabolic consequences of starch intake on serum glucose and insulin. This could then support the determination of an upper limit and optimum level of starch intake in cats, neither of which have been established.

Future research could assess serum glucose and insulin responses of healthy adult cats fed diet with a more equal macronutrient distribution to show clearer effects of alterations in starch content or chemistry on blood metabolites and hormones. Chapter 3's analysis of commercial diets was important for consumers, because these diets are purchased by cat owners. However, with the knowledge gained in the present MSc thesis, specific formulations with a high content of starch differing in its chemistry may yield

more definitive results on the impact of starch on energy expenditures and glucose and insulin responses.

In conclusion, although this MSc thesis did contribute to the understanding of how cats digest and metabolize starch, much of the domestic feline's physiology remains a mystery. This thesis uncovered some unexpected results that will serve to generate dietary recommendations by pet food companies. The pet food industry is consumer-driven, where raw and grain-free diets are marketed as superior. However, this thesis and other studies documented the ability of cats to metabolize dietary starch without short-term negative effects. Future research will uncover the cat's digestive physiology in relation to dietary starch. Such research will lead to improved health and longevity of cats around the world.

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Appendix 1. Power Analysis

Data used to calculate the power analysis came from the abstract "Effects of a high vs. low carbohydrate diet on fat and carbohydrate oxidation in cats" by Shoveller, A.K. et al., 2010 (energy expenditure, EE) and from the paper "Effects of six carbohydrate sources on diet digestibility and postprandial glucose and insulin responses in cats" by de Oliveira et al., 2008 (blood glucose and insulin concentrations). These papers are similar to the current study and will allow us to obtain a good approximation of sample size power.

The following 3 power analysis outputs were given for energy expenditure, blood glucose and blood insulin concentrations.

For EE:

One-Way ANOVA 12:47 Tuesday, February 5, 2013 1

Treatments = 3 CSS of Means = 12.5

Standard Deviation = 3.536 Alpha = 0.05

N per Group	Power
10	0.767
11	0.812
12	0.850
13	0.881
14	0.906
15	0.927
16	0.943
17	0.956
18	0.966
19	0.974

20	0.980
21	0.985
22	0.988
23	>.99
24	>.99
25	>.99
26	>.99
27	>.99
28	>.99
29	>.99
30	>.99

With our sample size of 19 cats we have 97.4% power.

For glucose:

One-Way ANOVA 12:47 Tuesday, February 5, 2013 2

Treatments = 3 CSS of Means = 198.06

Standard Deviation = 14.073 Alpha = 0.05

N per Group	Power
10	0.767
11	0.812
12	0.850
13	0.881
14	0.906
15	0.927
16	0.943
17	0.956
18	0.966
19	0.974
20	0.980

21	0.985
22	0.988
23	>.99
24	>.99
25	>.99
26	>.99
27	>.99
28	>.99
29	>.99
30	>.99

With our sample size of 19 cats we have 97.4% power.

For insulin:

One-Way ANOVA 12:47 Tuesday, February 5, 2013 3

Treatments = 3 CSS of Means = 64.92

Standard Deviation = 8.057 Alpha = 0.05

N per Group	Power
10	0.767
11	0.812
12	0.850
13	0.881
14	0.906
15	0.927
16	0.943
17	0.956
18	0.966
19	0.974
20	0.980
21	0.985

22	0.988
23	>.99
24	>.99
25	>.99
26	>.99
27	>.99
28	>.99
29	>.99
30	>.99

With our sample size of 19 cats we have 97.4% power.

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Appendix 2. Protein and Amino Acid Requirements of Cats

Protein

The current recommendation of protein intake in cats at maintenance is 20% of ME as protein (AAFCO, 2008), or 40 g protein/1,000 kcal ME (NRC, 2006). One theory regarding the current high protein requirement of felines is that they may not have a high protein demand, but rather a high glucose demand that must be met through gluconeogenesis (Eisert, 2011). Gluconeogenesis, the generation of glucose from amino acids (AA) and other non-carbohydrate substances, is the main energy providing pathway used in the feline species (Eisert, 2011). Felines have a high rate of gluconeogenesis to compensate for the lack of dietary sugar intake. Even with inclusion of carbohydrate in the diet, cats do not decrease rate of gluconeogenesis (Eisert, 2011). Glucose is the only energy substrate that can be used by certain tissues, including the brain, spinal cord, red blood cells, renal medulla, testes, uterus during pregnancy, and mammary glands during lactation (Eisert, 2011). Thus, because of the relatively low dietary glucose intake of cats, a high protein requirement is recommended for gluconeogenesis; currently, however, it appears that there is not consensus on the optimal level of dietary proteins for cats.

Researchers had previously considered that cats may have such a high protein requirement due to an inability to decrease protein oxidation (Rogers et al., 1977). However, cats were found to adapt their protein oxidation to the

amount of protein in the diet by comparing a high (HP) to a moderate protein diet (MP) (52 vs. 35% energy, respectively) using indirect calorimetry (Russel et al., 2002). Cats fed HP had a protein oxidation value of 23.0 ± 0.6 g/18h that was down-regulated to 15.6 ± 0.6 when fed the MP diet (Russel et al., 2002). This finding leaves the specific mechanistic reasons for the cat's innate high protein requirement unknown.

One important reason that cats require dietary protein is for the essential AA it provides. Protein is also essential for maintaining N balance and protein turnover, similar to other species. This is especially important during growth and also aging to combat age-associated lean body mass loss (LaFlamme, 2008). Cats need approximately 5.2 g/kg body mass of dietary protein per day (LaFlamme, 2008). However, maximal dietary protein is not beneficial. A popular consumer perspective is that the best dog or cat diet is one with the highest level of protein available. In humans, protein intake above dietary recommendations is excreted or stored as body fat; a storage system for protein does not exist (WHO, 2002). In addition, there may even be risk of certain AA toxicities such as branched chain, sulfur or aromatic AA that are required only at very low levels (WHO, 2002). It is unknown if this should be of concern in dogs or cats. Long-term excess protein intake is also very taxing on the kidneys and may cause lasting damage in humans (WHO, 2002); however, this has not yet been demonstrated long-term in dogs (Finco et al. 1992). Cats with existing kidney disease are recommended to be fed a diet lower in protein and P (Forrester and Kirk, 2009). Effects of dietary protein

above recommended guidelines have not been thoroughly studied in cats, however it is clear that some detrimental effects are present. Excess protein intake causes low P and Ca retention, which in turn causes much higher urinary excretion of P (Hashimoto et al., 1996). This leads to an imbalance in the Ca : P ratio which, if not corrected with dietary supplementation, would lead to bone mineral resorption (Hashimoto et al., 1996). High levels of dietary protein can also aggravate clinically severe hepatic disease or hepatic encephalopathy (Forrester and Kirk, 2009). Effects of excess protein are not well studied in cats, but appear to be important.

Urolithiasis and crystal formation in the feline urinary tract is a problem that has markedly increased in the United States over the past 30 years (Dijcker et al., 2011). Incidence rates of urolithiasis are estimated to be between 0.2 and 0.7% of cats (Lund et al., 1999). The 2 most common types of urinary crystallization are struvite urolithiasis and calcium oxalate urolithiasis (Dijcker et al., 2011). Main risk factors for struvite urolithiasis include basic urine and increased urinary Mg excretion, and risk factors for Ca oxalate urolithiasis include acidic urine and increased urinary calcium and oxalate excretion (Dijcker et al., 2011). These 2 types of crystals are opposite in some of their dietary recommendations: struvite uroliths are best combated through urine-acidifying diets and limiting Mg. Ca oxalate uroliths are reduced through increased dietary Mg to limit urinary Ca excretion and maintain a neutral or basic urine. Since the treatment for one increases the risk for the other, cats should be monitored regularly (Osborne et al., 2009).

One common proposed means of preventing urolithiasis is to feed high protein, low carbohydrate diets. Starch in diets has been linked to increased risk of struvite urolithiasis due to higher urinary excretion of Mg and higher urinary pH (Funaba et al., 2004). The beneficial effects of this diet for cats with Ca oxalate urolithiasis can be demonstrated by the reduction of urinary oxalic acid (Zentek and Shulz, 2004). The cat has low activity of the enzyme alanine:glyoxylate aminotransferase 1 (AGT1) in the mitochondria, responsible for conversion of glyoxylate to glycine (Dijcker et al., 2011). Glyoxylate must be removed from liver cells to prevent oxalate excretion and potential crystal formation through combination with Ca (Zentek and Shulz, 2004). The AGT1 activity is high in herbivorous species due to their high consumption of glyolates (precursors of oxalates) and carbohydrates, but is very low in cats due to their carnivorous nature (Dijcker et al., 2011). Sugars are a precursor of the peroxisomal glyoxylate pathway, and may overload the cat with hepatic oxalate causing high urinary oxalic acid (Dijcker et al., 2011). Zentek and Schultz (2004) compared 3 high protein/low carbohydrate with 3 low protein/high carbohydrate diets in cats with respect to urinary oxalic acid excretion. The protein sources in each of the diets were collagen tissue, horse meat, and soya isolate respectively, and the level of protein in the diets was altered through addition of rice and animal fat (77.6 vs. 22.6% DM CP collagen tissue, 64.2 vs. 27.3 % DM CP soya isolate, and 64.0 vs. 21.7% DM CP horse meat). Overall, protein intake was inversely correlated with oxalic acid excretion, indicating that high protein diets decrease the risk to develop Ca

oxalate urolithiasis. Oxalic acid excretion was highest with the low protein collagen tissue diet and lowest with the high protein horse meat diet (13.7 ± 4.3 vs. 0.9 ± 0.3 mg kg $BW^{-1}d^{-1}$, respectively; Zentek and Schultz, 2004).

Low protein/high carbohydrate intake might decrease the need for gluconeogenesis in the liver, and its precursors Gly and Ser. High Gly and Ser concentrations may cause a shift to oxalate synthesis through their participation in a negative feedback loop with AGT1 (Dijcker et al., 2011). Higher oxalate excretion did not equate to greater incidence of crystal formation; however, this causative effect is not ruled out due to the increasing incidence of hypercalciuria in cats that may lead to Ca oxalate urolithiasis (Hashimoto et al., 1996). Urinary oxalate excretion is noted to be the most critical risk factor for Ca oxalate urolithiasis, of greater importance than Ca excretion due to its higher potency (Dijcker et al., 2011). High protein intake also increases water intake, resulting in dilute urine and decreased risk of both types of crystal formation (Dijcker et al., 2011).

For these reasons it may be advisable that cats prone to urolithiasis should be fed high, but not excessive, protein diets with low carbohydrate content. Protein source is also important to consider because it will also affect urinary oxalate excretion and potentially crystallization with Ca. Whether it is the high protein or low carbohydrate element that decreases risk of urolithiasis has not yet been determined (Funaba et al., 2004). In fact, some controversy exists to whether high or low protein diets are best for managing Ca oxalate urolithiasis in cats (Lekcharoensuk et al., 2001). The

focus on protein inclusion level might not be correct. The explanation of Dijcker et al. (2011) of the involvement of sugars in the peroxisomal glyoxylate pathway points toward dietary starch as the potential true risk factor. Further research in this area of feline health is required.

Fortunately, feline nutritional science is progressing, and techniques for information discovery are being refined. Hendriks et al. (1997) examined the effectiveness of 2 methods to measure endogenous urinary N (EUN) excretion, because EUN is an important measure of basal protein metabolism and can be used to determine protein requirements. The first method measured N excretion from 6 cats fed a protein-free diet. The second method used the regression technique, which allows cats to be under more physiologically normal conditions while regression to zero protein intake was achieved by feeding 16 animals graded levels of protein (Hendriks et al., 1997). Feline EUN excretion was higher when cats were fed the protein-free diets compared to other animals, possibly due to the naturally higher excretion of N as urea, or due to the high AA demand of tissues causing protein breakdown that exceeds intake (Hendriks et al., 1997). Obligate carnivores do not have the ability to conserve nitrogen compared to other animals, due to their non-adaptive hepatic enzymes involved in AA catabolism (Hendriks et al., 1997); thus, the cat will lose N of catabolized AA when fed a protein-free diet.

One criticism of the protein-free diet technique is that the EUN excretion is not constant but rather declines with time (Hendriks et al., 1997). This

confounds results, as a lower EUN excretion value may be due to a longer period of being fed a protein-free diet, not because of protein requirement. Hendriks et al. (1997) found lower EUN excretion values when using the regression technique. The reason is unclear; however, catabolism of body AA for energy might be higher because of insufficient energy production with the protein-free diet, and thus EUN excretion is higher (Hendriks et al., 1997).

These techniques measuring protein requirement are important when determining the need of a full-protein diet for pet cats. The regression technique is a more accurate and appropriate measure of EUN excretion (Hendriks et al., 1997), because the animal is in a physiologically-normal state. This technique will give more accurate data for calculating true protein requirement for cats, which can then be used to formulate cat food.

Amino Acids

Felines have a dietary requirement for Arg (1.04% of DM minimum for maintenance; AAFCO, 2008), as they are unable to synthesize ornithine from Gln or Glu due to the low activity of pyrroline-5-carboxylate synthase and ornithine aminotransferase (Baker and Czarnecki-Maulden, 1991). In cats, Arg deficiency causes hyperammonemia, which can cause hypersalivation, hyperactivity, ataxia, hypothermia and even death (Baker and Czarnecki-Maulden, 1991). Symptoms of hyperammonemia in the cat may be seen after a single Arg-free meal (Baker and Czarnecki-Maulden, 1991). Canines also

exhibit some symptoms of hyperammonemia when deprived of dietary Arg, but to a far lesser extent than felines (Baker and Czarnecki-Maulden, 1991).

Cats require more sulfur-containing AA than dogs, in particular Met and Cys (0.62 vs. 1.1% DM minimum; AAFCO 2008), and are unable to use D-Met and DL-hydroxymethionine as efficiently as dogs (Baker and Czarnecki-Maulden, 1991). Both Met and Cys are important to promote acidic urine and prevent struvite urolithiasis, a condition of crystal formation in the urinary tract (Baker and Czarnecki-Maulden, 1991).

Inadequate levels of Met and Cys cause a requirement for dietary taurine, due, in part, to a high physiological demand and low cysteinesulfinic acid decarboxylase activity, which is necessary for converting Cys to hypotaurine (Baker and Czarnecki-Maulden, 1991). Taurine is important for proper nervous system excitability, neuroprotectivity and immunocompetency in the cat, and, in kittens, is necessary for proper neural development and has potential antioxidant effects (Baker and Czarnecki-Maulden, 1991). The current recommendation for dietary taurine supplementation in cats at maintenance is 0.10% DM minimum in extruded diets (AAFCO, 2008). Canned diets may be insufficient in dietary taurine, likely due to an element of heat processing that requires identification (Baker and Czarnecki-Maulden, 1991); thus, the requirement was raised to 0.20% DM minimum (AAFCO, 2008). The Cys is also used in the body to synthesize felinine, a branched chain sulfur AA excreted in the urine that may contribute to

territorial marking, as it is present in much higher concentrations in the urine of adult male cats (Baker and Czarnecki-Maulden, 1991).

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