Modelling Net Energy of Selected Commercial Diets Fed to Domestic Adult Cats

by

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ABSTRACT

MODELLING NET ENERGY OF SELECTED COMMERCIAL DIETS FED TO DOMESTIC ADULT CATS

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The ability of cats to metabolize carbohydrates is not definitively characterized. This thesis investigates carbohydrate digestibility and metabolism, and analyses the algorithms used to determine energy density and subsequent feeding recommendations for cats. Three commercial diets varying in carbohydrate level and source were compared. The modified Atwater equation was inaccurate at calculating metabolizable energy of commercial cat diets. Furthermore, carbohydrate type and level did not negatively affect macronutrient digestibility, and had minimal effects on postprandial glucose and insulin responses of cats. Last, the heat increment of feeding was used to model energy density on a net energy basis and develop equations that better estimate dietary energy availability for cats. These results suggest that despite the inherent carnivorous nature of domestic cats, they efficiently digest diets with significant contributions from carbohydrates. The energy required for utilizing these nutrients is similar to other monogastric species, although these processes take more time.
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List of Abbreviations

AAALAC: Association for Assessment and Accreditation of Laboratory Animal Care

AAFCO: Association of American Feed Control Officials

ADF: acid detergent fiber

AIC: Akaike Information Criterion

AOAC: Association of Official Analytical Chemists

ARMA: Autoregressive Moving Average Model

BMR: basal metabolic rate

BW: body weight

°C: degree Celsius

Ca: calcium

CaCO₃: calcium carbonate

CO₂: carbon dioxide

CF: crude fiber

CL: crude lipid

cm: centimetre

CP: crude protein

CS: Compound Symmetry

d: day(s)

dL: decalitre(s)

DE: digestible energy

DL-Met: DL-Methionine

DM: dry matter
DMD: dry matter disappearance

DP: degree of polymerisation

EE: energy expenditure

ELISA: Enzyme-linked Immunosorbent Assay

EME: estimated metabolizable energy

FEDIAF: European Pet Food Industry Federation

g: gram(s)

g: gravitational force

GE: gross energy

GI: glycemic index

GL: glycemic load

h: hour(s)

HCl: hydrochloric acid

HIF: heat increment of feeding

H_3PO_4: phosphoric acid

kcal: kilocalorie(s)

KCI: potassium chloride

kJ: kilojoule(s)

kg: kilogram(s)

L: litre(s)

m: metre(s)

ME: metabolizable energy

min: minute(s)
**mg:** milligram(s)

**mL:** millilitre(s)

**n:** sample size

**N:** nitrogen

**NDF:** neutral detergent fiber

**NE:** net energy

**NFE:** nitrogen-free extract

**NRC:** National Research Council

**NSP:** non-starch polysaccharide(s)

**O₂:** oxygen

**OM:** organic matter

**P:** phosphorus

**PGR:** perceived glycemic response

**PHNC:** Pet Health and Nutrition Centre

**R²:** coefficient of determination

**RFMR:** resting fed metabolic rate

**rMSPE:** root mean square percentage error

**RQ:** respiratory quotient

**SAS:** statistical analysis system

**SD:** standard deviation

**SEM:** standard error of the mean

**t:** time

**TDF:** total dietary fiber
UE: urinary energy

µIU: micro international unit(s)

USDA: United States Department of Agriculture

VCO$_2$: carbon dioxide production

VO$_2$: oxygen consumption

yr: year(s)
Chapter 1: Literature Review

1.1. Introduction

The domestic feline and its ancestors are nutritionally characterized as obligate carnivores (Bradshaw et al., 1996). However, the domestication of the cat has drastically altered the way that they are fed. Commercially available diets are fed to the majority of cats as they provide a convenient, economical, and balanced source of nutrition (Laflamme et al., 2008). The greatest difference between natural prey diets and commercial diets lies in the contribution of carbohydrates. Specifically, commercial diets contain significantly more carbohydrates than cats or their ancestors would theoretically consume in the wild (Verbrugghe and Hesta, 2017). Currently, an optimal level of carbohydrate intake to support health and well-being of domestic cats has not been established. Therefore, the nutritional effects of feeding carbohydrates to cats needs further investigation.

An estimated 59% of domestically owned cats in the United States are considered overweight or obese (APOP, 2016). This issue arises largely from overfeeding, and may be partially due to the unreliable equations that are used to meet regulatory guidelines for labeling the caloric content of pet foods (NRC, 2006). Having accurate estimates of the energy density of food is important to provide veterinary health care teams and pet owners with accurate feeding guidelines.

This review will examine the current literature regarding carbohydrate classification, carbohydrate inclusion in commercial feline diets, and the digestion and metabolism of carbohydrates by domestic cats. Furthermore, the methodologies used to determine energy density of commercial diets and subsequent feeding recommendations will be discussed.
1.2. Classification of Carbohydrates

Carbohydrates are organic molecules that consist of carbon, hydrogen, and oxygen ($\text{CH}_2\text{O}_n$), and have various physical and functional properties (NRC, 2006). Similar to other macronutrients, carbohydrates can be classified based on chemical structure and degree of polymerisation (DP) into three main groups; sugars ($1 \leq \text{DP} \leq 2$), oligosaccharides ($3 \leq \text{DP} \leq 9$), and polysaccharides ($\geq 10$ DP) (Table 1.1; Cummings and Stephen, 2007).

Sugars are comprised of monosaccharides (glucose, fructose, and galactose), disaccharides (maltose, lactose, sucrose, trehalose), and polyols (sugar alcohols) (Cummings and Stephen, 2007). Monosaccharides and polyols can be readily absorbed, while disaccharides require digestion by intestinal enzymes, i.e. disaccharidases (maltase, lactase, sucrase), prior to absorption in the small intestine (NRC, 2006).

Oligosaccharides exist as $\alpha$-glucans (malto-oligosaccharides), occurring primarily from starch hydrolysis, and non-$\alpha$-glucans such as fructo- and galacto-oligosaccharides (Cummings and Stephen, 2007). These molecules consist of three to ten monosaccharide units joined by glycosidic bonds (Pazur, 1970), and are resistant to enzymatic digestion (Slavin, 2013). Thus, they are fermented by microbial enzymes in the large intestine (Asp, 1995).

Polysaccharides are long chain carbohydrates, consisting of more than ten monosaccharide residues, and can be further divided into starch and non-starch polysaccharides (NSPs), based on digestibility and type of glycosidic linkage within the molecule (Stephan, 1983; Englyst, 1989; Bach-Knudsen, 1997). Starches are the principal carbohydrate in most extruded kibble diets, and exist as amylose and amylopectin chains joined by $\alpha$-glycosidic bonds (Asp, 1995). It is generally accepted that starches are completely digestible, though products that escape digestion and absorption in the small intestine, otherwise known as resistant starches, may be fermented in the
large intestine (NRC, 2006). Non-starch polysaccharides exist most abundantly as structural components of plant cell walls (cellulose, hemicellulose, and pectin), but also include storage polysaccharides, gums, mucilages, and hydrocolloids (Asp, 1995; Cummings and Stephen, 2007). Unlike starches, these molecules are linked by β-glycosidic bonds, and therefore cannot be digested in the small intestine by endogenous enzymes (Stephan, 1983).
Table 1.1. Classification of dietary carbohydrates (Adapted from Cummings and Stephen, 2007).

<table>
<thead>
<tr>
<th>Class</th>
<th>Subgroup</th>
<th>Principal Components</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sugars</td>
<td>Monosaccharides</td>
<td>Glucose, fructose, galactose</td>
</tr>
<tr>
<td></td>
<td>Disaccharides</td>
<td>Sucrose, lactose, maltose, trehalose</td>
</tr>
<tr>
<td></td>
<td>Polyols (Sugar alcohols)</td>
<td>Sorbitol, mannitol, lactitol, xylitol, erythritol, isomalt, maltitol</td>
</tr>
<tr>
<td>Oligosaccharides</td>
<td>α-glucans (Malto-oligosaccharides)</td>
<td>Maltodextrins</td>
</tr>
<tr>
<td></td>
<td>Non α-glucans</td>
<td>Fructo-oligosaccharides, galacto-oligosaccharides, raffinose, stachyose, polydextrose, inulin</td>
</tr>
<tr>
<td>Polysaccharides</td>
<td>Starch</td>
<td>Amylose, amylopectin</td>
</tr>
<tr>
<td></td>
<td>Non-starch polysaccharides</td>
<td>Cellulose, hemicellulose, pectin, plant gums, mucilages, hydrocolloids, glucomannans, β-glucans, arabinoxylans</td>
</tr>
</tbody>
</table>
1.3. Carbohydrates in Commercial Cat Food

The chemical composition of starches makes them useful in the formulation of commercial pet foods. Specifically, most domestic feline diets contain between 20 % and 40 % ME from carbohydrates, with a large contribution coming from starches (Villaverde and Fascetti, 2014). Starches are utilized to add structure to foods, retain moisture, or thicken formulations (Slade and Levine, 1989; Biliaderis, 1990). Most commonly, starches are used to maintain the shape and texture of dry, extruded kibble diets, one of the most common feeding methods in North America (Forrester and Kirk, 2009). The cooking process allows starch granules to be gelatinized, therefore activating their binding capacity and providing structural integrity to kibble (Lin et al., 1997; Svihus et al., 2005).

1.4. Carbohydrate Digestion in Domestic Felines

The physiological mechanisms of carbohydrate digestion in cats are well documented in the literature (Figure 1.1; Verbrugghe et al., 2017). Compared to other animals, cats have a reduced capacity for starch digestion. Carbohydrate digestion begins in the mouth by salivary amylase, with very low levels existing in the saliva of cats in contrast to other monogastrics (McGeachin and Akin, 1979). The level of amylase is highest in the pancreas, though activity in cats is much lower in comparison to other mammals (McGeachin and Akin, 1979). Pancreatic amylase is secreted into the small intestine, where the majority of starch digestion and absorption occurs (Kienzle, 1993). Furthermore, disaccharidases present in small intestine mucosa work to break down maltose, isomaltose, and sucrose, though again, activity is low in cats compared to other species (Kienzle, 1993). Once starches are digested into their monosaccharide components, they are readily absorbed in the small intestine (NRC, 2006). Glucose and galactose are transported from the intestinal lumen into the enterocyte by Na⁺/Glucose Co-Transporter 1 (SGLT1), while
fructose is transported by the Na+ independent GLUT5 transporter (Shirazi-Beechey et al., 2011). Once inside the enterocyte, both glucose and fructose are transported into the blood by GLUT2 (Kellett et al., 2008). The low pH of the hindgut and feces of cats indicates that poorly digestible carbohydrates pass through the small intestine to the colon, where they are fermented to short chain fatty acids through microbial fermentation (Kienzle, 1993). Overall, the digestive ability of cats can be influenced by a multitude of factors including diet type, ingredient and macronutrient composition, and processing conditions.

The variability in digestibility values in domestic cats can be attributed to ingredient differences and diet type. Kerr and colleagues (2011) determined that apparent total tract energy and macronutrient digestibility values were greater in cats fed both raw and cooked meat-based diets, compared to those fed a dry extruded diet. In a similar study, domestic cats fed 1-3 day old whole chicks had lower nutrient digestibility compared to cats fed canned or extruded commercial diets, while those fed ground chicken had significantly higher digestibility than cats fed the commercial diets (Kerr et al., 2014). However, all diets in the aforementioned studies were considered to be highly digestible. Additionally, apparent starch digestibility values were > 93% in cats fed six different diets containing 35% starch from various ingredient sources including corn, brewers rice, and lentils (deOliveira et al., 2008). Furthermore, digestibility values for dry matter, organic matter, crude protein, and crude fat were 84.5, 86.0, 82.0, and 95.3%, respectively in male cats fed a high carbohydrate diet (27% starch) (Theiss et al., 2004). This indicates that not only can carbohydrates efficiently be digested by cats and fermented by microbes, but that carbohydrate intake does not seem to alter digestibility of other macronutrients. It is likely that differences in macronutrient profiles and ingredient composition influence aspects of cat health, though the implications of feeding different macronutrient profiles on feline digestive and
metabolic ability requires further research.

The processing techniques commonly used to produce commercial diets also affect their ability to be digested. Tran et al. (2008) reported that extrusion increases the digestibility of carbohydrates and starches in particular. In cats, cooking corn starch increased digestibility from 79 to 88% (Morris et al., 1977). Furthermore, high temperature extrusion decreases resistant starch and increases rapidly digestible starch, therefore improving digestibility (Murray et al., 2001). Overall, the literature supports that carbohydrates, or more specifically starches, in adequately processed commercial diets can efficiently be digested by domestic felines.
Figure 1.1. Starch digestion and absorption in domestic felines. (1) Salivary amylase begins starch digestion in the mouth, and pancreatic amylase digests starches in the small intestine. (2) Disaccharidases on the small intestinal brush border break disaccharides into monosaccharides. (3) Glucose and galactose are transported into the enterocyte via Na$^+$/Glucose Co-Transporter 1 (SGLT1). (4) Fructose is transported into the enterocyte via GLUT5. (5) Glucose, galactose, and fructose exit the enterocyte via GLUT2, and enter circulation. (Adapted from Verbrugghe and Hesta, 2017).
1.5. Feline Glycemic Response

The carnivorous nature of felines makes them unique in their metabolism of carbohydrates. The glycemic response of domestic cats has been previously studied, with variable outcomes in response to dietary carbohydrate level and source (Jenkins et al., 1981; de-Oliveira et al., 2008). The glycemic response measures the rate of glucose appearance, and consequential insulin release, into the bloodstream in response to consumption of a meal (Jenkins et al., 1981) and can be influenced by both quality and quantity of carbohydrate (Foster-Powell et al., 2002). Foods can be characterized according to their postprandial glycemic effect using glycemic indices and glycemic load values (Foster-Powell et al., 2002).

In human literature, the glycemic index (GI) is described as the proportional glycemic response that a food produces, compared to a known control, usually white bread or glucose (Jenkins et al., 1981). Foods with a high glycemic index produce a higher and more significant glucose response in the first few hours postprandially compared to foods with a low GI (Jenkins et al., 1981). Therefore, glycemic indices can be used to predict glycemic response when a mixed diet response has not been determined. Although the glycemic index provides a measure of carbohydrate quality, it does not account for carbohydrate quantity in a food (Foster-Powell et al., 2002).

Glycemic load (GL) differs from GI, as it quantifies an overall glycemic effect of a food (Salmeron et al., 1997a; Salmeron et al., 1997b; Liu et al., 2000). It is defined as the product of the GI of a food, and the amount of carbohydrate in that food (Foster-Powell et al., 2002). Therefore, the GL assesses both quality and quantity of carbohydrate, and will be different depending on the amount of an ingredient consumed, even when those ingredients have identical GI’s. Evidently, foods with a high GL are expected to have a higher glucose response than low GL foods (Foster-
Powell et al., 2002). Overall, the GL gives a more accurate prediction of glucose response compared to the GI.

In dogs, both the amount and source of starch in a diet affect glucose and insulin responses (Carciofi et al., 2008). However, the effects of carbohydrate load and source on glycemic responses in the cat are less understood. Previously, low starch diets (low load) have been reported to decrease postprandial blood glucose, while high starch diets (high load) resulted in significantly elevated postprandial glucose concentrations in cats (Hewson-Hughes et al., 2011a). Similarly, serum glucose was significantly less in lean stable-weight cats fed a low carbohydrate diet compared to cats fed a high carbohydrate diet (Coradini et al., 2011). However, additional studies have suggested that starch does not have as pronounced of an effect on the glycemic response of cats compared to other species (Kienzle, 1994; Bouchard and Sunvold, 2000; de-Oliveira et al., 2008). In cats fed six diets differing in their exclusive starch source (corn, brewer’s rice, sorghum, peas, lentils, and cassava flour), only the corn diet stimulated a significant increase in postprandial glucose from baseline values (de-Oliveira et al., 2008). In other species, work has been conducted to understand how different processing techniques may alter the glycemic response to ingredients of complete diets, but is lacking in feline research. Clearly, more work in this area is needed.

Previously, blood glucose peaked at 10 h and 18 h postprandially for cats fed two diets of similar starch content (33 %) with significant starch contributions from either rice or sorghum and corn, respectively (Appleton et al., 2004). Furthermore, blood glucose has remained elevated for upwards of 24 h in cats fed a high carbohydrate diet (Farrow et al., 2013). These studies indicate that cats seem to reach peak glucose concentrations much slower than other monogastric species (Farrow et al., 2013) although it is critical to point out that most monogastric animals are omnivores. Overall, the observation of a postprandial peak and prolonged glycemic response in
domestic felines is consistent in the literature (Appleton et al., 2004; Coradini et al., 2011; Farrow et al., 2013) and must be considered when designing studies that measure the meal response.

Feline glycemic responses are usually measured in serum using repeated blood sampling or requiring the insertion of catheters (Reineke et al., 2010). These methods are not only invasive and stressful to cats, but also cannot report glucose values in real-time (Rebrin and Steil, 2000). Conversely, interstitial glucose can be measured using minimally invasive methods including iontophoresis (Garg et al., 1999; Tamada et al., 1999), hypodermic needles (Bantle and Thomas, 1997; Service et al., 1997), and ultrasound (Kost et al., 2000) to measure glucose in subcutaneous fluid and thus, are much less stress inducing. Continuous interstitial glucose monitoring systems were evaluated as an alternative to repeated blood sampling by Wiedmeyer et al. (2003), and were well correlated with whole blood glucose levels in cats, dogs, and horses. Furthermore, interstitial glucose has also been found to mimic blood glucose in multiple species including, rabbits, rats, and humans (Rebrin et al., 1999). Thus, interstitial glucose can be considered an appropriate and less invasive alternative to monitor glycemic responses in cats.

1.6. Indirect Calorimetry

Extracting energy from a substrate is achieved through complete oxidation, resulting in consumption of O₂, and production of CO₂ and water (Ferrannini, 1988). Indirect calorimetry is a technique used to measure the proportions of oxygen (O₂) consumption and carbon dioxide (CO₂) production for estimation of metabolic rate and substrate oxidation (Ferrannini, 1988). Indirect calorimetry operates under the assumptions that all oxygen is used to oxidize degradable substrates, and all CO₂ produced is recovered. Within these circumstances, it is possible to use measures of gas exchange to determine total energy production by the body (Ferrannini, 1988). Furthermore, data collected using this method can be used to calculate respiratory quotients (RQ),
heat increments of feeding (HIF), and specifics of macronutrient oxidation in animals (Ferrannini, 1988).

The RQ is defined by the ratio of CO$_2$ production (VCO$_2$) to oxygen consumption (VO$_2$), and responds to degree of substrate use in the body (McClave et al., 2003). Thus, it is a useful measure to determine which dietary macronutrient is being oxidized by an animal. An RQ of 0.7 indicates pure fat oxidation, while an RQ of 1.0 indicates pure carbohydrate oxidation (Lusk, 1928). Additionally, an RQ of 0.80 indicates oxidation of a mixed meal or protein (Ferrannini, 1988). These quotients are determined through stoichiometry, using the oxidation of 1 g of a substrate to determine the ratio of CO$_2$ produced to O$_2$ consumed. The stoichiometric equations of the three energy yielding substrates are as follows (Ferrannini, 1988):

$$1 \text{ g Glucose} + 0.746 \text{ L O}_2 \rightarrow 0.746 \text{ L CO}_2 + 0.6 \text{ g H}_2\text{O}$$

$$1 \text{ g Lipid} + 2.029 \text{ L O}_2 \rightarrow 0.746 \text{ L CO}_2 + 1.09 \text{ g H}_2\text{O}$$

$$1 \text{ g Protein} + 0.966 \text{ L O}_2 \rightarrow 0.782 \text{ L CO}_2 + 0.45 \text{ g H}_2\text{O}$$

The HIF, also commonly referred to as dietary-induced thermogenesis, can be described as the transient increase in metabolic rate attributed to consumption, digestion, absorption, and elimination of a meal (Chappell et al., 1997). Like RQ, the HIF is variable, and can be altered by dietary macronutrient content, diet processing conditions, and environmental temperature (LeGrow and Beamish, 1986; Collin et al., 2003). Therefore, the HIF is important to measure for an accurate representation of the effect of diet and macronutrient profile on energy expenditure (Moehn et al., 2005).

Previously, indirect calorimetry has been used to determine the differences in energy expenditure and macronutrient oxidation in cats fed various experimental diets. Previous work has suggested that when fed isocalorically, diet does not alter postprandial energy expenditure in cats.
(Lester et al., 1999; Russell et al., 2002; Nguyen et al., 2004; Gooding et al., 2015). However, respiratory quotients can be altered significantly due to differences in macronutrient intake (Lester et al., 1999; Russell et al., 2002; Nguyen et al., 2004; Gooding et al., 2015), suggesting that cats have the metabolic ability to alter macronutrient oxidation based on variable intake of nutrients (Gooding et al., 2015). Overall, indirect calorimetry is a valuable tool to investigate differences in nutrient and energy metabolism attributed to variable macronutrient intake.

1.7. Energy Systems

Energy density of diets can be assessed using multiple systems (Moehn et al., 2005). Common systems used to describe dietary energy include gross energy, digestible energy, metabolizable energy, and net energy (NRC, 2006; Figure 1.2).

Gross energy (GE) is defined as the energy released from complete combustion of a food in a bomb calorimeter (NRC, 2006), and is the least specific system used to evaluate dietary energy density. Alternatively, gross energy can be estimated using the chemical composition of a diet and standard values for each of the macronutrients (NRC, 2006). For fat, a heat of combustion of 8.7 – 9.5 kcal/g organic matter has been reported; though a value of 9.4 kcal/g fat is accepted for use in pet food (Kienzle et al., 2002). Depending on the chain length and degree of desaturation of fatty acids, the heat of combustion can vary within the reported range (Kienzle et al., 2002; Moehn et al., 2005; NRC, 2006). For protein, the heat of combustion ranges from 5.3-5.8 kcal/g organic matter (Kienzle et al., 2002), while a value of 5.7 kcal/g is commonly used in pet foods. Last, the heat of combustion of carbohydrates ranges from 3.7 - 4.3 kcal/g (NRC, 2006). In pet foods, a value of 4.1 kcal/g organic matter is accepted, which is used to describe both nitrogen-free extract (NFE; includes starches, sugars, and non-starch polysaccharides) and crude fiber (Kienzle et al., 2002; NRC, 2006). However, gross energy is not commonly labeled on pet foods, as it does not
accurately represent dietary energy that is available to the animal.

Digestible energy (DE) is described as the gross energy, less the energy lost in feces (Moehn et al., 2005). This system accounts for the digestibility of the feed and provides a measure of the potential energy available to be used by an animal (Moehn et al., 2005). However, determining fecal energy losses requires expensive and lengthy in vivo trials (Hervera et al., 2007). Alternative methods have been proposed to estimate the DE of pet foods, using the linear regression of crude fiber content to predict energy digestibility (Kienzle et al., 2006; Castrillo et al., 2009), however they are not commonly practiced.

Metabolizable energy (ME) is the difference between DE and the energy lost in urine and combustible gases, such as methane (Moehn et al., 2005). However, the energy loss through gases are negligible in companion animals, especially in cats, due to their carnivorous nature (Castrillo et al., 2009, Moehn et al., 2005; NRC, 2006). Currently, methods to predict urinary energy losses in both dogs and cats use digestible protein content of the feed as a parameter (NRC, 2006). Values of 1.25 and 0.9 kcal/g of digestible crude protein are used to estimate urinary energy losses of dogs and cats, respectively (NRC, 2006). It is generally rare for urine to be collected, as this method requires restriction in metabolic cages or adapted cages to ensure complete collection.

Last, net energy (NE) is determined by subtracting the heat increment of feeding (HIF) from the ME of a diet (Moehn et al., 2005). The HIF can be described as the energy lost during digestion, metabolism and excretion of food (Moehn et al., 2005). More specifically, the HIF results from the production of energy due to the exothermic reactions involved in the assimilation of food (Marshall, 1961). These reactions include the interconversion, transportation, and storage of food molecules throughout the body after ingestion. Thus, the proportion of ME attributed to the HIF is directly related to the plane of nutrition and varies according to the chemical
characteristics of the food being consumed (Marshall, 1961). Of all macronutrients, protein exhibits the greatest calorigenic effect, due to the deamination of the amino acids to be used as a source of energy, and the need to detoxify the ammonia by synthesis of urea or uric acid (Martin and Blaxter, 1965). Energy left after these processes are available to be used directly by the animal for maintenance, growth, and reproduction (NRC, 2006). Although NE is most precise measure of available energy for the animal, it is difficult to quantify (Castrillo et al., 2009).
Figure 1.2. Partitioning of dietary energy (Adapted from Case, 2011).
The first predictive equations to estimate dietary metabolizable energy included factors for nutrients based on their heat of combustion and overall digestibility, and corrected for possible energy losses through urine (NRC, 2006). Originally, factors of 4.1 kcal ME/g for protein and carbohydrates, and 9.3 kcal ME/g for fat were proposed (Rubner, 1885). This equation was later simplified by Atwater (1902), which used factors of 4 kcal ME/g for protein, 9 kcal ME/g for fat, and 4 kcal ME/g for carbohydrate (NFE). However, these factors were developed for human food, and include digestibility values of 98 %, 96 %, and 90 %, for carbohydrates, fats, and proteins, respectively (NRC, 2006). Thus, the original Atwater factors were modified to appropriately estimate ME of commercial pet foods (NRC, 2006). Today, the Atwater equations are used to predict ME values and develop feeding guidelines, and exist as traditional and modified:

Traditional: ME (kcal/kg) = \[4 \times \text{CP} \% + 4 \times \text{NFE} \% + 9 \times \text{crude fat} \%\] \times 10

(Atwater, 1902)

Modified: ME (kcal/kg) = \[3.5 \times \text{CP} \% + 3.5 \times \text{NFE} \% + 8.5 \times \text{crude fat} \%\] \times 10

(AAFCO, 1997)

Neither Atwater equation accurately predicts ME because the coefficients are often unreliable for pet foods (NRC, 2006). The National Research Council (NRC) has proposed additional equations that account for fiber and digestibility of energy while calculating an ME value of prepared cat foods, however they are not commonly used in practice or for package labels. Currently, the modified Atwater equation is accepted widely by American and European regulatory bodies, such as the Association of American Feed Control Officials (AAFCO), and the European Pet Food Industry Federation (FEDIAF), as a standard method to predict dietary ME of dog and cat foods (Castrillo et al., 2009).

In agricultural practices, dietary energy is commonly expressed in terms of net energy
(NE), which, aforementioned, is the most accurate representation of available energy (Castrillo et al., 2009). Models to predict NE using the nutrient content of complete diets have been developed for use with swine and cattle (Donker and Naik, 1979; Noblet et al., 1994), but to date, they do not exist for companion animals. As the accuracy of the current models used to predict dietary energy of commercial pet diets has proven to be variable, additional methods, such as net energy models, could potentially address this issue. Having accurate estimates of the energy density of food is necessary to develop accurate feeding guidelines, and address the issue of obesity due to overfeeding.
Chapter 2: Research Rationale and Objectives

While a range of literature exists investigating the mechanisms of starch digestion and metabolism in the domestic cat, less research has been directed towards the impact of carbohydrate ingredient source on macronutrient digestibility, glycemic response, and energy metabolism. Work that is being done to determine feline glycemic response often involves repeated blood sampling techniques (Reineke et al., 2010), and less invasive methodologies are only recently beginning to be investigated. Furthermore, much of the current literature investigating glucose and insulin responses of cats has been done in obese or diabetic subjects rather than healthy adults. Last, though it is well documented that the current energy algorithms in practice are not suitable for use in the domestic cat, only a few alternative methods have been proposed (NRC, 2006). Therefore, the objectives of this thesis are as follows:

1. Use both in vitro and in vivo digestibility techniques to determine if cats can efficiently digest diets differing in main carbohydrate sources (Chapter 3).

2. Measure the effects of three diets differing in their ingredient composition and main carbohydrate sources on respiratory quotient (RQ), energy expenditure (EE), interstitial glucose, serum glucose, and serum insulin in healthy adult cats (Chapter 4).

3. Calculate NE content of three commercial diets and develop models that can accurately predict dietary NE values for cats (Chapter 5).
Chapter 3: Digestibility is similar between commercial diets that provide ingredients with different perceived glycemic responses and the inaccuracy of using the modified Atwater calculation to calculate metabolizable energy¹,²

3.1. Abstract

Dietary starch is required for the production of a dry, extruded kibble; a common diet type for domesticated felines in North America. However, the amount and source of dietary starch may affect digestibility and metabolism of other macronutrients. The objectives of this study were to evaluate the effects of three commercial cat diets on in vivo and in vitro energy and macronutrient digestibility, and to analyze the accuracy of the modified Atwater equation. Dietary treatments differed in their perceived glycemic response (PGR) based on ingredient composition and carbohydrate content (34.1, 29.5, and 23.6 % nitrogen-free extract for High, Medium, and LowPGR, respectively). A replicated 3 × 3 Latin square design was used, with three diets and three periods. In vivo apparent protein, fat, and organic matter digestibility differed among diets, while apparent dry matter digestibility did not. Cats were able to efficiently digest and absorb macronutrients from all diets. Furthermore, the modified Atwater equation underestimates measured metabolizable energy by approximately 12 %. Thus, the modified Atwater equation does not accurately determine the metabolizable energy of highly digestible feline diets. Further research should focus on understanding carbohydrate metabolism in cats, and establishing an equation that accurately predicts the metabolizable energy of feline diets.


²A portion of these results were reported previously in: Berendt, K.D. 2014. Starch: an alternative energy source for cats. Univ. of Alberta, Edmonton.
3.2. Introduction

A trend towards pet foods that are organic and utilize novel ingredients is growing in the pet food industry. Pet food companies commonly advertise their product using terms such as “grain-free”, which is often combined with low carbohydrate claims. However, a grain-free diet might not be beneficial compared to a commercial diet that includes grains as a carbohydrate source, with a few exceptions. Overall, the ability of cats to metabolize carbohydrates is not conclusively defined, and research regarding optimum inclusion level and type of carbohydrate for the domestic cat is lacking. Although the domestic cat is an obligatory carnivorous species (Verbrugghe and Hesta, 2017), felines have displayed the physiological capability to successfully digest, absorb, and metabolize various carbohydrate sources, and that high carbohydrate inclusion levels (35 %) do not impair macronutrient digestibility (de-Oliveira et al., 2008). Furthermore, cats exhibit a metabolic ability to alter macronutrient oxidation based on variable intake of carbohydrates and fats (Gooding et al., 2015). This metabolic flexibility occurs in response to both level and source of macronutrient intake (Gooding et al., 2015).

There are many benefits of including different forms of carbohydrates in pet food formulations. Starch is required to maintain shape and texture of dry kibble, while the process of extrusion is the most cost-effective way to produce a stable, low moisture product that is resistant to microbial growth (Forrester and Kirk, 2009). Extrusion at high temperatures also increases the digestibility of carbohydrates and starches in particular (Tran et al., 2008). Consumers benefit from feeding kibble in terms of affordability, convenience, and assurance that their pets’ food will remain microbially safe for an extended period of time. Controversy exists regarding the impact of the long-term effects of feeding high carbohydrate diets (Verbrugghe and Hesta, 2017). However, research investigating the mechanisms of carbohydrate digestion and metabolism in cats fed
different ingredients sources has produced variable results.

The energy density of feline diets is commonly expressed as metabolizable energy (ME) (Livesey, 2001). Since routine ME measurements are not practical (AAFCO, 2012), generally accepted Atwater equations are used to predict ME values and develop feeding guidelines. These equations assign coefficients for the three macronutrients: protein, carbohydrate (measured as N-free extract (NFE)), and fat, and exist as traditional and modified:

Traditional: ME (kcal/kg) = \[4 \times CP \% + 4 \times NFE \% + 9 \times \text{crude fat} \%\] \times 10

(Atwater, 1916)

Modified: ME (kcal/kg) = \[3.5 \times CP \% + 3.5 \times NFE \% + 8.5 \times \text{crude fat} \%\] \times 10

(AAFCO, 1997)

Because the traditional Atwater equation has been found to overestimate ME (Kendall et al., 1982), a modified Atwater equation was developed and is currently used in industry to estimate dietary ME for companion animals (AAFCO, 1997). However, neither Atwater equation accurately predicts the ME of commercial pet foods, because the coefficients are unreliable (NRC, 2006). Because of the inaccuracy, the National Research Council (NRC) has suggested a more accurate method that accounts for crude fiber and digestibility of energy while calculating the ME value of prepared cat foods:

Step 1: GE (kcal) = (5.7 \times \text{g protein}) + (9.4 \times \text{g fat}) + 4.1 \times (\text{g NFE} + \text{g fiber})

Step 2: Percentage energy digestibility = 87.9 – (0.88 \times \text{percentage crude fiber in dry matter})

Step 3: DE (kcal/g) = (GE \times \text{percentage energy digestibility}/100)

Step 4: ME (kcal/g) = DE – (0.77 \times \text{g protein})

The NRC (2006) has also proposed an alternate equation to estimate percentage energy digestibility using total dietary fiber (TDF), rather than crude fiber:

22
Energy digestibility (%) = 95.6 – (0.89 \times \text{total dietary fiber} \text{% in dry matter})

However, past labeling practices of pet food do not require total dietary fiber to be reported and thus crude fiber is often used to approximate energy digestibility. Having accurate estimates of the energy density of food is important to provide practitioners and pet owners with accurate feeding guidelines.

The objectives of this study were to: 1) Measure the ME value of three diets differing in NFE content; 2) compare measured ME values to the predicted using the Atwater and modified Atwater equations; 3) use digestibility values (both in vitro and in vivo) to determine if cats can efficiently digest diets differing in perceived glycemic responses (PGR); and 4) compare in vivo and in vitro measures of dry matter disappearance. We hypothesized that 1) because the Atwater equations do not account for fiber or energy digestibility, measured and predicted ME values would differ within diet; and 2) due to adequate cooking/processing and high temperatures involved in extrusion, no diet will be significantly more digestible than another, with all being highly digestible.

3.3 Materials and Methods

All procedures were reviewed and approved by Procter and Gamble Pet Care’s Institutional Animal Care and Use Committee and were in accordance with the United States Department of Agriculture (USDA) and the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) guidelines. The Animal Utilization Protocol was 013-9127 (Protocol date: 3/7/2013).

3.3.1. Animals

Twelve cats (6 neutered males, 6 spayed females) of similar age (4.9 ± 1.2 yr) and weight (4.4 ± 0.8 kg) were used for the present study. Cats were previously acclimated to housing facilities
and metabolic cages. Cats received physical veterinary exams to ensure health prior to and during the study.

3.3.2. Experimental Diets and Design

Since the glycemic response to each diet was not measured, the term perceived glycemic response (PGR) was used to characterize the diets in the present study. The PGR refers to the glycemic response expected from glycemic indices of carbohydrate sources determined in human studies. Diets were selected for containing ingredients that are hypothetically expected to elicit high, medium, and low glycemic responses, and named based on their respected PGR. Three diets were studied (Table 3.1): HighPGR: Purina ONE Chicken and Rice (Nestlé, St. Louis, MO), MediumPGR: Iams Kitten Proactive Health (Procter & Gamble, Cincinnati, OH), and LowPGR: Innova Dry Adult Cat Food (Procter & Gamble, Cincinnati, OH). Purina ONE Chicken and Rice was chosen as the HighPGR diet due to its high inclusion of Brewer’s rice, a high glycemic index (GI) grain (Jenkins et al., 1981; Carciofi et al., 2008). Iams Kitten Proactive health was comprised of ingredients including corn meal and sorghum, that both elicit a lower glycemic response than ingredients such as Brewer’s rice (de-Oliveira et al., 2008). Lastly, Innova Dry Adult Cat Food was predicted to have the lowest PGR because of the use of barley, a low GI carbohydrate (Jenkins et al., 1988).

The experimental design was a replicated 3 × 3 Latin square, with three diets and three periods, with each cat randomly receiving every diet and all diets equally represented within each period. Each period lasted 10 d, with 5 d of acclimation to diet immediately followed by 5 d of collections. Cats were fasted overnight and weighed prior to feeding the morning of d 1 and 6. Maintenance energy requirements for each cat were based on historical feeding and body weight records. Diet allowance was determined based on the calculated energy density (modified Atwater
calculation; AAFCO, 1997) of each diet. Cats were fed to maintain body weight (BW). Cats were fed once at 07:00 daily and food refusals were collected and weighed at 13:00. Cats had *ad libitum* access to fresh water.

During the acclimation (d 1 to 5), cats were housed in a one-room, free-living, group environment (13.94 m²) in an environmentally controlled facility (22°C; 50 – 60 % relative humidity) with a 12 h light:12 h dark cycle and natural light through windows. Cats were placed in individual metabolism cages (0.61 m length × 0.61 m width x 0.62 m height; Suburban Surgical Company, Wheeling, IL) for feeding each day from 06:30 until 13:00, and were then moved back into their free-living environment.

During the collection periods (d 6 to 10), cats were housed in individual stainless-steel metabolism cages for total collection. Two labeled urine collection bottles per cat fitted with screened funnels and containing 10 mL HCl as preservative were placed under each cage. Feces and urine collections started at 08:00 on d 6. Feces were collected, weighed, and scored using a scale of 0-5 with 0 as no stool, 1 as watery liquid, and 5 as extremely dry (The Iams Company standard operating procedure for collections and scoring). Feces were frozen in bags for each cat at -16°C until analysis. Urine bottles were emptied daily into bottles for each cat and refrigerated at 3°C until analyses. Clinical observations were recorded, but none were noted throughout the study.

3.3.3. Chemical Analyses and Calculations

Urine composites were mixed thoroughly and two 50-mL urine sub-samples were prepared for subsequent analyses. An aliquot of each cat’s fecal composite was freeze-dried. Diet and freeze-dried feces were ground to fine particle matter using a hand grinder and analyzed. Proximate analyses were completed in triplicate for each of the three experimental diets and fecal samples.
using AOAC procedures (AOAC, 1997). Ether extract was analyzed following acid hydrolysis (954.02), and dry matter (DM) was determined by vacuum drying at 100°C for 24 h (934.01). Dietary and urinary nitrogen were determined by oxidation using a crude protein/nitrogen (CP/N) analyzer (990.03; Leco Corp., St Joseph, MI) and crude protein (CP) calculated. Crude fiber (CF) was analyzed through a ceramic fiber filtration method (962.09). Acid detergent fiber (973.18) and neutral detergent fiber (2002.04) were subsequently analyzed. Starch content was approximated as N-free extract (NFE), calculated as:

\[
\text{NFE} (%) = 100 - \text{protein} (%) - \text{fat} (%) - \text{fiber} (%) - \text{ash} (%) - \text{moisture} (%)
\]

(AAFCO, 1997) (979.10)

Ash was measured after exposure to 550°C for 4 h (942.05), P through spectrophotometry (964.06), and Ca by atomic absorption spectrometry with electrothermal furnace (968.08). Gross energy (GE) of diets, feces, and urine samples were determined by bomb calorimetry (C-2000; IKA Staufen, Germany). The digestible energy (DE) from each diet was calculated by subtracting energy lost in feces from the determined GE intake. The ME intake of each diet was measured by subtracting energy in feces and urine from GE intake.

3.3.4. Dry Matter Disappearance (In-Vitro Energy Digestibility)

The 3-step in vitro energy digestibility technique described by Huang et al. (2003) was used to quantify digestible energy. In vitro DM digestibility was calculated by deducting the residue DM from the sample DM followed by division by the sample DM. Organic matter digestibility, protein digestibility, and fat digestibility were calculated using similar methods. The in vitro energy digestibility was calculated using the following formula:

\[
\text{In Vitro Energy Digestibility} = \frac{(\text{sample DM} \times \text{sample GE}) - (\text{residue DM} \times \text{residue GE})}{(\text{sample DM} \times \text{sample GE})}
\]
3.3.5. Statistical Analyses

Data were analyzed using the GLIMMIX procedure of SAS (version 9.3, SAS Inst., Cary, NC, USA) with cat as experimental unit, cat and period as random effects, and diet as a fixed effect. Means were separated using the least significant difference with a Tukey-Kramer test for multiple comparisons. Statistical significance was declared at \( P < 0.05 \). Data were reported as least-squares means \( \pm \) SEM.

3.4. Results

3.4.1. Diet Composition

When diets were analyzed, differences were detected in protein content, crude fat, and available lysine, with small differences in ash, acid detergent fiber (ADF) and neutral detergent fiber (NDF) (Table 3.1). The differences in nutrient levels were expected and occurred due to differences in ingredient composition of these diets, which were clearly labeled and the basis of inclusion.

3.4.2. Bodyweight, Food and Energy Intake, and Urinary/Fecal output

Cats fed the LowPGR diet excreted most feces on an as-is basis, followed by cats fed the MediumPGR diet, and fecal excretion was lowest for cats fed the HighPGR diet \( (P = 0.05; \) Table 3.2). Fecal output of DM was greater for cats fed the MediumPGR diet than cats fed the HighPGR diet \( (P = 0.02, \) Table 3.2), and both diets did not differ from the LowPGR diet. For all three diets, fecal scores of cats were within the ideal range (Kerr et al., 2012). Urinary N was greater \( (P < 0.01, \) Table 3.2) for the LowPGR diet than for the Medium and the HighPGR diet. Fecal output was a function of feed intake, and when output was normalized based on intake, differences no longer existed.
Body weight was similar among groups at the beginning of the study \((P > 0.05)\), and did not change throughout the study \((P > 0.05; \text{data not shown})\). Daily food intake was greater for cats receiving the Medium and LowPGR diets \((P < 0.001)\) than for cats on the HighPGR diet (Table 3.3). The amount fed was higher for the Medium \((45.0 \, \text{g/d})\) and LowPGR \((45.1 \, \text{g/d})\) diets compared to the HighPGR \((41.8 \, \text{g/d})\) diet \((P < 0.001; \text{Table 3.3})\) for cats fed the LowPGR diet, intermediate for cats fed the MediumPGR diet and lowest for the HighPGR diet. Specific nutrient intakes are presented in Table 3.3.

3.4.3. *In Vivo and In Vitro Digestibility*

Dry matter digestibility was similar among diets, whereas OM digestibility was greater \((P = 0.03, \text{Table 3.3})\) for cats fed the HighPGR diet than for cats fed the MediumPGR diet, and neither treatment differed from cats fed the LowPGR diet. Protein digestibility was greatest \((P < 0.01, \text{Table 3.3})\) in cats fed the LowPGR diet, followed by cats fed the HighPGR diet, and it was lowest for cats fed the MediumPGR diet. Fat digestibility was greater \((P < 0.01, \text{Table 3.3})\) for cats fed the Low and MediumPGR diets than for cats fed the HighPGR diet. Dry matter disappearance (DMD) was greatest for cats fed the LowPGR diet, followed by cats fed the HighPGR diet, and then cats fed the MediumPGR diet. Since DMD was determined using *in vitro* energy digestibility, only one measurement was recorded, and we can thus not comment on statistical significance of these differences; however, the DMD digestibility values of 92.7, 91.1, and 90.7 % for the Low, High and MediumPGR diets, respectively, were similar to *in vivo* measurements of DM digestibility (Table 3.3). On average, *in vitro* DMD overestimated apparent DM digestibility by 5.0 %.
3.4.4. Energy

Per unit of feed, the GE, urinary energy (UE), and measured ME value were greatest \( (P < 0.001; \text{Table 3.4}) \) for cats fed the LowPGR diet. The GE and measured ME value were greatest for the LowPGR diet \( (P < 0.001) \), followed by the MediumPGR diet, and lowest for the HighPGR diet. The UE was greatest for cats fed the LowPGR diet \( (P < 0.001) \), with intermediate values for cats fed the HighPGR diet, and lowest for cats fed the MediumPGR diet.

3.4.5. Measured ME Versus Calculated ME

Calculated ME using both the traditional and modified Atwater equations was compared with analyzed ME (Table 3.5). The ME value calculated using both ME equations followed the same ranking as for the measured ME value. Because only one number per diet can be calculated, we could not assess this numerical ranking statistically. The measured ME values were greater than both calculated ME values, using both the traditional and modified Atwater equations, for all three diets (Table 3.5). Thus, both modified and traditional Atwater equations underestimate ME. The modified Atwater equation underestimated measured ME values by approximately 12 % for all diets (11.9, 10.8, and 13.6 % for High, Medium and LowPGR, respectively). The traditional Atwater equation had the closest prediction to the measured ME values (2.0, 1.5, and 4.6 % below measured ME for High, Medium and LowPGR, respectively) (Table 3.5). Though not included in our hypotheses, ME and GE were also calculated using the NRC equations that account for crude fiber. This method underestimated GE by 2.7 % on average (5.8, 1.0 and 1.4 % below measured GE for High, Medium and LowPGR, respectively), and underestimated ME by 8 % on average (11.3, 6.0, and 7.8 % below measured ME for High, Medium and LowPGR, respectively). Therefore, the NRC equations can predict GE more accurately than ME.
3.5. Discussion

This study was the first to show similarity among in vivo and in vitro measures of digestibility, and that high-quality diets have similar digestibilities regardless of ingredient selection. Similar to previous literature, the modified Atwater equation does not accurately predict measured ME content of the cat diets investigated in the present study (Kendall et al., 1982; NRC, 2006).

The observed differences in intake among diets were expected. We offered food isocalorically and intake differences were due to food refusals that may have occurred when cats reached a level of carbohydrate intake close to their “carbohydrate ceiling” (Hewson-Hughes et al., 2011). This ceiling is a level of carbohydrate intake beyond which food intake ceases, suggesting that cats may only be able to metabolize ingested carbohydrate up to a definitive level. This ceiling occurs at approximately 300 kJ/day of carbohydrate (Hewson-Hughes et al., 2011); however, the ceiling of intake was roughly 244 kJ/day (58.4 kcal/d; as 4.18 kJ equates to 1 kcal) in the present study. Indeed, other variances in individual cats, experimental methods, or the environment may have contributed to the lower ceiling observed in the present study. Other studies indicated that when provided ad libitum access to feed, cats fed high fat or high protein diets, rather than high carbohydrate diets, are more prone to weight gain (Backus et al., 2007; Coradini et al., 2011). These studies support the existence of a “carbohydrate ceiling”, resulting in lower intakes of high carbohydrate diets compared with diets high in fat or protein (Hewson-Hughes et al., 2011). Notably, neutral detergent fiber (NDF) and crude fiber (CF) did not impact food consumption in the present study, supporting the conclusion that intake was affected by differences in total NFE.

Differences in protein digestibility in the present study may have been related to dietary protein intake, similar to other species. For example, apparent crude protein digestibility, and
nitrogen (N) intake increased with increasing dietary protein level in mink (Jiang et al., 2015). Similarly, horses consuming 865 g/d of crude protein increased their CP digestibility 5 % compared to horses consuming 840 g/d (Graham-Theirs and Bowen, 2011). However, calculating crude protein measurements and apparent digestibility values has various limitations that need to be considered.

Studies in pigs have indicated that the apparent digestibility of fat increases with increasing amounts of fat in the diet (Kane et al., 1981). This increase supports results in the present study, as fat digestibility was higher for the MediumPGR and LowPGR diets that contained approximately 20 % fat compared to the HighPGR diet with 10.8 % fat. Furthermore, lipid type and processing conditions affect apparent fat digestibility. In rats, liquid oils are absorbed more readily than solid triacylglycerols (Wang et al., 2011). Notably, lipid type may have contributed to fat digestibility differences observed in the present study. While all diets contained saturated fat from animal sources, the Medium and LowPGR diets contained unsaturated fats from fish oil and sunflower oil, respectively. Non-hydrogenated palm oil was retained at 99.6% in rats (Wang et al., 2011) indicating inclusion of non-hydrogenated oils in the Medium and LowPGR diets may have contributed to the greater fat digestibility values in these diets compared to the HighPGR diet.

Apparent OM digestibility measurements approximated carbohydrate digestibility in the present study. Previously, apparent OM digestibility correlated negatively to fiber content of dog and cat foods (Earle et al., 1998). The addition of 40 % apple pomace, a high fiber ingredient, to a meat-based diet with 0 % crude fiber decreased OM digestibility from 85.5 to 56.9 % in cats (Fekete et al., 2001). However, crude fiber content did not seem to influence apparent OM digestibility in the present study based on the lack of correlation between the two variables. This indicates that our results may not be biologically relevant, or that all diets were below a
physiological maximum for fiber. Regardless, these OM digestibly measurements indicate that, despite the inherent carnivorous nature of domestic cats, they can efficiently digest diets with significant contributions from carbohydrates.

Our digestibility values are similar to those reported in literature. In both intact and neutered male cats fed a high carbohydrate diet (27 % starch), dry matter, organic matter, crude protein, and crude fat exhibited digestibilities of 84.5, 86.0, 82.0, and 95.3 %, respectively (Theiss et al., 2004). Furthermore, apparent starch digestibility values were > 93 % in cats fed six different diets containing 35 % starch (de-Oliveira et al., 2008). The results of these studies agree with the present study demonstrating that starch can be digested readily by cats, and does not reduce digestibility of other macronutrients.

Dry matter disappearance was also measured in vitro to allow us to compare to our in vivo measurements (Table 3.3). The numerical trend among diets was the same as in vivo measurements suggesting that in vitro digestion may be used to predict in vivo digestibility allowing for rapid screening of novel ingredients or processing methods prior to placing an in vivo study.

The results of the fecal and urine analyses varied minimally among diets. Previous research indicates an increase in urinary N excretion when N intakes are increased beyond theoretical requirements (Marini and Van Amburgh 2003; Tomé and Bos, 2000). In the present study, daily UE excretion did not differ, but cats fed the LowPGR diet had the greatest urinary N excretion. Cats consuming the LowPGR diet had the greatest protein intake, resulting in the greatest UE loss per kg of diet. Our study diets exceeded the recommended protein contribution of 18 % DM for adult cats, and the measurements of urinary N indicate that protein was supplied in excess of the cats’ protein requirements (AAFCO, 2014). Additionally, fecal score did not differ among treatments and were within the ideal score, indicating that our cats had a solid function and health
of the gastrointestinal tract, nutrient absorption, and a consistent colonic environment (Kerr et al., 2012).

In the present study, ME values calculated using both Atwater and modified Atwater equations underestimated measured ME values among all diets. The discrepancy mimics research, and indicates that the modified Atwater calculation is inappropriate for calculating a ME value to determine daily food allowance for cats. In dogs, the modified Atwater equation underestimated the ME value of low-ash poultry meal by 15% (Yamka et al., 2007). Furthermore, predicted ME values underestimated in vivo values of dog food with ME values above 3.6 kcal ME/g DM (Castrillo et al., 2009; Kienzle et al., 1998; Laflamme, 2001), which corresponds to most dry extruded pet foods currently on the market. Conversely, the modified Atwater equation has accurately predicted ME value in some studies. Digestibility studies of commercial and non-commercial pet foods predicted ME values with an error of 0.16% for dogs and 1.57% for cats (Hall et al., 2013). Those studies included both wet and dry foods of varying quality, with lower dry matter, fat, carbohydrate, and energy digestibility compared with the present study diets. Additionally, Hall et al. (2013) calculated ME using a correction factor for energy lost in urine (0.86 × g protein absorbed). This correction may explain why calculated energy density was more accurate than predicted values in the present study.

While the modified Atwater equation may accurately predict energy values for less digestible pet foods, its use for high quality diets may underestimate true energy value of feed to calculate feeding requirements. This discrepancy is a prevalent issue in today’s society, as the market share of premium and super-premium pet foods is growing disproportionately to other segments of products that are lower in price and purported quality (Combelles, 2004). Moreover, pet food has generally improved in quality and digestibility since the Atwater equations were first
developed. Thus, using these current standards to calculate ME contents of diets may cause overfeeding and subsequent weight gain of pets due to a caloric surplus.

Since cat foods tend to be more energy dense than dog foods, we recommend using the traditional Atwater to calculate ME for all cat diets. Alternative approaches may also be considered to predict dietary ME more accurately, such as the in vitro DMD assay or the NRC ME prediction equations. Notably, a recent labeling policy has been approved that requires pet food manufacturers to report total dietary fiber (Bureau of Nutritional Sciences, 2017). With the adoption of this policy, standards may begin to shift towards using the NRC predictive ME equations that include total dietary fiber instead of the Atwater models. Regardless, various calculations and theories have been proposed as better predictors of diet ME value than the modified or traditional Atwater equations (NRC, 2006), but a new standard for regulatory guidance has yet to be determined.

In conclusion, the present study supported previous research that cats can efficiently digest diets with major contributions from carbohydrates. Despite statistical differences in digestibility among diets, all diets were highly digestible. Data from in vitro and in vivo digestibility methods demonstrated that in vitro techniques can potentially be used in place of in vivo studies to rapidly screen novel ingredients or processing methods. Furthermore, we confirmed that the modified Atwater equation does not give an accurate estimate of ME of high quality pet foods and may not be appropriate for any cat diet due to the overall higher energy density as compared to dog foods. It is recommended that the traditional Atwater coefficients be used to calculate diet ME values to avoid inaccurate feeding guidelines and subsequent weight gain in pets until a more accurate equation to estimate dietary ME for cat foods is established.
3.6. Tables and Figures

Table 3.1. Analyzed nutrient composition of the three experimental diets differing in perceived glycemic response (PGR). The PGR refers to the expected theoretical glycemic response that would result from consumption of each diet.

<table>
<thead>
<tr>
<th>Item</th>
<th>HighPGR</th>
<th>MediumPGR</th>
<th>LowPGR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture, %</td>
<td>7.16</td>
<td>6.76</td>
<td>5.31</td>
</tr>
<tr>
<td>Ash, %</td>
<td>6.36</td>
<td>6.31</td>
<td>6.38</td>
</tr>
<tr>
<td>Crude protein(^5), %</td>
<td>38.02</td>
<td>35.86</td>
<td>42.06</td>
</tr>
<tr>
<td>Ether extract, %</td>
<td>15.7</td>
<td>22.2</td>
<td>22.9</td>
</tr>
<tr>
<td>Crude fat, %</td>
<td>10.83</td>
<td>20.02</td>
<td>20.42</td>
</tr>
<tr>
<td>Nitrogen-free extract, %</td>
<td>34.1</td>
<td>29.5</td>
<td>23.6</td>
</tr>
<tr>
<td>Starch, %</td>
<td>36.75</td>
<td>30.72</td>
<td>23.56</td>
</tr>
<tr>
<td>Crude fiber, %</td>
<td>1.17</td>
<td>1.78</td>
<td>2.58</td>
</tr>
<tr>
<td>Acid detergent fiber, %</td>
<td>1.88</td>
<td>2.95</td>
<td>2.43</td>
</tr>
<tr>
<td>Neutral detergent fiber, %</td>
<td>7.36</td>
<td>12.58</td>
<td>10.57</td>
</tr>
<tr>
<td>Available lysine, %</td>
<td>1.62</td>
<td>1.91</td>
<td>2.80</td>
</tr>
<tr>
<td>GE, kcal/kg</td>
<td>4,916</td>
<td>5,253</td>
<td>5,462</td>
</tr>
<tr>
<td>Calculated ME, kcal/kg(^6)</td>
<td>3,752</td>
<td>4,081</td>
<td>4,137</td>
</tr>
</tbody>
</table>

\(^1\) Each diet was analyzed in triplicate. \(^2\) HighPGR was Purina ONE Chicken and Rice (Nestlé, St. Louis, MO) containing as 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\(_3\)PO\(_4\), CaCO\(_3\), caramel color, choline chloride, and salt. \(^3\) MediumPGR was Iams Kitten Proactive Health (Procter & Gamble, Cincinnati, OH) containing as 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, CaCO₃, brewer’s dried yeast, DL-Met, and salt. LowPGR was Innova Dry Adult Cat Food (Procter & Gamble, Cincinnati, OH) containing as 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. Percentage N \times 6.25. Calculated with modified Atwater equation (AAFCO, 1997): ME (kcal/kg) = [3.5 \times CP (%) + 3.5 \times NFE (%) + 8.5 \times crude fat (%)] \times 10.
Table 3.2. Feces and urine characteristics of cats fed three experimental diets differing in perceived glycemic response (PGR). The PGR refers to the expected theoretical glycemic response that would result from consumption of each diet.

<table>
<thead>
<tr>
<th></th>
<th>HighPGR</th>
<th>MediumPGR</th>
<th>LowPGR</th>
<th>SEM(^1)</th>
<th>(P)-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fecal characteristics</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fecal score(^2)</td>
<td>4.0</td>
<td>4.0</td>
<td>3.7</td>
<td>0.06</td>
<td>0.229</td>
</tr>
<tr>
<td>Fecal output (wet), g/d</td>
<td>12.2(^c)</td>
<td>15.5(^b)</td>
<td>16.1(^a)</td>
<td>3.95</td>
<td>0.050</td>
</tr>
<tr>
<td>Fecal DM, %</td>
<td>39.7</td>
<td>37.3</td>
<td>36.8</td>
<td>1.03</td>
<td>0.328</td>
</tr>
<tr>
<td>Fecal output (DM), g/d</td>
<td>4.8(^b)</td>
<td>5.8(^a)</td>
<td>5.5(^{ab})</td>
<td>0.20</td>
<td>0.022</td>
</tr>
<tr>
<td>Fecal output (wet) g/d: Energy</td>
<td>0.07</td>
<td>0.08</td>
<td>0.08</td>
<td>0.01</td>
<td>0.369</td>
</tr>
<tr>
<td>Intake, kcal EME(^3/d)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fecal output (wet) (g)/100 g</td>
<td>30.8</td>
<td>36.7</td>
<td>37.3</td>
<td>1.65</td>
<td>0.267</td>
</tr>
<tr>
<td>DM intake</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fecal energy (kcal g d(^{-1}))</td>
<td>3576</td>
<td>3555</td>
<td>3478</td>
<td>24.7</td>
<td>0.243</td>
</tr>
<tr>
<td><strong>Urine characteristics</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urine energy (kcal/g d(^{-1}))</td>
<td>155</td>
<td>143</td>
<td>172</td>
<td>9.16</td>
<td>0.437</td>
</tr>
<tr>
<td>Urine N (mg/mL)</td>
<td>3.52(^b)</td>
<td>3.36(^b)</td>
<td>4.12(^a)</td>
<td>0.10</td>
<td>0.003</td>
</tr>
</tbody>
</table>

\(^{a-b}\)Within a row, means without a common superscript differ \((P < 0.05)\).

\(^1\)Pooled SEM. Means were based on 12 cat observations per diet.

\(^2\)Scores based on the following scale: 1 = watery, liquid that can be poured; 2 = unformed stool; 3 = soft, moist, formed stool; 4 = dry, well-formed stool; 5 = dry, hard pellets.

\(^3\)EME: Estimated metabolizable energy; calculated using the modified Atwater equation (AAFCO, 1997).
Table 3.3. Intake and total tract digestibility of nutrients in cats of three experimental diets differing in perceived glycemic response (PGR). The PGR refers to the expected theoretical glycemic response that would result from consumption of each diet.

<table>
<thead>
<tr>
<th></th>
<th>HighPGR</th>
<th>MediumPGR</th>
<th>LowPGR</th>
<th>SEM¹</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Food and nutrient intake (as-fed)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Food intake, g/d</td>
<td>42.8ᵇ</td>
<td>45.0ᵃ</td>
<td>45.1ᵃ</td>
<td>1.2</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Protein intake, g/d</td>
<td>14.9ᵇ</td>
<td>15.0ᵇ</td>
<td>17.6ᵃ</td>
<td>0.49</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Fat intake, g/d</td>
<td>6.7ᶜ</td>
<td>10.0ᵇ</td>
<td>10.3ᵃ</td>
<td>0.37</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Fiber intake, g/d</td>
<td>0.8ᶜ</td>
<td>1.1ᵇ</td>
<td>1.4ᵃ</td>
<td>0.05</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>NFE² intake, g/d</td>
<td>14.6ᵃ</td>
<td>13.3ᵇ</td>
<td>10.6ᶜ</td>
<td>0.45</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Energy from NFE (kcal/d)³</td>
<td>58.4</td>
<td>53.2</td>
<td>42.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GE intake, kcal/d</td>
<td>210.2ᶜ</td>
<td>236.4ᵇ</td>
<td>246.2ᵃ</td>
<td>6.90</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td><strong>In vitro DMD⁴, %</strong></td>
<td>91.1</td>
<td>90.7</td>
<td>92.7</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**In vivo Digestibility**

<table>
<thead>
<tr>
<th></th>
<th>DM, %</th>
<th>OM, %</th>
<th>Protein, %</th>
<th>Fat, %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>87.6</td>
<td>90.9ᵃ</td>
<td>88.7ᵇ</td>
<td>92.9ᵇ</td>
</tr>
<tr>
<td></td>
<td>86.2</td>
<td>89.5ᵇ</td>
<td>87.3ᶜ</td>
<td>95.4ᵃ</td>
</tr>
<tr>
<td></td>
<td>87.0</td>
<td>90.3ᵇ ab</td>
<td>91.4ᵃ</td>
<td>95.0ᵃ</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.03</td>
<td>0.49</td>
<td>0.25</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.031</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

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

¹Pooled SEM. Means were based on 12 cat observations per diet.

²NFE = N-free extract.

³Caloric value calculated by multiplying NFE intake by 4 kcal, noting that 1g of carbohydrate provides 4 kcal of energy (Osborne and Voogt, 1978).

⁴Dry matter disappearance. Determined using in vitro simulated digestion procedure (Huang et al., 2003).
Table 3.4. Intake and excretion (as is basis and per 100g) of energy of cats of three experimental diets differing in perceived glycemic response (PGR). The PGR refers to the expected theoretical glycemic response that would result from consumption of each diet.

<table>
<thead>
<tr>
<th></th>
<th>HighPGR</th>
<th>MediumPGR</th>
<th>LowPGR</th>
<th>SEM(^1)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fecal energy, kcal/d</td>
<td>18.97</td>
<td>23.12</td>
<td>21.37</td>
<td>0.82</td>
<td>0.116</td>
</tr>
<tr>
<td>Urinary energy, kcal/d</td>
<td>11.42(^c)</td>
<td>11.25(^bc)</td>
<td>13.86(^a)</td>
<td>0.41</td>
<td>0.011</td>
</tr>
<tr>
<td>GE, kcal/100 g diet</td>
<td>491.6(^c)</td>
<td>525.3(^b)</td>
<td>546.9(^a)</td>
<td>3.2</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Fecal energy, kcal/100 g diet</td>
<td>44.37</td>
<td>50.38</td>
<td>47.46</td>
<td>1.6</td>
<td>0.353</td>
</tr>
<tr>
<td>Urinary energy, kcal/100 g diet</td>
<td>19.84(^b)</td>
<td>17.31(^c)</td>
<td>20.02(^a)</td>
<td>0.4</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Measured ME, kcal/100 g diet</td>
<td>426.0(^c)</td>
<td>457.4(^b)</td>
<td>478.7(^a)</td>
<td>3.6</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

\(^a\)\(^c\) Within a row, means without a common superscript differ (P < 0.05).

\(^1\)Pooled SEM. Means were based on 12 cat observations per diet.

\(^2\)Calculated with modified Atwater equation (AAFCO, 1997): ME (kcal/kg) = \([3.5 \times CP (\%) + 3.5 \times \text{NFE (\%)} + 8.5 \times \text{crude fat (\%) }\] \times 10.)
Table 3.5. Comparison of measured ME and GE with ME and GE calculated using the Atwater, modified Atwater and NRC equations, and resulting caloric surplus per day for three experimental diets differing in perceived glycemic response (PGR) fed to cats. The PGR refers to the expected theoretical glycemic response that would result from consumption of each diet.

<table>
<thead>
<tr>
<th></th>
<th>HighPGR</th>
<th>MediumPGR</th>
<th>LowPGR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Measured GE, kcal/kg as fed</td>
<td>4,916(^c)</td>
<td>5,253(^b)</td>
<td>5,469(^a)</td>
</tr>
<tr>
<td>Calculated GE, kcal/kg as fed</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NRC(^3)</td>
<td>4,631</td>
<td>5,201</td>
<td>5,390</td>
</tr>
<tr>
<td>GE surplus per day, %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NRC(^3)</td>
<td>5.8</td>
<td>1.0</td>
<td>1.4</td>
</tr>
<tr>
<td>Measured ME, kcal/kg as fed</td>
<td>4,259(^c)</td>
<td>4,574(^b)</td>
<td>4,787(^a)</td>
</tr>
<tr>
<td>Calculated ME, kcal/kg as fed</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Modified Atwater(^1)</td>
<td>3,752</td>
<td>4,081</td>
<td>4,137</td>
</tr>
<tr>
<td>Traditional Atwater(^2)</td>
<td>4,176</td>
<td>4,505</td>
<td>4,565</td>
</tr>
<tr>
<td>NRC(^4)</td>
<td>3,778</td>
<td>4,301</td>
<td>4,413</td>
</tr>
<tr>
<td>ME surplus per day (kcal/d)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Modified Atwater</td>
<td>21.7</td>
<td>22.2</td>
<td>29.3</td>
</tr>
<tr>
<td>Traditional Atwater</td>
<td>3.6</td>
<td>3.1</td>
<td>10</td>
</tr>
<tr>
<td>NRC(^4)</td>
<td>21.1</td>
<td>12.1</td>
<td>16.4</td>
</tr>
<tr>
<td>ME surplus per day, %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Modified Atwater</td>
<td>11.9</td>
<td>10.8</td>
<td>13.6</td>
</tr>
<tr>
<td>Traditional Atwater</td>
<td>2.0</td>
<td>1.5</td>
<td>4.6</td>
</tr>
<tr>
<td>NRC(^4)</td>
<td>11.3</td>
<td>6.0</td>
<td>7.8</td>
</tr>
</tbody>
</table>

\(^1\)Calculated with modified Atwater equation (AAFCO, 1997): ME (kcal/kg) = \([3.5 \times \text{CP} \% + 3.5 \times \text{NFE} \% + 8.5 \times \text{crude fat} \%] \times 10.\)

\(^2\)Calculated with traditional Atwater equation (Atwater, 1902): ME = \([4 \times \text{CP} \% + 9 \times \text{crude fat} \% + 4 \times \text{NFE} \%] \times 10.\)

\(^3\)Calculated using the NRC equation (NRC, 2006): GE (kcal) = \((5.7 \times \text{g protein}) + (9.4 \times \text{g fat}) + 4.1 \times (\text{g NFE} + \text{g fiber}).\)

\(^4\)Calculated using the NRC equation (NRC, 2006): ME (kcal/g) = DE – (0.77 \times \text{g protein}).
Chapter 4: Carbohydrate level and source have minimal effects on the feline glycemic response and the benefits of using interstitial glucose and calorimetry measurements as alternatives to repeated blood sampling1,2

4.1. Abstract

The carnivorous nature of the domestic cat makes it unique in its metabolism of carbohydrates. The cats’ glycemic response has been previously studied, with variable outcomes in response to carbohydrate level and source, but is an important response to understand how to control glycemia. The objectives of this study were to determine the respiratory quotient (RQ) and energy expenditure (EE) of cats fed three commercial diets differing in carbohydrate content and source and to compare continuous interstitial glucose monitoring with the postprandial serum glucose and insulin response. Domestic shorthair cats (n = 19, 10 males, 9 females) of similar age (4.3 ± 0.48 yr) and ideal body condition score (3.3 ± 0.6 on a 9-point scale) were used. Animals were fed, once a day, one of three commercial diets that differed in their perceived glycemic response (PGR; 34.1, 29.5, and 23.6 % nitrogen-free extract for High, Medium, and LowPGR, respectively) in a replicated incomplete 3 × 3 Latin square design. The three periods consisted of 8 d of adaptation to the diet, followed by 21 h calorimetry measurements and 20 h real-time interstitial glucose measurements on day 9. On d 10, postprandial serum glycemic and insulinemic responses were assessed. Body weight and metabolizable energy intake did not differ among treatments. Energy expenditure in the fasted state did not differ among treatments, while postprandial EE was highest for the HighPGR diet compared to the MediumPGR and LowPGR diets. Cats revealed a prolonged postprandial glucose and insulin response compared to other

1A version of this manuscript will be submitted to a scientific journal (Authors: N.J. Asaro, K. Berendt, R. Zijlstra, J. Brewer, and A.K. Shoveller)

2A portion of these results were reported previously in: Berendt, K.D. 2014. Starch: an alternative energy source for cats. Univ. of Alberta, Edmonton.
monogastric animals, yet diet effects were minimal. Overall, interstitial glucose measures were less variable than serum glucose measurements, and followed a parallel pattern to RQ. Therefore, going forward, calorimetry and continuous interstitial glucose monitoring should be considered as less invasive alternatives to repeated blood sampling.
4.2 Introduction

Dry commercial diets formulated for domestic cats include carbohydrates to ensure product stability and limit microbial growth (Forrester and Kirk, 2009). However, as domestic cats are obligate carnivores (Verbrugghe and Hesta, 2017), their mechanisms of starch digestion, absorption and metabolism are not entirely understood. Much of the current work investigating glucose and insulin responses of cats has been done in obese or diabetic subjects. Findings concerning glucose metabolism in healthy adult cats are variable among studies, though the observation of a prolonged glycemic response compared to other monogastric animals is consistent (Appleton et al., 2004; Coradini et al., 2011; Farrow et al., 2013). The postprandial glycemic response measures the rate of glucose appearance, and consequential insulin release, into the bloodstream in response to consumption of a meal (Jenkins et al., 1981) and is often measured with repeated blood sampling. In dogs, both the amount and source of dietary starch affects the postprandial glucose and insulin response (Carciofi et al., 2008). However, this effect does not seem as significant in cats as compared to other monogastric species (Kienzle et al., 1994; Bouchard and Sunvold, 2000; de-Oliveira et al., 2008). To determine the glycemic response, repeated measures of serum glucose and insulin are commonly used. However, more recently, real-time interstitial glucose measurements have been introduced as a less invasive alternative to repeated blood sampling (Aussedat et al., 2000).

Indirect calorimetry is a technique that measures exchange of respiratory gases to enable calculation of energy and macronutrient metabolism. Data collected using this method can be used to determine respiratory quotient (RQ), energy expenditure (EE), and specifics of macronutrient oxidation in animals (Ferrannini, 1988). The RQ determines which dietary macronutrient is being oxidized by an animal. An RQ of 0.7 indicates mainly fat oxidation, while an RQ of 1.0 indicates
mainly carbohydrate oxidation (Lusk, 1928). Previously, cats have been found to alter macronutrient oxidation based on differences in dietary intake, reflected in measures of RQ (Hoenig et al., 2007; Green et al., 2008; Gooding et al., 2014). Furthermore, carbohydrate intake, or glucose load, alters postprandial glucose response and macronutrient oxidation in cats; though type of carbohydrate was not investigated (Gooding et al., 2014). Conversely, macronutrient intake does not seem to affect energy expenditure in cats fed isocaloric provisions (Lester et al., 1999; Green et al., 2008; Gooding et al., 2014).

The objectives of this study were to measure the effects of three diets differing in nutrient profile, ingredient composition, and their perceived glycemic response (PGR) on respiratory quotient (RQ) and energy expenditure (EE) in cats. Further, this study had the objective to compare continuous interstitial glucose monitoring and postprandial serum glucose and insulin responses in cats fed the three experimental diets. We hypothesized that; 1) energy expenditure would not differ between cats fed similar daily amounts of dietary energy, regardless of different diet compositions; 2) RQ will be highest for the HighPGR diet, due to the high inclusion of starch and inclusion of ingredients with high glycemic responses; 3) interstitial glucose will behave similarly to serum glucose; 4) based on glycemic responses of the included ingredients (Carciofi et al., 2008), cats fed the HighPGR diet will exhibit the highest glycemic response relative to the Medium and LowPGR diets.

4.3 Materials and Methods

All procedures were reviewed and approved by Procter & Gamble Pet Care’s Institutional Animal Care and Use Committee (AUP 013-9127; Protocol date: 3/7/2013) and were in accordance with USDA and AAALAC guidelines.
4.3.1. Animals, Diets and Experimental Design

Domestic shorthair cats \((n = 19, 10 \text{ males}, 9 \text{ females})\) of similar age \((4 - 5 \text{ yr})\) and body condition score \((3 – 4.5 \text{ on a 9-point scale})\) were used. 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 had been acclimated to calorimetry chambers (Gooding et al., 2012) and also trained to accept repeated (up to eight per day) saphenous vein blood draws (Lockhart et al., 2011). Cats were assigned to diets in a \(3 \times 3\) Latin square, with cats cycling through all diets in three periods in six complete and one incomplete \(3 \times 3\) Latin square.

The term perceived glycemic response (PGR) was used to characterize the diets in the present study. The PGR refers to the expected theoretical glycemic response that would result from consumption of each diet (Asaro et al., 2017). This estimate was determined by looking at the known glycemic indices, from human nutrition research, of the main carbohydrate containing ingredients included in each diet. Feline glycemic responses to common dietary carbohydrate sources were also considered when selecting diets (Carciofi et al., 2008). However, though the glycemic response is suggestive of glycemic index, it is not determined against a known control and therefore no glycemic index can be calculated in the current study, but deserves further research. Three commercial diets were selected for study based on their content of ingredients predicted to elicit high, medium, and low glycemic responses (Chapter 3; Table 3.1). Purina ONE Chicken and Rice (Nestlé, St. Louis, MO, USA) was chosen as the HighPGR diet due to its high inclusion of Brewer’s rice, known to elicit a high glycemic response (Jenkins et al., 1981; Carciofi et al., 2008; de-Oliveira et al., 2008). Iams Kitten Proactive Health (Procter & Gamble, Cincinnati, OH, USA) was comprised of ingredients including corn meal and sorghum, that both elicit a lower
glycemic response than ingredients such as Brewer’s rice, and was termed MediumPGR (Bouchard and Sunvold, 2001; Carciofi et al., 2008; de-Oliveira et al., 2008). Last, Innova Dry Adult Cat Food (Procter & Gamble, Cincinnati, OH, USA) was predicted to have the lowest PGR because of the use of barley, which has previously been found to elicit a low glycemic response (Jenkins et al, 1988), and its higher relative inclusion of protein. Diet analyses were completed as described in Chapter 3 (Table 3.1). Cats were fed to maintain body weight (BW) using individual historical data of energy intake. Dietary metabolizable energy (ME) allowance was calculated using the modified Atwater calculation, though it was later determined that the modified Atwater equation consistently underestimated true ME of the experimental diets (Asaro et al., 2017). Cats were subjected to an 8-d pre-feeding schedule followed by calorimetry and glucose measures. Food intake and orts were recorded daily, and BW was recorded on d 9 of each period. The same schedule was used for all 3 periods of the study.

4.3.2. Experimental Procedures

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 and the swinging door was locked from 16:00 to 07:00. Artificial lighting schedule followed a pattern of 12 h light beginning at 06:30 and 12 h darkness beginning at 18:30 although the room also had a full wall of windows so they also had natural light. 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 disinfected weekly with Nolvasan (Allivet, St. Hialeah, FL) and daily cleaning was performed.

Cats were divided into four groups of five and one group of four to accommodate rotation through the 5 calorimetry chambers. Cats began the study with an 8-d pre-feeding period on one
of the three diets. Day 1 for each group of cats was staggered to ensure each group received a consistent 8-d of pre-feeding before indirect calorimetry measurements on d 9. Cats were placed in individual cages for feeding at 07:00 each morning and given 60 min to eat before they were removed and left-over food was weighed.

At 06:00 on d 9, subcutaneous interstitial glucose sensors (Guardian Continuous Monitoring Devices, Medtronic Diabetes, Northridge, CA) were implanted between the ninth and last rib of each cat. Sensor cannulae were 14 mm, with an attached $4.17 \times 3.56 \times 0.94$ cm transmitter lying 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 of the sensor and dressing. After interstitial glucose sensor placement, the first group of cats was placed in the calorimetry chambers for 21-h to measure respiratory gas exchange. The calorimetry chambers (Qubit Systems, Kingston, ON, Canada) were composed of Plexiglas and measured $53.3 \times 53.3 \times 76.2$ 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 was large enough to provide enough separation between areas used for feeding, sleeping and elimination (Gooding et al., 2012). Indirect calorimetry techniques were used to measure EE and RQ, and indirect calorimetry conduct was performed as described by Gooding et al. (2014). Energy expenditure was calculated using the abbreviated Weir equation; \((EE \text{ kcal/d}) = [3.94 \times \text{O}_2 \text{ exchange (L/h)} + 1.11 \times \text{CO}_2 \text{ exchange (L/h)}] \times 24 \text{ h}]\) (Weir, 1912), and RQ was calculated as the ratio of CO$_2$ production to O$_2$ consumption. On a daily basis, the chambers and water bowls were disinfected and the litter, litter boxes, toys, hammock and beds were removed and cleaned. Analyzers (CO$_2$ and O$_2$) were calibrated at 06:45 and 18:00. Interstitial glucose measurements were automatically
recorded every 5 min. These real-time measurements were used to create a 20 h curve of interstitial glucose concentrations for each of the cats. Calibration of interstitial glucose monitors 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 an 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. The detection limit was 40 mg/dL and this value was recorded when the measurement was equal to or below this limit.

On d 10, sequential blood sampling was completed to determine postprandial serum glucose and insulin response. Blood samples (1 mL) were taken from the saphenous vein 15 min before feeding (06:45, fasting) and at 30, 60, 120, 240, 360, 480, and 600 min after feeding. Sampling was started on the right rear leg, moving dorsally for each subsequent blood draw, and switched to the left rear leg on sample 4, or before if necessary, depending on the individual cat. Cats were kept in individual cages in a procedure room for the duration of the blood draws (05:45-16:15) for constant monitoring. Blood was obtained using the BD Safety-Lok Vacutainer (Becton, Dickinson and Company, Franklin Lakes, NJ), 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 a Thermo Scientific IEC Centra GP8 centrifuge (Fisher Scientific Company, Ottawa, ON) at 1862 × g and -4°C for 15 min after clotting to collect the serum. Serum aliquots were stored at -20°C until analysis. Serum glucose analysis was completed through colorimetric measurements with a Beckman Coulter AU480 automated chemistry analyzer (Indianapolis, IN). Serum insulin analysis was completed using a feline ELISA kit (Mercodia Inc., Winston Salem, NC). Peak glucose and insulin concentrations and times were calculated from mean serum concentrations among cats at each time point for each diet.
4.3.3. Statistical Analyses

Statistical power was calculated prior to the study with SAS (version 9.3, SAS Institute Inc., Cary, NC, USA) using data from Shoveller et al. (2010). For the population size of 19 cats, statistical power was found to be 97.4% for energy expenditure, serum insulin and glucose concentrations.

Data were analyzed using the proc MIXED function of SAS version 9.3 with cat as the experimental unit, cat and period as random effects, diet as a fixed effect, and time as a repeated measure. Body weight was included as a covariate in the serum glucose and insulin analyses. Repeated measures analyses were performed for RQ, EE, serum glucose, serum insulin and interstitial glucose over time. The autoregressive moving average model (ARMA 1,1) covariance structure was used in the analysis of interstitial glucose, while the compound symmetry (CS) structure was used in the analysis of RQ, EE, serum glucose, and serum insulin, as they were found to have the lowest values for Akaike Information Criterion (AIC).

When the effect of diet was significant ($P < 0.05$), means were separated using the least significant difference with a Tukey-Kramer test for multiple comparisons. If $0.05 < P < 0.10$, a trend was declared. Data were reported as least-squares means ± SEM.

4.4. 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 interstitial glucose 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 20 h instead of the planned 22 h.
In period 2, data was not recorded past the 10.5 h calibration for four cats due to software malfunction. Two of these cats were fed the LowPGR diet, one was fed the MediumPGR diet, and one was fed the HighPGR diet. The data that was collected in the 12 h previous to calibration was included in our analyses. In period 3, the calorimetry software did not record the first 12 h of data for one cat fed the LowPGR diet. Therefore, the sample size was reduced to 18 cats for our calorimetry measurements.

4.4.1. Food and Energy Intake

On an as-fed basis, daily food intake was higher for the HighPGR diet (45.6 g/d), than for the MediumPGR (37.9 g/d) and LowPGR (40.0 g/d) diets ($P = 0.02$). However, cats were fed isocalorically (155.8, 154.5, 154.3 kcal/d for High, Medium and LowPGR, respectively) and therefore ME intake (kcal ME/d) did not differ among diets (Table 4.1; $P = 0.98$).

4.4.2. Indirect Calorimetry

Fasted RQ was greater for cats fed the HighPGR diet ($P = 0.004$) than cats fed the MediumPGR and LowPGR diets. Immediately following meal feeding, the RQ increased until approximately 360 min, stayed at plateau, and then declined from 660 min to the end of the calorimetry period (Figure 4.1). The RQ in the fed state was greater (Table 4.2; $P < 0.001$) for cats fed the HighPGR diet than for cats fed the MediumPGR and LowPGR diets. The EE in the fasted state did not differ ($P = 0.160$) among diets. In the fed state, EE was greater in the HighPGR diet than the MediumPGR diet and LowPGR diet (Table 4.2; $P < 0.001$).

4.4.3. Interstitial and Serum Measurements

Mean 20 h interstitial glucose concentration was greater for the HighPGR diet than for the Medium and LowPGR diets ($P = 0.003$) (Table 4.3). Occasionally, sensors recorded a reading of
40 mg/dL with little variation during the entire period. This reading may indicate that interstitial glucose levels were below the detection limit of 40 mg/dL. Multiple readings of 40 mg/dL occurred for four cats in period 1, two cats in period 2, and one cat in period 3. Of these, four instances occurred in cats fed the LowPGR diet, two for the MediumPGR diet, and one for the HighPGR diet. However, low glucose readings were consistent among the same cats, and as such, were not a concern.

Peak serum glucose concentrations occurred in the fasted state (-15 min), decreased immediately post meal feeding and then reached plateau for the three diets (Figure 4.3). Mean 600-min serum glucose concentrations did not differ among treatments (Table 4.3) or between the fasted or fed states. Greater body weight resulted in greater serum glucose (P < 0.001). Time, included as a covariate, also affected serum glucose (P < 0.001). Diet and time did not interact (P = 0.112).

Serum insulin concentrations tended to increase postprandially before reaching plateau (Figure 4.4). Fasted measurements of serum insulin did not differ among diets (P = 0.468). In the fed state, serum insulin was greater for the HighPGR than for the MediumPGR diet (P < 0.001), and did not differ from the LowPGR diet. Body weight was a significant covariate, indicating that greater body weight resulted in greater serum insulin (P < 0.05). Time also affected serum insulin (P < 0.001). Diet and time interacted (P < 0.001). Average peak time for insulin was 60 min postprandially for the LowPGR diet, followed by the MediumPGR (240 min) and HighPGR diets (360 min). Peak insulin concentrations of the HighPGR and LowPGR diets were greater than for the MediumPGR diet (P = 0.004).
4.5. Discussion

The results from the present study suggest that healthy cats fed a diet high in starch content (36.75%) exhibit a higher RQ compared to cats fed diets with lower starch content (30.72% and 23.56%). Second, consumption of a high PGR diet containing starch (36.75%) with significant contributions from brewer’s rice, results in elevated interstitial glucose, but not serum glucose, compared to cats fed diets lower in starch, and comprised of ingredients predicted to elicit a lower glycemic response. Third, carbohydrate source and level have a minor effect on postprandial serum glucose and insulin responses in cats. Last, EE was greatest in diets predicted to have a high glycemic response compared to diets predicted to have a low glycemic response. Overall, the current results support the value of using real-time interstitial glucose monitors or calorimetry to examine glucose metabolism, and that carbohydrate content and ingredient selection may affect postprandial energy expenditure in cats.

Observed differences in both fasted and fed RQ between diets were similar to those reported in the literature. Previously, mean fasted RQ was higher in cats fed a high carbohydrate diet than those fed a high fat diet (Gooding et al., 2014). Furthermore, baseline RQ was higher in cats fed a high carbohydrate diet (38.1% carbohydrate, 27.5% protein) than cats fed a high protein diet (24.7% carbohydrate, 45.2% protein) (Hoenig et al., 2007). Additionally, cats had lower RQ and therefore increased levels of fat oxidation with greater dietary fat (Lester et al., 1999; Gooding et al., 2014). The lower RQs of cats fed the Medium and LowPGR diets in the present study suggest that our cats adapted comparably to previous studies, by increasing fat oxidation in response to greater dietary fat content and fat intake. Similarly, the greater mean fed RQ in cats fed the HighPGR diet implies increased carbohydrate oxidation compared to cats fed the Medium and LowPGR diets. This modulation of RQ according to diet composition is consistent with the
literature and indicates that lean healthy cats can adapt macronutrient utilization to alterations in dietary macronutrient intake (Hoenig et al., 2007; Gooding et al., 2015).

In the present study, RQ values in both the fed and fasted states were close to 0.7, indicating that despite the high carbohydrate content of the diets, cats tended to oxidize fat (Lusk, 1928). However, feeding high carbohydrate diets may increase lipogenesis and synthesis of triacylglycerols in cats (Gooding et al., 2014). Previously, cats gained more weight after consuming a high carbohydrate diet compared to a high fat diet, likely due to the increased lipogenesis and fatty acid storage in adipose tissue (Gooding et al., 2014). Though body weight was not different among treatment groups, cats in the present study were likely exhibiting increased fat synthesis and subsequent oxidation in response to the high carbohydrate content of the experimental diets, therefore explaining our low observed RQ values.

Respiratory quotients (RQs) for all diets followed a biphasic response, peaking twice throughout the calorimetry period (Figure 4.1). A similar pattern has been observed in dogs and pigs. However, the response in the present study was significantly delayed in contrast to other species. In dogs, a peak in RQ was observed within the first 30 min of feeding, and again at 75 min postprandially (Diamond and LeBlanc, 1987). The first peak in RQ may be attributed to stimulation of the sympathetic nervous system, coupled with increased respiratory rate that may have occurred with the excitement of the feeding process (Diamond and LeBlanc, 1987). The second peak in RQ observed in dogs coincides with the digestive phase, indicating an increase in carbohydrate oxidation when food is digested and absorbed (Diamond and LeBlanc, 1987). Furthermore, in pigs, RQ began to increase 25 min postprandially, and remained elevated up to 80 min post feeding (Stombaugh and Grifo, 1977). Because the cats in the present study did not exhibit a rapid increase in RQ directly after feeding, it is likely that the first peak in RQ coincides
with the digestive phase, similar to that of dogs. The second peak in RQ coincides with calibration of the chambers, therefore it possible that this peak is attributed to excitement of cats when researchers entered the room (Rand et al., 2002). This is notable, as disruptions were minimal when cats were in chambers, indicating that human interaction can significantly affect rates of glucose oxidation and energy expenditure especially in well-socialized cats.

The lack of difference in fasted EE among diets is consistent with previous research, both in cats and other species. In two studies where cats were fed diets varying in macronutrient composition, fasted EE was not different among treatments (Lester et al., 1999; Gooding et al., 2014). In dogs fed both low and high carbohydrate diets, resting EE was not different (McKnight et al., 2014). Additionally, fasting heat production was not different in growing pigs fed three diets differing in protein, fat, and starch content (Heo et al., 2014). Similar heat production is expected, as animals in a post-absorptive state resort to glycogen and lipid stores as the main sources of energy (Jequier et al., 1987). Furthermore, lean body mass or fat-free mass is directly correlated to energy expenditure (Cunningham, 1991). Since body weight was not different among treatment groups, it is not surprising that there were not differences in fasted energy expenditure.

The differences in postprandial EE observed in the present study were not anticipated, nor consistent with the limited previous research. In cats, EE was not altered by differences in macronutrient intake when fed isocaloric provisions (Lester et al., 1999; Gooding et al., 2014). Similarly, in dogs fed two diets either low or high in carbohydrate content relative to fat, postprandial EE was not different at any time throughout the course of a 23-hour calorimetry period (McKnight et al., 2014). Meal size and frequency, not dietary macronutrient content, are factors that can influence EE (LeBlanc and Diamond, 1986; Pouteau et al., 2000). Previously, EE decreased due to overfeeding (Pouteau et al., 2000), while feeding multiple times daily increased
dietary induced thermogenesis in dogs (LeBlanc and Diamond, 1986). Because cats in the present study were fed once daily, and provisions were restricted to maintenance requirements, we did not expect to see differences in postprandial EE. Differences in postprandial EE may be explained by increased lipogenesis in response to high carbohydrate intake. The correlation between carbohydrates and lipogenesis has been observed in multiple species. In omnivorous fish, increased lipid biosynthesis is related to increased dietary carbohydrate intake (Hemre et al., 2002). Similarly, in human infants, metabolic rate was positively correlated with carbohydrate intake, presumably as a consequence of increased fat synthesis (Sauer et al., 1986). It is probable that differences in carbohydrate content and resulting intake of the experimental diets in the present study stimulate variable lipid synthesis, and therefore different postprandial EE among treatments. Furthermore, the calculated ME from which feeding recommendations were developed was previously proven to be inaccurate (Asaro et al., 2017). When caloric intake was reanalyzed using the true ME of the diets (Chapter 3), it was noticed that cats were unintentionally offered non-isocaloric meals, with more energy being consumed by cats fed the HighPGR diet (Table 4.1). It is likely that these unintended differences contributed to the observed differences in postprandial EE among diets and supports the fact that postprandial energy expenditure is in part related to the total calories consumed (Apfelbaum et al., 1971).

Interstitial glucose has not been well studied in the domestic cat. Feline glycemic responses are usually measured using repeated blood sampling, often requiring catheter placement (Reineke et al., 2010). These methods are not only invasive and stressful to cats, but also cannot yield values in real-time (Rebrin and Steil, 2000). Stress has been found to cause temporary hyperglycemia in cats (Rand et al., 2002) and therefore blood glucose concentrations may be overestimated due to stress-induced changes (Wiedmeyer et al., 2003). Continuous interstitial glucose monitoring
systems were first evaluated in cats by Wiedmeyer et al. (2003) and were well correlated with whole blood glucose levels (Wiedmeyer et al., 2003). Additionally, interstitial glucose has been found to mimic blood glucose in multiple species including, rabbits, rats, and humans (Rebrin et al., 1999). As expected, interstitial glucose followed a parallel pattern to RQ in the present study, peaking twice throughout the total calorimetry period (Figure 4.2). The first peak in interstitial glucose begins approximately 6 h postprandially, and corresponds with the first peak in RQ. Similarly, the second peak in interstitial glucose coincides with the second peak in RQ. As discussed previously, it is possible that the first peak is due to the effect of feeding and digestion, while the second peak is likely due to the excitement of having people enter the room (Rand et al., 2002). Though not strongly correlated ($\rho = 0.28$; data not shown), the relationship between RQ and interstitial glucose may yield an alternative, less invasive technique to determine feline glycemic response.

The preprandial elevation in serum glucose observed in the present study was unexpected. Based on glucose response studies conducted in multiple monogastric species, serum glucose was anticipated to peak postprandially (Appleton et al., 2004, Galgani et al., 2006; Carciofi et al., 2008; de-Oliveira et al., 2008; Farrow et al., 2013). In dogs (Carciofi et al., 2008) and humans (Galgani et al., 2006), serum glucose peaked at approximately 60 min postprandially, and returned to baseline (measures taken at time 0) levels by 180 min postprandially. Previously, blood glucose peaked at 10 h (rice) and 18 h (sorghum/corn) postprandially for cats fed two diets of similar starch content (33%) with significant starch contributions from either rice or sorghum and corn (Appleton et al., 2004). Furthermore, blood glucose has remained elevated for upwards of 24 h in cats fed a high-carbohydrate diet (Farrow et al., 2013). These studies indicate that cats seem to reach peak glucose concentrations more slowly than other monogastric omnivorous species (Farrow et al.,
A 10 h blood sampling period may not have been long enough to capture peak serum glucose concentrations in the cats. Additionally, it is likely that similar to the interstitial measurements, serum glucose peaked preprandially due to meal anticipation, resulting in transient hyperglycemia (Rand et al., 2002). However, it is worth noting that serum glucose concentrations among all diets did not deviate more than 15 mg/dL between baseline and peak measurements. This agrees with previous literature that cats have a low and more gradual glucose response compared to other monogastric species (Appleton et al., 2004).

The serum insulin concentrations in the present study are consistent with the literature. Previously, consumption of either a low carbohydrate or high carbohydrate diet did not affect serum insulin in the fed and fasted states in healthy adult cats (Coradini et al., 2011). Additionally, serum insulin did not differ between cats fed high fat and high carbohydrate diets (Gooding et al., 2014). However, in the present study, mean serum insulin, but not serum glucose, was different among cats fed dietary treatments. It was expected that these two variables would be dependent on one another (de-Oliveira et al., 2008; Farrow et al., 2013). The present results suggest that factors other than starch level and ingredient source affect insulin release in cats. Farrow and colleagues (2013) found that cats consuming high protein diets tended to reach peak insulin faster than those fed high starch or high fat diets. Similarly, in the present study, serum insulin peaked fastest in cats fed the LowPGR diet, which also contained the largest proportion of protein. This pattern may be due to the stimulation of insulin release by amino acids, such as arginine, leucine, and alanine (Yasuda et al., 2011). Conversely, consumption of low protein diets has been found to decrease time to peak insulin compared to low starch diets in cats (Verbrugghe et al., 2010). Overall, postprandial insulin patterns in cats are inconsistent among studies, suggesting that insulin secretion is not entirely regulated by plasma glucose, and further, that insulin is not the sole driver
of glucose uptake. Clearly this area requires further research especially in light of the propensity for cats to develop Type 2 Diabetes (Henson and O’Brien, 2006).

The findings that serum glucose and serum insulin did not increase with PGR were unexpected. The postprandial serum glucose and insulin concentrations suggest that differences between diets are not as distinct in cats compared to other species. Similar to our results, in cats fed six diets that differed in their starch ingredient source (corn, brewer’s rice, sorghum, peas, lentils and cassava flour), only the corn diet stimulated a significant increase in postprandial glucose from baseline values (de-Oliveira et al., 2008). Furthermore, previous studies support the conclusion that starch has a minimal effect on the glucose and insulin responses of cats (Kienzle et al., 1994; Bouchard and Sunvold, 2000). This metabolic anomaly may be associated with cats’ enzymatic ability to preferentially use amino acids for energy, or potentially due to the delayed digestion and absorption of carbohydrates in cats (Kienzle et al., 1994; Morris, 2001). However, the regulation of carbohydrate metabolism in cats is relatively unknown and requires further investigation.

In conclusion, while RQ, EE, and interstitial glucose behaved as expected, serum glucose and insulin concentrations presented variability that suggests their lack of scientific value. The results of the present study indicate that cats have a prolonged postprandial glucose and insulin response compared to other monogastric species, when consuming diets with starch ranging from 24 – 36 %. Furthermore, the present study suggests that starch source and inclusion level have minimal effects on postprandial glucose and insulin responses in lean adult cats. Overall, interstitial glucose proved to be less variable than serum glucose measurements, and should be considered as a non-invasive measure that additionally can more robustly define the real-time glucose response to a meal. For these reasons, calorimetry and interstitial glucose measurements
are suggested to be superior to repeated serum glucose measurements, as they require fewer animals to investigate metabolism, and should be considered over traditional methods for future research.
4.6. Tables and Figures

Table 4.1. Body weight and food and energy intake for cats consuming three experimental diets differing in perceived glycemic response (PGR). The PGR refers to the expected theoretical glycemic response that would result from consumption of each diet.

<table>
<thead>
<tr>
<th>Variable</th>
<th>HighPGR</th>
<th>MediumPGR</th>
<th>LowPGR</th>
<th>SEM$^1$</th>
<th>$P$-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body Weight (kg)</td>
<td>4.99</td>
<td>4.95</td>
<td>4.94</td>
<td>0.34</td>
<td>0.99</td>
</tr>
<tr>
<td>Food intake as fed (g/day)</td>
<td>45.6$^a$</td>
<td>37.9$^b$</td>
<td>40.0$^b$</td>
<td>2.68</td>
<td>0.02</td>
</tr>
<tr>
<td>Calculated ME$^2$ Intake (kcal/day)</td>
<td>155.8</td>
<td>154.5</td>
<td>154.3</td>
<td>10.08</td>
<td>0.98</td>
</tr>
<tr>
<td>True ME Intake (kcal/d)$^3$</td>
<td>230.7$^a$</td>
<td>173.9$^b$</td>
<td>196.2$^b$</td>
<td>12.71</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

$^a$ Within a row, means without a common superscript differ ($P < 0.05$). $^1$Pooled SEM. Means were based on 19 cat observations per diet. $^2$Calculated with modified Atwater equation (AAFCO, 1997): ME (kcal/kg) = [3.5 × CP (%) + 3.5 × NFE (%) + 8.5 × crude fat (%)] × 10. $^3$Calculated using analyzed ME from Chapter 3.
Table 4.2. Indirect calorimetry measurements of cats consuming three experimental diets differing in perceived glycemic response (PGR) at different time intervals. The PGR refers to the expected theoretical glycemic response that would result from consumption of each diet.

<table>
<thead>
<tr>
<th>Variable</th>
<th>HighPGR</th>
<th>MediumPGR</th>
<th>LowPGR</th>
<th>SEM</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>RQ</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean Fasting$^2$</td>
<td>0.77$^a$</td>
<td>0.75$^b$</td>
<td>0.75$^b$</td>
<td>0.007</td>
<td>0.004</td>
</tr>
<tr>
<td>Mean Postprandial$^3$</td>
<td>0.79$^a$</td>
<td>0.76$^b$</td>
<td>0.76$^b$</td>
<td>0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>EE (kcal/kg BW/d)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean$^4$</td>
<td>43.3$^a$</td>
<td>41.8$^b$</td>
<td>41.6$^b$</td>
<td>0.28</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mean Fasted</td>
<td>42.1</td>
<td>40.9</td>
<td>39.8</td>
<td>1.18</td>
<td>0.160</td>
</tr>
<tr>
<td>Mean Postprandial</td>
<td>43.3$^a$</td>
<td>41.8$^b$</td>
<td>41.6$^b$</td>
<td>0.29</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

$^a-c$ Within a row, means without a common superscript differ ($P < 0.05$). $^1$Pooled SEM. Means were based on 18 cat observations per diet. $^2$Fasting measurements were taken at $t = -60$ and $t = -30$ min. $^3$Fed (postprandial) measurements were taken at $t = 0$ min onward. $^4$Mean of total calorimetry period (fasted and fed measurements).
Table 4.3. Continuous/Real-time interstitial glucose monitoring and pre- and postprandial serum glucose and insulin response of cats consuming three experimental diets differing in perceived glycemic response (PGR). The PGR refers to the expected theoretical glycemic response that would result from consumption of each diet.

<table>
<thead>
<tr>
<th>Variable</th>
<th>HighPGR</th>
<th>MediumPGR</th>
<th>LowPGR</th>
<th>SEM</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interstitial glucose</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20 h (mg/dL d⁻¹)</td>
<td>71.9ᵃ</td>
<td>64.3ᵇ</td>
<td>59.0ᵇ</td>
<td>2.64</td>
<td>0.003</td>
</tr>
<tr>
<td>Serum glucose</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>600 min (mg/dL d⁻¹)</td>
<td>95.2</td>
<td>96.0</td>
<td>94.6</td>
<td>1.31</td>
<td>0.559</td>
</tr>
<tr>
<td>Fasting² (mg/dL d⁻¹)</td>
<td>100.8</td>
<td>105.1</td>
<td>107.3</td>
<td>2.46</td>
<td>0.173</td>
</tr>
<tr>
<td>Fed³ (mg/dL d⁻¹)</td>
<td>94.4</td>
<td>94.7</td>
<td>92.8</td>
<td>1.33</td>
<td>0.298</td>
</tr>
<tr>
<td>Peak (mg/dL d⁻¹)</td>
<td>100.8</td>
<td>105.1</td>
<td>107.3</td>
<td>2.57</td>
<td>0.074</td>
</tr>
<tr>
<td>Serum insulin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>600-min (µIU/mL d⁻¹)</td>
<td>10.4ᵃ</td>
<td>7.2ᵇ</td>
<td>9.5ᵃ</td>
<td>0.68</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fasting (µIU/mL d⁻¹)</td>
<td>6.3</td>
<td>5.3</td>
<td>6.5</td>
<td>0.77</td>
<td>0.468</td>
</tr>
<tr>
<td>Fed (µIU/mL d⁻¹)</td>
<td>10.9ᵃ</td>
<td>7.5ᵇ</td>
<td>9.8ᵃ</td>
<td>0.75</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Peak (µIU/mL d⁻¹)</td>
<td>13.6ᵃ</td>
<td>9.2ᵇ</td>
<td>14.7ᵃ</td>
<td>1.26</td>
<td>0.004</td>
</tr>
</tbody>
</table>

ᵃ-c Within a row, means without a common superscript differ (P < 0.05). ¹Pooled SEM. Means were based on 19 cat observations per diet. ²Fasting measurements were taken at t = -15 min prior to meal feeding. ³Fed measurements were taken at t = 0 min onward.
Figure 4.1. Respiratory quotient of cats consuming three experimental diets differing in perceived glycemic response (PGR): high, medium and low PGR. The PGR refers to the expected theoretical glycemic response that would result from consumption of each diet. Measurements were taken in 30-min intervals. Cats (n = 19) were fed at t = 0 min and equipment calibration occurred at t = 630 min (vertical dotted line).
Figure 4.2. Interstitial glucose concentrations of cats consuming three experimental diets differing in perceived glycemic response (PGR): high, medium and low PGR, over a measurement period of 20 h. The PGR refers to the expected theoretical glycemic response that would result from consumption of each diet. Cats (n=19) were fed at t = 0 min, but monitors did not start recording until 120 min post feeding (vertical dotted line).
Figure 4.3. Serum glucose concentrations of cats consuming three experimental diets differing in perceived glycemic response (PGR): high, medium and low PGR, after eight sequential blood draws ($P < 0.591$). The PGR refers to the expected theoretical glycemic response that would result from consumption of each diet. Cats ($n=19$) were fed at $t=0$ min.
Figure 4.4. Serum insulin concentrations of cats consuming three experimental diets differing in perceived glycemic response (PGR): high, medium and low PGR, after eight sequential blood draws ($P < 0.591$). The PGR refers to the expected theoretical glycemic response that would result from consumption of each diet. Cats ($n=19$) were fed at $t = 0$ min.
Chapter 5: Modelling net energy of commercial cat diets

5.1. Abstract

Net energy (NE) accounts for the proportion of energy expenditure attributed to the digestion, absorption, and metabolism of ingested food. Net energy is the most accurate system to represent the proportion of ingested energy available for tissue level metabolism. For companion animals, including cats, models to predict NE density of food do not exist. The objectives of this study were to measure the heat increment of feeding (HIF) in cats, and to model dietary NE of commercial diets. In total, 19 domestic shorthair cats (10 males, 9 females) of similar age (4.3 ± 0.48 yr) and body condition (3.3 ± 0.6 on a 9-point scale) were used. Cats were fed one of three commercial diets once a day. Diets differed in ingredients that had different perceived glycemic response (PGR (Chapter 3); 34.1, 29.5, and 23.6 % nitrogen-free extract for High, Medium, and LowPGR, respectively). Diet order was based on an incomplete replicated $3 \times 3$ Latin square design, with three diets and three periods. Each period consisted of 8 d of adaptation to the diet, followed by calorimetry measurements on day 9. On the calorimetry days, animals were placed in chambers and fed 1 hour thereafter. Cats remained in indirect calorimetry chambers for 21 h to measure CO$_2$ production and O$_2$ consumption, and energy expenditure was calculated. Net energy was determined as the difference between metabolizable energy (ME, determined in vivo in Chapter 3) and the HIF. Analyzed NE was highest for the LowPGR diet, followed by the Medium and HighPGR diets ($P < 0.001$). In addition, NE models were developed using ME, and one of four dietary parameters (crude protein (CP), lipid (CL), fiber (CF), and starch). Models 1-4 and 5-8 were developed using HIF values from 0-2 h and 0-21 h, respectively. Energy expenditure in the fasted (-1 - 0 h) state did not differ between diets.

$^1$A version of this manuscript will be submitted to a scientific journal (Authors: N.J. Asaro, W.D. Mansilla, J.P. Cant, D.J. Seymour, J. Brewer, and A.K. Shoveller)
Energy expenditure in the fed state (0 - 21 h) was highest in cats fed the HighPGR diet compared to cats fed the MediumPGR and LowPGR diets (43.3, 41.8, 41.6 kcal/kg BW/d for High, Medium and LowPGR, respectively) \((P < 0.001)\). In the first 2 h postprandial, the HIF amounted to \(1.74 \pm 0.17\%\) of ME (average for all diets; \(P = 0.13\)). Over the full calorimetry period, the heat increment of feeding accounted for \(20.9 \pm 1.52\%\) of ME (average for all diets; \(P = 0.50\)). Of the 8 models developed, the models \(NE = (0.941 \times ME) + (0.519 \times CP)\), which was developed using HIF from 0 – 2 h, fit our observed data best. In conclusion, using HIF to model energy density on an NE basis accounts for similar amounts of HIF as other species and can be calculated to provide a more accurate estimate of dietary energy availability for cats.
5.2. Introduction

Net energy (NE) models have been developed for use with multiple agricultural species such as swine and cattle (Donker and Naik, 1979; Noblet et al., 1994), but to date, they do not exist for the domestic cat. The pet food industry currently uses the modified Atwater equation to estimate the metabolizable energy (ME) content of pet foods (NRC, 2006; Asaro et al., 2017). This equation assigns coefficients to three macronutrients – protein, fat, and carbohydrate (calculated as nitrogen-free extract; NFE) – to predict the ME content of a diet (NRC, 2006). However, these equations result in inaccurate predictions of dietary energy content (NRC, 2006). Developing models to accurately predict the available energy in food intended for cats is critical to provide consumers with optimal feeding recommendations (Asaro et al., 2017).

Net energy can be described as the ME minus the heat increment of feeding (HIF) (Moehn et al., 2005). The HIF, also commonly referred to as diet-induced thermogenesis, is variable, and can be altered by dietary macronutrient content, diet processing conditions, and environmental temperature (LeGrow and Beamish, 1986; Collin et al., 2003). Therefore, the HIF is important to measure for an accurate representation of dietary energy directly available to the animal (Moehn et al., 2005). The HIF can be measured through indirect calorimetry, using measures of oxygen and carbon dioxide exchange to calculate energy expenditure of animals (Ferrannini, 1988). Compared to ME, which accounts for fecal and urinary energy losses, NE is more complex to quantify. To overcome this issue, mathematical models have been developed that predict the NE content of feed using diet composition variables (Donker and Naik, 1979; Noblet et al., 1994). Unlike ME models, NE gives a precise estimate of the energy directly available for use by an animal (Moehn et al., 2005). Noblet et al. (1994) proposed a model to estimate the NE of pig feed, using macronutrient composition and ME content of the complete diet as parameters. This model
provides a superior prediction of the energy content of a feed, and allows for increased accuracy of formulation and subsequent feeding recommendations.

The objectives of this study were to calculate NE content of three diets differing in macronutrient profile, ingredient composition, and perceived glycemic responses (PGR), and to propose new models that can accurately predict NE of domestic cat diets. We hypothesized that 1) parameters used for NE models in other monogastric species can be used to predict NE of domestic cat diets; and 2) due to differences in macronutrient profile, ingredient composition and PGR, HIF would be different between diets.

5.3. Materials and Methods

5.3.1. Animals and Housing

All procedures were reviewed and approved by Procter & Gamble Pet Care’s Institutional Animal Care and Use Committee and were in accordance with the United States Department of Agriculture (USDA) and the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) guidelines (Study number: 013-9127; Protocol date: 3/7/2013). Nineteen domestic shorthair cats (10 males, 9 females) of similar age (4.3 ± 0.48 yr) and body condition score (3.3 ± 0.6 on a 9-point scale) were used. Cats were housed at the Pet Health and Nutrition Centre at Procter & Gamble Pet Care (Lewisburg, OH) and received physical exams by an accredited veterinarian to ensure health before and during the study. Cats had been appropriately acclimated to calorimetry chambers according to Gooding et al. (2012) previous to the present study.

Cats were housed in free-living group environments with room enrichment including perches, toys, beds, scratching posts, and climbing apparatus. Cats could choose to go outside in a fully fenced area through a swinging door during daylight hours. The swinging door was locked
from 16:00 to 07:00. The lighting schedule was 12 h:12h light:dark beginning at 06:30. The housing room was equipped with a full wall of windows which provided cats with natural light. Room temperature was kept at 22°C, and relative humidity was 50 to 60%. Water was provided \textit{ad libitum} via automatic waterers for the duration of the study. Surfaces, including walls and windows, were cleaned weekly with Nolvasan disinfectant (Allivet, St. Hialeah, FL) and daily cleaning was performed.

5.3.2. Experimental Diets and Design

Three diets were selected containing ingredients that are predicted to elicit high, medium, and low glycemic responses, and named based on their respected PGR (Chapter 3). The PGR refers to the expected glycemic response that would hypothetically result from consumption of each diet. This estimate was determined by accounting for the glycemic indices of the main carbohydrate sources included in each diet, determined from studies in humans, and the glycemic response of these ingredients previously measured in companion animals (Jenkins et al., 1981; Jenkins et al., 1988; Carciofi et al., 2008; de-Oliveira et al., 2008). Three diets were studied (Chapter 3; Table 3.1): HighPGR: Purina ONE Chicken and Rice (Nestlé, St. Louis, MO, USA), MediumPGR: Iams Kitten Proactive Health (Procter & Gamble, Cincinnati, OH, USA), and LowPGR: Innova Dry Adult Cat Food (Procter & Gamble, Cincinnati, OH, USA). Purina ONE Chicken and Rice was chosen as the HighPGR diet due to its high inclusion of Brewer’s rice, known to elicit a high glycemic response (Jenkins et al., 1981; Carciofi et al., 2008; de-Oliveira et al., 2008). Iams Kitten Proactive health was comprised of ingredients including corn meal and sorghum, that both elicit a lower glycemic response than ingredients such as Brewer’s rice (Bouchard and Sunvold, 2001; Carciofi et al., 2008; de-Oliveira et al., 2008). Last, Innova Dry Adult Cat Food was predicted to have the lowest PGR because of the use of barley, which has previously been found to elicit a low
glycemic response (Jenkins et al., 1988), and its higher relative inclusion of protein. Diet analyses were completed as described in Chapter 3 (Table 3.1).

Cats were assigned to diets based on a $3 \times 3$ Latin square with six complete squares and one incomplete square. Each period lasted 9 d with 8 d of adaptation to the diet, and 1 d of calorimetry measurements for a consecutive 22 h. Cats were fed to maintain BW using individual historical data of energy intake. Cats were placed in individual cages for feeding at 07:00 each morning and given 60 min to eat before orts were removed and weighed. Dietary ME was calculated using the modified Atwater calculation (AAFCO, 1997), and feed allowance was determined based on this estimation.

5.3.3. Indirect Calorimetry

On d 9 of each period, cats were placed in calorimetry chambers for a 22-h measurement of respiratory gas exchanges. The calorimetry chambers (Qubit Systems, Kingston, ON, Canada) were composed of Plexiglas and measured $53.3 \times 53.3 \times 76.2$ 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 chambers were large enough to provide enough separation between areas used for feeding, sleeping and elimination (Gooding et al., 2012). Indirect calorimetry techniques were used to measure energy expenditure and respiratory quotient (RQ) and indirect calorimetry conduct was performed as described by Gooding et al. (2014). Daily, the chambers and water bowls were disinfected and the litter, litter boxes, toys, hammock, and beds were removed and cleaned.

On study days, cats were placed in the chambers and after 30 min of gas equilibration, two fasting gas exchange measurements were taken on each cat at 60 and 30 min prior to feeding to establish fasted, resting CO$_2$ (VCO$_2$) production and O$_2$ consumption (VO$_2$). At time 0, cats were
fed their individual assigned diet. Thereafter, VO$_2$ and VCO$_2$ were measured in 30 min intervals. The calorimeter was open circuit and ventilated with room air being drawn through at a rate between 5 and 10 L/min depending on the weight of individual cats.

Calibration of the analyzers and mass flow meters was performed prior to each day of calorimetry measurements 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. After 22 h, the cats were removed from chambers and placed back into their rooms.

5.3.4. Analyses

Levels of O$_2$ and CO$_2$ in the respiration chambers were measured with infrared O$_2$ and CO$_2$ analyzers (Qubit Systems, Kingston, ON, Canada). Rate of airflow was measured using a mass flow meter. The analyzed ME and nutrient profiles of diets are described in Chapter 3 (Table 3.1).

Energy expenditure was segmented by time point, similar to Gooding et al. (2016). Postprandial EE, fed EE, return to fasted EE, and late fasted EE were measured from 0 to 5.5 h, 5.5 to 10.5 h, 10.5 to 15.5 h, and 15.5 h to 21 h post feeding, respectively. Since a clear increase in EE was observed between 0 and 2 h, the average EE during the first 2 h post feeding was also analyzed.

In the present study, basal metabolic rate (BMR) could not be measured. Measurements of basal metabolic rate require an animal to be in a postabsorptive state, and not have undergone significant activity (Kleiber, 1975). However, eliminating activity in an animal study requires restraining of animals, which would increase stress and lead to inaccurate measurements. Thus, resting fed metabolic rate (RFMR) was used to approximate BMR. The RFMR is defined as the lowest observed value of energy expended by an animal in a fed state which otherwise meets the
criteria for basal metabolism (NRC, 2006).

5.3.5. Calculations

Energy expenditure was calculated using the abbreviated Weir equation (Weir, 1912):

\[
EE \text{ (kcal/d)} = [3.94 \times O_2 \text{ exchange (L/h)} + 1.11 \times CO_2 \text{ exchange (L/h)}] \times 24 \text{ h}
\]

RQ was calculated as the ratio of CO₂ production to O₂ consumption. The NE of the diets was determined as the ME per 100 g dry matter, minus HIF associated with consuming the same amount of food (Case, 2011). Heat increment of feeding was calculated for the first 2 h post feeding and the entire calorimetry period (0 – 21 h), as the area under the curve of postprandial EE above the RFMR. Area under the curve was calculated using the linear trapezoidal rule (Wolever and Jenkins, 1986).

5.3.6. Modelling Net Energy and Statistical Analyses

Correlations between calculated NE and analyzed ME were performed using PROC CORR in SAS (version 9.3, SAS Institute Inc., Cary, NC, USA). Our proposed NE models were developed using both the HIF from 0 – 2 h postprandially, and the HIF for the complete calorimetry period (0-21 h). Coefficients chosen minimized residual sum of squares between predicted and calculated NE. Coefficients of determination (R²) and rMSPE values were calculated in Microsoft Excel (2017) and numerically compared to determine which model fit our observed data best.

Statistical power was calculated prior to the study with SAS (version 9.3, SAS Institute Inc., Cary, NC, USA) using data from Shoveller et al. (2010). For the population size of 19 cats, statistical power was found to be 97.4 % for energy expenditure. Body weight, feed intake, and HIF data were analyzed using the PROC MIXED of SAS with individual cats as the experimental unit, cat and period as random effects, and diet as a fixed effect. Repeated measures analyses were performed for EE over time using the compound symmetry covariance structure, because it had
the lowest Akaike Information Criterion (AIC). Energy expenditure was pooled from all diets, and differences in EE across time were compared against lowest fasted EE using the Dunnett test (SAS Version 9.4). Means were separated using the PDIF option of SAS with a Tukey-Kramer test for multiple comparisons. A $P$-value < 0.05 was used to declare statistical significance. Data were reported as least-squares means ± SEM.

5.4. Results

In period 2, data were not recorded past the 10.5 h calibration for 4 cats due to software malfunction. Two of these cats were fed the LowPGR diet, one was fed the MediumPGR diet, and one was fed the HighPGR diet. The data that were collected in the 12 h previous to calibration were included in our analyses. In period 3, the calorimetry software did not record the first 12 h of data for one cat fed the LowPGR diet. Data from this cat were not included for HIF calculation.

5.4.1. Diet Composition, Body Weight and Feed Intake

The composition of the three test diets differed in macronutrient content (Chapter 3; Table 3.1). Over the duration of the study, body weight did not differ among dietary treatments ($P = 0.99$). On an as-fed basis, daily food intake was higher for the HighPGR treatment than the MediumPGR and LowPGR treatments (Chapter 4; Table 4.1; $P = 0.02$). However, ME intake did not differ between diets ($P = 0.98$), as intended. Differences in energy intake would have required inclusion of energy intake on the HIF. Indeed, the effect of volume of food should be pursued in the future.
5.4.2. *Indirect Calorimetry*

Following feeding, EE increased until 1.5 h, decreased between 2 and 4 h, remained constant until 20.5 h, then increased until the end of the calorimetry period \((P < 0.05; \text{Figure 5.1})\). In the fasted state (-1 to 0 h), EE did not differ between dietary treatments \((P = 0.160)\). There were no differences in RFMR between dietary treatments \((P = 0.89)\). In the first 2 h postprandially, EE was not different between dietary treatments \((P = 0.148)\). In the postprandial state (0 to 5.5 h), EE did not differ between dietary treatments \((P = 0.167)\). In the fed state (5.5 to 10.5 h), EE was highest in cats fed the HighPGR diet, intermediate in cats fed the MediumPGR diet, and lowest in cats fed the LowPGR diet \((P < 0.001)\). In the return to fasted state (10.5 – 15.5 h), EE was higher in cats fed the HighPGR and LowPGR diets than cats fed the MediumPGR diet \((\text{Table 5.1}; P < 0.001)\). In the late fasted state (15.5 – 21 h), EE was higher in cats fed the HighPGR diet compared to cats fed the Medium and LowPGR diets \((P < 0.001)\). Over 21 h, EE was higher in cats fed the HighPGR diet compared to cats fed the Medium and LowPGR diets \((P < 0.001)\).

For the first 2 h postprandially, the HIF per 100 g of diet (DM basis) was higher for MediumPGR than the Low and HighPGR dietary treatments \((\text{Table 5.2}; P = 0.01)\). For the complete calorimetry period (0 – 21 h), the HIF per 100 g of diet on a DM basis did not differ among dietary treatments \((P = 0.127)\). Over the first 2 h postprandially, HIF amounted to 1.58 %, 2.03 % and 1.60 % of ME intake for the High, Medium and LowPGR diets, respectively, and did not differ among dietary treatments \((P = 0.13; \text{Table 5.2})\). Over the whole calorimetry period, (0 – 21 h) the HIF was 21.7 %, 21.6 % and 19.5 % of ME intake for the High, Medium and LowPGR diets, respectively, and did not differ among dietary treatments \((P = 0.50; \text{Table 5.2})\).
5.4.3. Net Energy and Proposed Models

Net energy models were created with HIF values taken from 0 – 2 h (Table 5.3; equations 1-4), and 0 – 21 h (Table 5.3; equations 5-8). Models developed using the HIF values from 0 – 2 h were able to better predict analyzed NE compared to models developed using the HIF values from 0 – 21 h. Of the eight suggested models to predict NE, the model $NE = (0.941 \times ME) + (0.519 \times CP)$ had the highest $R^2$ and lowest rMSPE, and therefore fit our observed data best (Table 5.3). Furthermore, of the various macronutrient inputs used to model net energy, including CP as a variable maximized accuracy of the model.

5.5. Discussion

The present study is the first to propose equations that estimate NE values of domestic cat diets. Though indirect calorimetry has previously been used to analyze EE, this study is original in quantifying the HIF as a proportion of ME intake in cats. Furthermore, to our knowledge, this is the first study to follow cats for a prolonged time post feeding, and determine changes in EE at different points throughout the 21 h calorimetry period. The results of this study were novel, and resulted in the development of equations that have potential to alter determinations of dietary energy supply and the resultant feeding recommendations for adult cats.

The observed measurements of EE are similar to those reported in literature (Lester et al., 1999; Russel et al., 2002; Nguyen et al., 2004; Center et al., 2011; Gooding et al., 2015). In 16 neutered normal-weight cats, the RFMR was approximately 39 ± 1 kcal/kg/d (Nguyen et al., 2006). In cats fed both high fat and high carbohydrate diets, fasted EE ranged from 44 to 47 kcal/kg/d, and fed EE ranged from 43 to 51 kcal/kg/d (Gooding et al., 2015). Furthermore, Center et al. (2011) found daily resting EE to be 36 ± 7.7 kcal/kg/day in cats with underweight, normal and overweight body condition scores. These similarities suggest that our results are
valid, and appropriate to use to predict NE supply for the domestic cat in general.

The duration of elevated postprandial EE in the present study is similar to that of other species, both monogastrics and ruminants. In sheep, EE increased by 40 – 80 % during consumption of a meal, and persisted for up to 2 h before rapidly declining to rates similar to those recorded in a fasted state (Graham, 1965). In fur seals and sea lions, metabolism peaked approximately 3 h after a meal, and returned to fasting levels between 6 and 10 h (Rosen and Trites, 1997). Furthermore, diet-induced thermogenesis in humans lasted for 4.8 h and 5.8 h after consumption of processed-food meals and whole-food meals, respectively (Barr and Wright, 2010). Certainly, variances in animal size, meal composition, and experimental methods may contribute to differences in HIF among species. Thus, it was not surprising to see a 2 h postprandial energy response in cats in the present study.

Heat increment of feeding from 0 – 2 h was lower than values for terrestrial herbivores and omnivores. Although HIF values have not previously been reported for the domestic cat, the HIF accounts for up to 30 % of ME intake in other mammals and birds (Smith et al., 1978). However, in other carnivorous endotherms, HIF values appear to be much lower. In house wrens (Chappell et al., 1997), harbor seals (Ashwell-Erickson and Elsner, 1981), and penguin chicks (Janes and Chappell, 1995), the HIF amounted to 6.3 %, 4.7 %, and 10.0 % of ingested ME, respectively. Furthermore, Kendall et al. (1983) found that cats have significantly lower costs to maintain body weight compared to Beagles on a metabolic body weight basis. Interestingly, HIF values in this study were similar to those reported in carnivorous fish. In salmonids fed complete diets, the HIF was less than 3 % of ingested ME (Smith et al., 1978). Furthermore, due to the low energy cost of protein metabolism in fish, the NE of protein is higher for salmonids than mammals and birds (Smith et al., 1978). This similarity suggests that low energy production in the postprandial
state results in analogous HIF values among carnivorous species. Perhaps like carnivorous fish, cats have a low energy cost of protein metabolism (Smith et al., 1978). The unique relationship between protein metabolism and energy production in cats may explain why our HIF values are considerably lower than those reported in other mammals, but similar to carnivorous fish.

When HIF was taken from 0 - 21 h and expressed as a proportion of ME, the present results were comparable to those in other mammals. In pigs, the HIF for starch, sugars, and digestible crude protein represented 29 % of ME content on average (Noblet et al., 1994). In ruminants, the HIF is variable and can represent 30 – 70 % of ME intake; though microbial fermentation and rumination contribute largely to these costs (Armstrong, 1956). However, by taking the complete calorimetry period into account, measurements cannot necessarily be categorized as HIF, which is usually measured in a postprandial rather than a fasted state. Furthermore, models (equations 4-8) created using the HIF values from 0 - 21 h fit our data poorly. Perhaps including an increased number of experimental diets would result in equations that more accurately fit the observed NE values. Regardless, it is suggested that the observed increase in EE at the end of the calorimetry period is a characterizing feature of cat nutrient metabolism in general, as it was consistently observed on all diets. Aforementioned, the costs associated with protein metabolism are higher compared to other macronutrients (Raben et al., 2003). Perhaps, domestic cats are increasing protein catabolism or deposition at the end of the calorimetry period. Investigation of the timing and specifics of nutrient metabolism in cats may elucidate this unexplained increase in EE.

This study was limited in the ability to parameterize variables in our models. Net energy prediction equations have been developed for other species that parameterize all of the dietary macronutrients in a single equation (Noblet et al., 1994; Donker and Naik, 1979). However, as
the present study only employed three diets, only one macronutrient input, in combination with ME, could be parameterized at a time. Additionally, this study was the first to express HIF as a proportion of ME for cats, restricting the ability to comment on the accuracy of the developed prediction equations for an independent set of data until more work is completed. Further research should focus on testing the accuracy of these equations to predict NE values of additional diets.

In conclusion, NE, which accounts for energy spent in the digestion, absorption and metabolism of nutrients, is a more accurate measure of energy directly available to an animal (NRC, 2006). Expressing energy density on an NE basis could allow a more accurate feeding recommendation than what is currently utilized for commercial feeding recommendations, and limit the provision of excess calories to cats. Thus, we proposed multiple equations to estimate a NE value and recommend the equation $NE = (0.941 \times ME) + (0.519 \times CP)$ to determine NE of complete diets for domestic cats, because it yielded the best fit to observed values.
5.6. Tables and Figures

Table 5.1. Indirect calorimetry measurements for cats consuming a single meal of three experimental diets differing in perceived glycemic response (PGR) at different time intervals. The PGR refers to the expected theoretical glycemic response that would result from consumption of each diet. Fasting measurements were taken at t = -60 and t = -30 min, and fed measurements were taken at t = 0 min onward.

<table>
<thead>
<tr>
<th>Variable</th>
<th>HighPGR</th>
<th>MediumPGR</th>
<th>LowPGR</th>
<th>SEM1</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>EE (kcal/kg d⁻¹)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RFMR²</td>
<td>36.5</td>
<td>36.1</td>
<td>35.4</td>
<td>2.18</td>
<td>0.89</td>
</tr>
<tr>
<td>Overall (0-21h)</td>
<td>43.3ᵃ</td>
<td>41.8ᵇ</td>
<td>41.6ᵇ</td>
<td>0.29</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fasted (-1-0h)</td>
<td>42.1</td>
<td>40.9</td>
<td>39.8</td>
<td>1.18</td>
<td>0.160</td>
</tr>
<tr>
<td>Postprandial (0-5.5h)</td>
<td>41.7</td>
<td>41.5</td>
<td>40.7</td>
<td>1.54</td>
<td>0.167</td>
</tr>
<tr>
<td>Immediate Postprandial (0-2h)</td>
<td>43.4</td>
<td>44.5</td>
<td>42.4</td>
<td>1.78</td>
<td>0.148</td>
</tr>
<tr>
<td>Fed (5.5-10.5h)</td>
<td>42.2ᵃ</td>
<td>41.1ᵇ</td>
<td>39.9ᶜ</td>
<td>0.47</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Return to Fasted (10.5-15.5h)</td>
<td>44.7ᵃ</td>
<td>42.4ᵇ</td>
<td>43.6ᵃ</td>
<td>0.60</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Late Fasted (15.5-21h)</td>
<td>44.1ᵃ</td>
<td>41.3ᵇ</td>
<td>42.0ᵇ</td>
<td>0.56</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

ᵃ-c Within a row, means without a common superscript differ (P < 0.05). ¹Pooled SEM.

Means were based on 18 cat observations per diet. ²RFMR: resting fed metabolic rate.
Table 5.2. Metabolizable energy and heat increment used to determine NE/100 g diet. Heat increment of feeding (HIF) is presented as AUC from 0-2 h postprandial, and 0-21 h postprandial. The perceived glycemic response (PGR) refers to the expected theoretical glycemic response that would result from consumption of each diet.

<table>
<thead>
<tr>
<th>Name</th>
<th>HighPGR</th>
<th>MediumPGR</th>
<th>LowPGR</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ME, kcal/100 g diet (DM)&lt;sup&gt;1&lt;/sup&gt;</td>
<td>458.9&lt;sup&gt;c&lt;/sup&gt;</td>
<td>490.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>505.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.85</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>HIF</strong>&lt;sup&gt;0-2 h&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HIF (% of ME)</td>
<td>1.58</td>
<td>2.03</td>
<td>1.60</td>
<td>0.25</td>
<td>0.13</td>
</tr>
<tr>
<td>HIF, kcal/100 g diet (DM)</td>
<td>5.82&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.87&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.56&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.00</td>
<td>0.01</td>
</tr>
<tr>
<td>Measured NE, kcal/100 g diet (DM)</td>
<td>453.0&lt;sup&gt;c&lt;/sup&gt;</td>
<td>481.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>499.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.00</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>NE/ME (DM) %</td>
<td>98.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>98.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>98.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.21</td>
<td>0.02</td>
</tr>
<tr>
<td><strong>HIF</strong>&lt;sup&gt;0-21 h&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HIF (% of ME)</td>
<td>21.7</td>
<td>21.6</td>
<td>19.5</td>
<td>2.15</td>
<td>0.50</td>
</tr>
<tr>
<td>HIF, kcal/100 g diet (DM)</td>
<td>77.6</td>
<td>94.6</td>
<td>79.3</td>
<td>9.20</td>
<td>0.127</td>
</tr>
<tr>
<td>Measured NE, kcal/100 g diet (DM)</td>
<td>381.3&lt;sup&gt;c&lt;/sup&gt;</td>
<td>396.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>426.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.20</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>NE/ME (DM) %</td>
<td>83.1</td>
<td>80.7</td>
<td>84.3</td>
<td>1.91</td>
<td>0.161</td>
</tr>
</tbody>
</table>

<sup>a-c</sup> Within a row, means without a common superscript differ ($P < 0.05$).

<sup>1</sup>DM = dry matter; From Asaro et al., 2017.

<sup>2</sup>Pooled SEM. Means were based on 18 cat observations per diet.
Table 5.3. Proposed NE models and associated $R^2$ and rMSPE using heat increment of feeding (HIF) values from 0-2 h postprandial, and 0-21 h postprandial.

<table>
<thead>
<tr>
<th>Proposed Model&lt;sup&gt;1&lt;/sup&gt;</th>
<th>$R^2$</th>
<th>rMSPE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Using HIF&lt;sub&gt;(0-2 h)&lt;/sub&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. NE = (0.941 × ME) + (0.519 × CP)</td>
<td>0.975</td>
<td>2.97</td>
</tr>
<tr>
<td>2. NE = (0.992 × ME) - (0.170 × CL)</td>
<td>0.973</td>
<td>3.14</td>
</tr>
<tr>
<td>3. NE = (0.995 × ME) - (0.0002 × Starch)</td>
<td>0.972</td>
<td>3.21</td>
</tr>
<tr>
<td>4. NE = (0.984 × ME) + (0.246 × CF)</td>
<td>0.972</td>
<td>3.21</td>
</tr>
<tr>
<td>Using HIF&lt;sub&gt;(0-21 h)&lt;/sub&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5. NE = (0.605 × ME) + (2.60 × CP)</td>
<td>0.337</td>
<td>25.1</td>
</tr>
<tr>
<td>6. NE = (0.843 × ME) – (0.431 × CL)</td>
<td>0.283</td>
<td>27.1</td>
</tr>
<tr>
<td>7. NE = (0.826 × ME) – (0.0001 × Starch)</td>
<td>0.294</td>
<td>27.0</td>
</tr>
<tr>
<td>8. NE = (0.800 × ME) + (6.386 × CF)</td>
<td>0.296</td>
<td>26.9</td>
</tr>
</tbody>
</table>

<sup>1</sup>Energy in kcal/kg dry matter, nutrients in g/kg dry matter; CP: crude protein, CL: crude lipids, CF: crude fiber.)
**Figure 5.1.** Average energy expenditure of cats fed 3 experimental diets. Measurements were taken in 30 min intervals. Cats \((n = 19)\) were fed at \(t = 0\) min. Vertical dotted lines separate fasted, immediate postprandial, postprandial, fed, return to fasted, and late fasted time points, respectively.
Chapter 6: Summary and Conclusions

This research expands on our understanding of the effects of carbohydrate level and source on the digestive and metabolic processes of domestic cats. Along with building on the current breadth of knowledge, this work revealed new conclusions around predictions of energy density and how to apply novel methodologies to measure physiological responses.

The first experiment found that cats can efficiently digest diets with major contributions from carbohydrates, and confirmed that the modified Atwater equation does not give an accurate estimate of ME for high-quality commercial diets. This research found a quantifiable estimate of the inaccuracy of the modified Atwater equation, and concluded that using this equation provides a 12% caloric surplus to cats on average. Furthermore, this study was the first to show similarity among in vivo and in vitro measures of digestibility, and that high-quality extruded diets have similar digestibility regardless of ingredient selection. The similarities between in vitro and in vivo digestibility values in the present study suggest the potential of using in vitro techniques prior to, or in place of in vivo studies.

The second experiment built on previous research to confirm that cats have prolonged glucose and insulin responses compared to other monogastric species when consuming diets relatively high in starch. Furthermore, the results of this study suggest that starch source and inclusion level have minimal effects on postprandial glucose and insulin responses in adult lean cats. While RQ, EE, and interstitial glucose behaved as expected, serum glucose and insulin presented variability that emphasized their lack of scientific value for investigation of the feline glycemic response. Interstitial glucose measures proved to be less variable than serum measurements, and therefore can potentially be used as a minimally-invasive measure to define the real-time glucose response to a meal. Additionally, RQ and interstitial glucose presented
similar patterns in their curves. Though not statistically correlated, the relationship between RQ and interstitial glucose may be an alternative, less invasive technique to investigate the feline glycemic response. Overall, the current results support the value of using real-time interstitial glucose monitors or calorimetry to examine glucose metabolism, and that cats have a low and more gradual glucose response compared to other monogastric species.

The last experiment was the first to quantify the HIF as a proportion of ME intake in cats. We concluded that the HIF in cats is much lower than other monogastric species, but similar to other carnivorous species. This research was unique as it followed cats for a prolonged time post feeding, and investigated differences in energy production at various stages of metabolism. The most significant contribution to research that came from this work was the development of equations to predict dietary NE of commercial feline diets. These equations have the potential to drastically alter how we determine dietary energy density and the resultant feeding recommendations for adult cats. Feline obesity is a prevalent issue largely caused by overfeeding, and the unreliable equations that underestimate the true energy content of a diet. Thus, precision feeding using NE models, rather than the inaccurate ME models currently in place, has the potential to play a preventative role in addressing feline obesity.

Though this work advanced knowledge in terms of carbohydrate digestibility and metabolism in domestic cats, there were limitations within the research. First, because diets differed in various macronutrients, and not solely carbohydrate content, we cannot be certain that the differences observed are directly related to carbohydrate level and source. However, because we wanted to look at commercial diets, we were unable to control dietary macronutrient levels in our experimental diets. It would be useful for further research to conduct studies with diets that are constant in fat and protein, and variable in terms of carbohydrate level. This would allow one
to more accurately comment on the effects of carbohydrates on study outcomes. Additionally, the use of human interstitial glucose sensors in Chapter 4 resulted in instances of inaccurate readings, as the lower limit of sensors of 40 mg/dL were not low enough for some cats. Use of sensors with a lower detection limit may be more appropriate for future feline research. Last, in Chapter 5, we were limited to parameterize one dietary parameter in combination with ME. Had more experimental diets been used, we could have potentially developed a single equation to estimate dietary NE using multiple dietary parameters, similar to other species. Regardless, the work done in this thesis allows us to determine which nutrient is most useful in conjunction with ME to predict dietary NE.

Further research should focus on exploring the use of alternative, non-invasive methods to investigate digestibility, glycemic response, and other physiological processes in the domestic cat, as this work has visibly highlighted the possibility that they may be acceptable. Additionally, determining energy density of commercial diets using NE rather than ME should be considered going forward, as it is clearly a more accurate representation of available dietary energy, and creates the opportunity for precision feeding. As the equations developed in the present study can only be validated for use with our experimental diets, research should aim to develop a NE model that can remain accurate when applied to a wide variety of commercial feline diets. Overall, an optimal level of carbohydrate intake to support health and well-being of domestic cats has not been established, though the body of research is constantly increasing. Nevertheless, the exact nutritional, metabolic, and physiological effects of feeding carbohydrates to cats requires further investigation.
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