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EVALUATING GLUCOSE-BASED CARBOHYDRATES FOR USE IN CANINE DIETS

BY

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DISSERTATION

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ABSTRACT

Extruded diets remain the most common diet format fed to dogs. Even though dogs are classified as Carnivora, carbohydrates make up 20-50% of most extruded diets. The majority of carbohydrates are in the form of starch, providing a readily available energy source. Other carbohydrate forms include dietary fiber, oligosaccharides, and resistant starches (RS) that may elicit a multitude of health benefits. Previous research has focused on the composition of traditional carbohydrate-based ingredients, but little has been done as regards the composition of various whole ingredient, fractionated ingredient, or single-source carbohydrate sources.

The overall objective of this research was to evaluate glucose-based carbohydrate sources for use in canine diets, including ingredient chemical compositional analyses, effects on apparent total tract macronutrient digestibility, and effects on fecal characteristics, microbiota composition, and fermentative end-products. Our first aim was to determine the chemical composition of carbohydrate sources commonly incorporated into pet foods. We evaluated various whole grains, processed grains, grain fractions, and pseudocereal grains. Our second aim was to evaluate the effects of graded concentrations of polydextrose on apparent total tract macronutrient digestibility, fecal characteristics, fecal fermentative end-products, and fecal microbial populations in healthy adult dogs. Polydextrose was fed at 0, 0.5, 1, or 1.5% of the diet DM. Our third aim was to determine the effects of RS, naturally from whole grains, on apparent total tract macronutrient digestibility, fecal characteristics, and fecal fermentative end-products in healthy adult dogs fed a baked whole grain-containing biscuit treat. Dogs received either 0, 10, or 20 g biscuits/d (estimated to be 0, 2.5, or 5 g RS/d) that were fed within their daily caloric allowance. Our fourth aim was to determine the effects of graded concentrations of

a corn-based RS source on apparent total tract macronutrient digestibility, fecal fermentative end-products, and fecal microbial populations in healthy adult dogs. Dogs were fed 0, 1, 2, 3, or 4% of diet DM of Hi-maize 260®, a corn-based ingredient that consists of 60% RS and 40% digestible starch.

In our first aim, we determined that whole grains and grain fractions contain many essential nutrients including amino acids and minerals, and may provide a significant source of fiber. Because the chemical composition of grains varied greatly depending on their respective fractions (i.e., barley flake vs. malted barley vs. steamed rolled barley) and among grain type (i.e., barley flake vs. brown rice vs. canary grass seed), pet food professionals must consider both factors when formulating diets. In our second aim, we determined that polydextrose appeared to be moderately fermentable, which was evident by the increased ($P<0.05$) fecal SCFA concentrations, primarily acetate and propionate, and by the decrease ($P<0.05$) in fecal pH, without affecting food intake or fecal output. The inclusion of polydextrose also decreased ($P<0.05$) some protein catabolites, in particular fecal indole concentrations. Fecal *C. perfringens* concentrations were decreased ($P<0.05$) by including polydextrose in the diet, but other bacteria were unaffected. In our third aim, we determined that fat digestibility decreased ($P<0.05$) with whole grain treat consumption (i.e., increased RS consumption), but apparent total tract dry matter, organic matter, and crude protein digestibilities were not affected. Fecal fermentative end-products, including short- and branched-chain fatty acids, ammonia, phenols, and indoles, and microbial populations were not affected. In our fourth and final aim, we determined that dietary RS linearly decreased ($P<0.05$) apparent total tract dry matter, organic matter, crude protein, fat, and gross energy digestibilities, and fecal pH was linearly decreased ($P < 0.05$) with increased RS consumption. Fecal output was linearly increased ($P < 0.05$) with increased RS

consumption. Fecal scores and fecal fermentative end-product concentrations, including ammonia, short-chain fatty acids, branched-chain fatty acids, phenols, and indoles were not affected by RS consumption. Predominant bacterial phyla present in all dogs included Firmicutes, Bacteroidetes, Fusobacteria, Proteobacteria, Actinobacteria, and Tenericutes. Although few bacterial taxa were altered by RS consumption, fecal *Faecalibacterium* were increased ($P < 0.05$) with increased RS consumption.

This research provided a compositional database of whole ingredient, fractionated ingredient, and single-source carbohydrate sources, which may potentially impact future pet food formulations. This research provided knowledge about the use of non-digestible, glucose-based carbohydrate sources, including polydextrose, whole grain-containing RS and dietary fiber, and corn-based RS, for use in canine diets. Based on the current results, the most prominent beneficial effects were observed in healthy dogs when fed 1.5% polydextrose. However, more research is needed to determine an optimal dose of whole grains, grain fractions, or single-source RS that may elicit a beneficial response when fed to dogs.

DEDICATION

This dissertation is dedicated to my parents, Jack and Sandra Beloshapka, my fiancé R. Michael Salwan, and all those who helped further my career.

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CHAPTER 1: INTRODUCTION

A healthy gastrointestinal (GI) tract is important for the overall health of dogs. The GI microbiome is a very complex ecosystem in which the host's microbes play an integral role in a variety of body systems including: creating a defense barrier against invading pathogens (i.e., salmonella); aiding in digestion, fermentation, and energy harvest from the diet; providing nutritional support for enterocytes; and activating the development of the host immune system (Suchodolski, 2011a). It is well known that diet can alter gut microbiota and overall GI health. Previous research has demonstrated the benefits of feeding dietary fiber and prebiotics to dogs, including an increase in butyrate, acetate, propionate, lactate and total short-chain fatty acid (SCFA) production (Vickers et al., 2001), an increase in fecal bifidobacteria, and a decrease in fecal phenol and indole concentrations (Swanson et al., 2002a; 2002b; Flickinger et al., 2003). Similarly, resistant starch (RS) is a readily fermentable carbohydrate source that can modulate the gut microbial composition and fecal SCFA concentrations, which has been well demonstrated in pigs, humans, and rodents (Bird et al., 2007; McOrist et al., 2011; Haenen et al., 2013; Tachon et al., 2013). Feeding whole grains, which contain RS when minimally processed (Dust et al., 2004; Hernot et al., 2008) or the feeding of purified RS sources, may have similar beneficial effects on the canine gut, but has not been well tested. Furthermore, other glucose-based carbohydrates, such as polydextrose, may possess properties that benefit GI health.

The major objectives of this research were to: 1) determine the chemical composition of carbohydrate sources commonly incorporated into pet foods; 2) determine the effects of RS, naturally from whole grains, on apparent total tract macronutrient digestibility, fecal characteristics, fecal microbiota, and fecal fermentative end-products in healthy adult dogs; 3)

evaluate the effects of graded concentrations of polydextrose on apparent total tract macronutrient digestibility, fecal characteristics, fecal fermentative end-products, and fecal microbial populations in healthy adult dogs; and 4) determine the effects of graded concentrations of a corn-based RS source on apparent total tract macronutrient digestibility, fecal fermentative end-products, and fecal microbial populations in healthy adult dogs. From this research, we hoped to gain knowledge about the use of novel non-digestible carbohydrate sources, including polydextrose, whole grain-containing RS and dietary fiber, and corn-based RS, for use in canine diets. More specifically, this research may identify a minimum dose by which these compounds beneficially alter indicators of GI health, including the bacterial taxa and fecal fermentative end-products affected, with potential impact on pet food formulation.

Previous research has focused on the composition of traditional carbohydrate-based ingredients, but little has been done as regards the composition of various whole or fractionated carbohydrate sources. Experiment 1 was performed to determine the starch, amino acid, and macromineral composition of various whole grains, processed grains, and grain components used in pet foods. It was hypothesized that whole grains and their fractions would contain > 20% dietary fiber or RS that may improve GI health. Additionally, the whole grains were expected to provide more essential amino acids and macrominerals than grain fractions, demonstrating the nutritive value of whole grains.

Many novel non-digestible carbohydrates possess prebiotic activity in humans, but have not been tested for use in dogs. Experiment 2 was performed to evaluate the effects of graded concentrations of polydextrose (Sta-Lite®) on apparent total tract digestibility, fecal characteristics, microbial populations (using qPCR), and fermentative end-product concentrations in healthy adult dogs. Polydextrose was fed at 0, 0.5, 1, or 1.5% of the diet DM.

These dietary concentrations were based on previous literature and its likely inclusion in practice considering cost and GI tolerance. It was hypothesized that increased inclusion of polydextrose would decrease fecal pH, increase fecal SCFA concentrations, decrease fecal phenol and indole concentrations, and beneficially alter fecal microbial populations by increasing *Lactobacillus* spp. and *Bifidobacterium* spp. and decreasing *Clostridium perfringens* and *Escherichia coli*.

Although whole grains are commonly fed to dogs, there is no published research evaluating their effects in dogs. Experiment 3 was designed to evaluate the effects of feeding a baked whole grain-containing treat on apparent total tract digestibility, fecal microbiota (using qPCR), and fermentative end-product concentrations in healthy adult dogs. It was hypothesized that the RS present in the whole grains would increase fecal *Lactobacillus* spp. and *Bifidobacterium* spp.; decrease fecal pH; decrease fecal protein metabolite concentrations, namely ammonia, branched-chain fatty acids (BCFA), phenols, and indoles; and increase fecal SCFA concentrations without affecting stool quality in healthy adult dogs.

Due to the high variability and difficulty in quantifying RS from whole grains, using RS obtained from a single-source may be useful in identifying an effective dose of RS to feed to dogs. To identify an RS dose that is well-tolerated, yet elicits a beneficial effect on fecal fermentative end-products and microbial populations, Experiment 4 was performed. This experiment was designed to evaluate graded concentrations (0, 1, 2, 3, and 4% of diet DM) of Hi-maize 260®, a corn-based ingredient that consists of 60% RS and 40% digestible starch, on apparent total tract macronutrient digestibility, fecal fermentative end-product concentrations, and fecal microbial populations (using 16S rRNA gene amplicon-based Illumina sequencing) in healthy adult dogs. It was hypothesized that RS would: 1) not affect fecal characteristics or apparent total tract macronutrient digestibility; 2) increase fecal SCFA and beneficial microbial

communities (i.e., *Blautia*, *Lachnospira*, *Veillonella*, *Megasphaera*, and *Faecalibacterium*) in a linear fashion; and 3) decrease fecal phenol, indole, ammonia, and potentially pathogenic microbial communities (i.e., *Clostridium*, *C. hiranonis*, and *Fusobacterium*) in a linear fashion.

CHAPTER 2: LITERATURE REVIEW

The domestic canine (*Canis familiaris*) is classified as a member of the order Carnivora, with ancestral species consuming diets that consisted predominantly of meat. However, over time, the modern dog has evolved to consume a more omnivorous diet, and extruded diets remain the most common diet format fed to dogs (Case et al., 1995; Laflamme et al., 2008; Phillips-Donaldson, 2013). Even though dogs are classified as Carnivora, carbohydrates make up 20-50% of most extruded diets (Bradshaw, 2006). The majority of carbohydrates are in the form of starch, providing a readily available energy source. Other carbohydrate forms include dietary fiber, oligosaccharides, and resistant starches (RS) that may elicit a multitude of health benefits. Previous research has focused on the composition of traditional carbohydrate-based ingredients, but little has been done in regards to the composition of various whole ingredient, fractionated ingredient, or single-source carbohydrate sources. More developed knowledge of source, chemical composition, physicochemical properties, and dose of fiber or other non-digestible carbohydrates is warranted. All of the aforementioned factors greatly influence cost, gastrointestinal (GI) tolerance, and health-promoting effects of the ingredients. This information can help pet food formulators to best utilize ingredients and maximize the benefits of the carbohydrate source(s) used.

Currently, the United States pet product industry is > $55 billion/year, with nearly half ($22 billion) coming from pet food alone (Taylor, 2011; Phillips-Donaldson, 2012; APPMA, 2013). According to the 2013-2014 American Pet Products Association survey, 68% of U.S. households own a pet. Within the population, 45.3 million U.S. households own a cat and 56.7 million households own a dog (APPMA, 2013). Pets continue to offer companionship and health

benefits (i.e., decreased blood pressure, increased physical activity, etc.; Risely-Curtiss, 2010) to their owners, leaving many owners seeking the best nutrition possible for their companions. Furthermore, there has been a continued public perception that grains or other carbohydrates are not the best ingredients for pet food (Aldrich, 2012; Taylor, 2014). Therefore, more research is needed to educate both pet owners and pet food formulators on potential benefits of feeding whole ingredient, fractionated ingredient, or single-source carbohydrate sources to dogs.

DIETARY FIBER

To date, a multitude of definitions for dietary fiber exist worldwide (Institute of Medicine, 2001). According to the American Association of Cereal Chemists (AACC), dietary fiber is defined as the edible parts of plants or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine (AACC, 2009). An even more descriptive definition of dietary fiber was proposed by the Food and Nutrition Board of the Institute of Medicine to encompass both the physical characteristics and physiological effects in humans. This definition includes 3 parts: (1) dietary fiber, which is defined as nondigestible carbohydrates and lignin, intrinsic and intact in plants; (2) functional fiber, which consists of isolated, nondigestible carbohydrates having beneficial physiological effects in humans; and (3) total fiber, which is the sum of dietary fiber and functional fiber (Institute of Medicine, 2001). One group of widely studied functional fibers are the prebiotics. As defined, prebiotics are non-digestible food ingredients that are 1) resistant to gastric acidity, hydrolysis by mammalian enzymes, and GI absorption; 2) fermented by intestinal microbiota; and 3) able to selectively stimulate the growth and/or activity of those intestinal bacteria that contribute to the host's health and well-being (Gibson and Roberfroid,

1995; Gibson et al., 2004; Roberfroid et al., 2010; and Gibson et al., 2010). Currently, there are three established classes of prebiotics (e.g., fructans, galactooligosaccharides, and lactulose), but others may exist.

The benefits of consuming dietary fiber dates back to 430 BC, in which the improved laxative effects of coarse wheat were compared to those of refined wheat (Slavin, 1987). Today, a multitude of research exists on the benefits of consuming dietary fiber and/or prebiotics that go far beyond laxation. In humans, consumption of the recommended intake of dietary fiber (25-35 g fiber/d for adults) has been associated with many health benefits, including decreased risk for type 2 diabetes mellitus, obesity, cardiovascular disease, colon cancer, and improved immunity due to changes in gut microbial populations (Otten et al., 2006; Kaczmarczyk et al., 2012). Additionally, dietary fiber increases fecal bulk, aids in laxation, and promotes regularity. The main driver for dietary fiber inclusion in canine diets does not revolve around improving cardiovascular health or decreasing the risk of colon cancer because these diseases have a low incidence in that species. Dietary fiber is used, however, to maintain stool quality and GI health, which may enhance overall health and longevity. Previous dietary fiber-based research in dogs has focused on a few key indices of GI health, often resulting in decreased fecal pH and concentrations of putrefactive compounds [e.g., phenols, indoles, branched-chain fatty acids (BCFA)], increased concentrations of fecal short-chain fatty acids (SCFA; acetate, propionate, and butyrate), and alterations in gut bacterial populations (Vickers et al., 2001; Swanson et al., 2002a; 2002b; Flickinger et al., 2003; Propst et al., 2003; Zentek et al., 2003; Middelbos 2007a; 2007b; Beloshapka et al., 2012; 2013). Dietary fibers often increase concentrations of beneficial (*Bifidobacterium*, *Lactobacillus*, *Blautia*, *Lachnospira*, *Veillonella*, *Megasphaera*, and *Faecalibacterium* spp.) and decrease concentrations of potential pathogenic bacteria

(*Clostridium* spp.*,* *Escherichia coli,* and *Fusobacterium* spp.*)*, improving gut health (Vickers et al., 2001; Swanson et al., 2002a; 2002b; Flickinger et al., 2003; Propst et al., 2003; Zentek et al., 2003; Grieshop et al., 2004; Middelbos et al., 2007a; 2007b; Beloshapka et al., 2012; 2013; Panasevich, unpublished data).

Dietary fibers and prebiotics have been a consistent area of interest in the pet food industry over the past several decades. In addition to improving GI health and gut microbiota, it has been observed that including dietary fibers in companion animal diets have also led to improved immune function and weight loss, that may decrease the incidence of obesity and diabetes mellitus, which continue to be a concern in the pet population (Middelbos et al., 2007b; de Godoy et al., 2013). The benefits of other non-digestible carbohydrates, including short-chain fructooligosaccharides, inulin-type fructans, galactooligosaccharides, mannanoligosaccharides, yeast cell wall extracts, resistant starch (RS), and polydextrose also have been studied in adult dogs (Vickers et al., 2001; Swanson et al., 2002a; 2002b; Flickinger et al., 2003; Propst et al., 2003; Grieshop et al., 2004; Middelbos et al., 2007a; 2007b; Knapp et al., 2008; Kanakupt et al., 2011; Beloshapka et al., 2012). However, some of this research focused only on tolerance or *in vitro* effects and little has been noted for appropriate dosage and behavior of some of these fibers *in vivo*.

GUT MICROBIOME

Many of the beneficial effects of functional fibers and other non-digestible carbohydrates are thought to come from the GI microbiota. It has been well documented that the gut microbiome is a complex and diverse ecosystem, maintaining a variety of roles that contribute to overall health. Gut microbiota (1) develop and maintain GI immunity, (2) contribute to fecal

biomass to ultimately aid in laxation, (3) produce organic acids, which provide energy for GI epithelial cells and induce apoptosis in pre-cancerous cells, (4) improve mineral absorption, and (5) inhibit pathogen adhesion to GI epithelia (Ouwehand et al., 2005; Vieira et al., 2013; Moloney et al., 2014; Zeng et al., 2014). The gut microbial ecosystem can be influenced by many factors, including diet, lifestage, environment, disease status, or medication (Chen et al., 2014). While many intestinal microbes prefer carbohydrates, some ferment protein when abundant in the diet and/or colon (Macfarlane and Macfarlane, 2012). The fermentation of protein produces putrefactive compounds, such as phenols, indoles, ammonia, and biogenic amines, which may be detrimental to colon health (Cummings and Macfarlane, 1991; Smith and Macfarlane, 1997). One of the benefits of consuming a fermentable fiber or prebiotic can be observed by the production of SCFA (acetate, propionate, and butyrate) and lactate. These acids are the preferred energy source of colonocytes (Kim et al., 1982; Scheppach and Weiler, 2004). Additionally, they are the primary luminal anions in humans and other omnivores, resulting in a decreased colonic pH. This decrease in pH creates an optimal environment for some bacteria to proliferate, but inhibits others, such as pathogenic *E. coli* or *Salmonella* spp. (Topping and Clifton, 2001). Furthermore, the gut microbial ecosystem has the ability to stimulate the differentiation and proliferation of epithelial cells (Kim et al., 1982; Nepelska et al., 2012). Gut microbiota fermentation of non-digestible oligosaccharides has not only been observed to modulate bacterial populations, but also to improve calcium absorption and bone density in adolescent girls and growing rats (Abrams et al., 2005; Weaver et al., 2011). Certain species of commensal bacteria and potentially beneficial bacteria are needed for the regulation of immune responses, such as inflammatory bowel disease (IBD), and by reducing the pro-inflammatory immune markers as well as limiting the outgrowth of potentially pathogenic bacterial species

(Konikoff and Denson, 2006; Vieira et al., 2013). Therefore, a balanced gut microbial ecosystem is imperative to overall host health and well-being.

High-throughput methods of microbial evaluation

In the past, knowledge regarding GI tract (GIT) microbiota was primarily obtained using culture-based techniques. Over the past decade, molecular techniques have dramatically changed the research landscape and have greatly enhanced our understanding of the composition, dynamics, and functionality of the host-microbiota ecosystem in dogs and cats (Ritchie et al., 2008; Desai et al., 2009; Suchodolski, 2011a; 2011b; Swanson et al., 2011; Barry et al., 2012). Because only a fraction of the organisms present in the GIT can be cultured and studied, progress in the field was greatly hindered until the recent availability of molecular assays. Multiple DNA-based, culture-independent methods for microbiome analysis have recently emerged and may be useful tools to effectively identify and quantify microbial populations. Several molecular tools based on the microbial 16S rRNA gene are available, including quantitative PCR (qPCR); fluorescent in situ hybridization (FISH); gel-based techniques such as restriction fragment length polymorphism (RFLP) analysis, denaturing gradient gel electrophoresis (DGGE), and temperature gradient gel electrophoresis (TGGE); and sequencing techniques such as 454 pyrosequencing (Roche Applied Science, Indianapolis, IN, USA), Illumina (Illumina Inc., San Diego, CA, USA) sequencing, and Sanger sequencing. The next-generation sequencing techniques (i.e., 454 pyrosequencing, Illumina) are also commonly used for shotgun sequencing and functional assessment. These methods are briefly summarized in Table 2.1 (Kerr et al., 2013). This area of study is always evolving and techniques that were novel just a few years ago (i.e., 454 pyrosequencing) are quickly being replaced by more refined and/or economical

techniques (i.e., Illumina). Furthermore, the results obtained through these high-throughput methodologies continue to indicate that GI microbial analysis in the past was not nearly as ostensive as was once thought. At that time, many research projects focused on dietary fiber and non-digestible carbohydrates measured a few microbial groups linked to health (i.e., *Bifidobacterium* spp., *Lactobacillus* spp.) or representing potential pathogens (i.e., *Clostridium perfringens*, and *Escherichia coli*). It is now known that the canine gut harbors hundreds of microbial species, the *Bifidobacterium* and *Lactobacillus* genera only represent approximately 1% of sequences in the gut, and that *C. perfringens* and *E. coli* are commensal bacteria present in healthy people and animals. Given these recent developments, the biological relevance of culture-based and qPCR-based studies performed in the past must be interpreted cautiously. As technological advancements continue, our view and understanding of the GI microbiota and the criteria we use to evaluate the potential benefits of dietary ingredients must continue to evolve.

Canine fecal microbiome

It is well documented that the GIT of mammals is densely populated with microorganisms, with an approximate density of 10^{11} to 10^{12} colony forming units/mL of digesta in the canine colon (Samal et al., 2011). In general, bacterial density and diversity increase from the stomach to small intestine to colon. Predominant bacterial phyla present in the canine GIT include Firmicutes, Bacteroidetes, Proteobacteria, Fusobacteria, and Actinobacteria, (Suchodolski, 2011a; Suchodolski et al., 2008, 2009; Middelbos et al., 2010; Handl et al., 2011; Swanson et al., 2011; Beloshapka et al., 2013). While each individual's normal GI microbial population is unique, major population shifts may be indicative of health status or disease state, and major shifts may occur with GI disease (Jia et al., 2010; Chaban et al., 2012; Suchodolski et

al., 2012b). More specifically, previous research in this area indicated that many cases of IBD have resulted in reduced microbial diversity (Xenoulis et al., 2008; Suchodolski et al., 2012a; 2012b). Although the predominant bacterial phyla present in the canine GIT include Firmicutes, Bacteroidetes, Proteobacteria, Fusobacteria, and Actinobacteria, the prevalence depends on GIT region, sample type (GI digesta vs. GI mucosa vs. feces), and dog (Suchodolski, 2011a; Suchodolski et al., 2008, 2009; Middelbos et al., 2010; Handl et al., 2011; Swanson et al., 2011). Recent experiments have used next-generation sequencing to evaluate the effects of dietary fibers and non-digestible carbohydrates. Shifts in unconventional bacterial groups, such as increases in *Blautia*, *Lachnospira*, *Veillonella*, *Megasphaera*, and *Faecalibacterium* and decreases in *Clostridium, C. hiranonis*, and *Fusobacterium*, which have been observed with increased dietary fiber or prebiotics in recent dog and cat studies (Beloshapka et al., 2013; Hooda et al., 2013; Panasevich, unpublished data), may provide more insight into the mechanisms responsible for the physiological changes occurring in the gut.

While the gut microbiome of both dogs and cats is not well defined, the feline gut microbiome has been characterized to a much lesser degree. Hooda et al. (2013) evaluated the fecal microbiome of growing kittens fed diets with altered dietary protein:carbohydrate ratios using 454 pyrosequencing. One of two diets were fed, either high-protein/low-carbohydrate (HPLC; n = 7) or moderate-protein/moderate-carbohydrate (MCMC; n = 7), and feeding started with the mothers during gestation and continued after weaning. Fresh fecal samples were collected from all kittens at 8, 12, and 16 weeks of age. These researchers concluded that a total of 384, 588 sequences were obtained, with an average of 9,374 sequences per sample. These researchers also concluded that the most abundant bacterial phyla were Firmicutes (MPMC: 8 wk: 80% of sequences; 12 wk: 73% of sequences; 16 wk: 71% of sequences; HPLC: 8 wk: 75%

of sequences; 12 wk: 79% of sequences; 16 wk: 78% of sequences), Actinobacteria (MPMC: 8 wk: 18% of sequences; 12 wk: 26% of sequences; 16 wk: 28% of sequences; HPLC: 8 wk: 8% of sequences; 12 wk: 5% of sequences; 16 wk: 5% of sequences), Fusobacteria (MPMC: 8 wk: 0.1% of sequences; 12 wk: 0.2% of sequences; 16 wk: 0.1% of sequences; HPLC: 8 wk: 0.1% of sequences; 12 wk: 0.1% of sequences; 16 wk: 0.1% of sequences), Bacteroidetes (MPMC: 8 wk: 0.6% of sequences; 12 wk: 0.6% of sequences; 16 wk: 0.2% of sequences; HPLC: 8 wk: 0.6% of sequences; 12 wk: 0.7% of sequences; 16 wk: 0.2% of sequences), and Proteobacteria (MPMC: 8 wk: 1% of sequences; 12 wk: 0.1% of sequences; 16 wk: 0.3% of sequences; HPLC: 8 wk: 4% of sequences; 12 wk: 3% of sequences; 16 wk: 3% of sequences). Major bacterial genera detected in kittens fed HPLC diet included *Collinsella* (3.9-6.7% of sequences), *Faecalibacterium* (5.0-7.2% of sequences), *Eubacterium* (6.8-8.4% of sequences), *Fusobacterium* (10.4-11.1% of sequences), *Blautia* (7.2-9.8% of sequences), and *Clostridium* (14.4-18.1% of sequences). Major bacterial genera detected in kittens fed MPMC diet included a higher abundance of *Megasphaera* (17.9-33.0% of sequences), *Bifidobacterium* (12.0-20.8% of sequences), and *Mitsuokella* (7.6-10.1% of sequences). That study provided a good framework for how dietary macronutrient ratios can alter gut microbial populations (Hooda et al, 2013).

Previous studies of dietary influences on the canine microbiome have used 454 pyrosequencing. Beloshapka et al. (2013) evaluated the effects of gut microbial populations of healthy adult dogs when fed raw meat diets with or without inulin or yeast cell wall extracts (YCW), assessed using 454 pyrosequencing. Six healthy adult beagle dogs (mean age = 5.5 years; mean BW = 8.5 kg) were randomly allotted to 1 of 6 dietary treatments: (1) beef control; (2) beef + 1.4% inulin DMB; (3) beef + 1.4% YCW DMB; (4) chicken control; (5) chicken + 1.4% inulin DMB; and (6) chicken + 1.4% YCW DMB. These researchers concluded that a total

of 358,693 sequences were obtained, with an evaluation of 4000 randomly selected sequences used to provide diversity estimates. These researchers also concluded that predominant bacterial phyla present in all dogs included Fusobacteria (~43% of sequences), Firmicutes (~37% of sequences), Bacteroidetes (10-15% of sequences), Proteobacteria (5% of sequences), and Actinobacteria (2-3% of sequences). These researchers concluded that predominant bacterial genera included *Fusobacterium* (16-36% of sequences), *Cetobacterium* (8-33% of sequences), *Clostridium* (12-21% of sequences), and *Bacteroides* (6-18% of sequences; Beloshapka et al., 2013). However, while advancements continue to be made in order to further develop the current knowledge of canine gut microbial populations, research continues to utilize traditional molecular techniques. Additionally, progress has also been made to determine the effects of fiber supplementation on the canine gut microbial ecosystem, but other novel nondigestible carbohydrates have yet to be adequately studied.

POLYDEXTROSE

Polydextrose is a polysaccharide synthesized by random polymerization of glucose, sorbitol, and a suitable acid catalyst at a high temperature and partial vacuum. It is composed of many different glycosidic linkages, but the 1,6-glycosidic linkage is predominant in this polymer (Allingham, 1982; Lahtinen et al., 2010). Polydextrose has an average degree of polymerization (DP) of 12 and an average molecular weight of 2,000, ranging anywhere from 162 to 20,000 kDa (Craig et al., 1998; Craig, 2008).   It is a water-soluble, low-calorie bulking agent that is currently used in a variety of foods, including baked goods, functional beverages, and diabetic products (Jie et al., 2000; Probert et al., 2004; Fava et al., 2007; Mäkivuokko et al., 2007). Because polydextrose is a randomly bonded polysaccharide, it is resistant to mammalian

enzymes, which allows for most of the substrate to pass through the body unabsorbed (Figdor and Bianchine, 1983). Polydextrose is resistant to digestion and absorption in the small intestine and is partially fermented in the large intestine, and therefore is a suitable dietary fiber source that has been observed to modulate indices of GI health both *in vitro* and *in vivo*.

Effects of polydextrose on fecal fermentative end-products and microbiota

Polydextrose has been studied *in vitro* using human fecal inoculum and in dogs used as a model for humans, with results suggesting that it possesses prebiotic properties. Probert et al. (2004) evaluated the prebiotic potential of polydextrose by adding 1% (wt/vol) or 2% (wt/vol) to an anaerobic medium using human fecal inoculum. Six substrates were tested in that study including: (1) polydextrose (Litesse Ultra, a high grade form of polydextrose), (2) lactitol monohydrate, (3) a lactitol monohydrate:polydextrose (50:50) blend, (4) short-chain fructooligosaccharides, (5) polydextrose (using a pooled inoculum), and (6) oligofructose (using a pooled inoculum) for a period of 48 h. For test substrates 1-4, the same healthy human donated a sample for inoculation; test substrates 5 and 6 were inoculated using a pooled sample from four healthy human donors. Those researchers concluded that SCFA concentrations, namely acetate and butyrate [acetate: 1% polydextrose: 29 mM (initial) to 46 mM; 2% polydextrose: 78 mM (initial) to 104 mM; butyrate: 1% polydextrose: 32 mM (initial) to 52 mM; 2% polydextrose: 48 mM (initial) to 57 mM], were numerically increased with the addition of polydextrose. Probert et al. (2004) also assessed bacterial populations using FISH. These researchers observed an increase ($P=0.01$) in bifidobacteria in all vessels (6.91 log cells/mL of culture fluid vs. 7.99 log cells/mL of culture fluid, 8.04 log cells/mL of culture fluid, 7.88 log cells/mL of culture fluid) during the first fermentation, but lactobacilli were not within the detection limits (a value of $<10^6$

is below detection limit) of the technique. Additionally, in order to determine the species of bifidobacteria present, these researchers used PCR and species-specific primers after adaptation to the substrate. When polydextrose was added at 1% (wt/vol), 5 species of bifidobacteria were identified: *B. adolescentis, B. bifidum, B. catenulatum, B. longum,* and *B. dentium*. When polydextrose was added at 2% (wt/vol), 7 species of bifidobacteria were identified: *B. adolescentis, B. angulatum, B. bifidum, B. catenulatum, B. longum, B. infantis,* and *B. dentium* (Probert et al., 2004). In another *in vitro* study, Mäkivuokko et al. (2007) examined the effects of adding polydextrose to dark chocolate using two *in vitro* simulation techniques. The first was a gastric and small intestinal digestion simulation adapted from Fuller (1991), while the second was an automated four-stage colon simulator (Mäkivuokko et al., 2005). Those researchers concluded that production of acetate [four-stage treatment mean; baseline: 25.8 μM; cocoa mass only: 29 μM; cocoa mass + 2% polydextrose: 57.3 μM], butyrate [four-stage treatment mean; baseline: 16.3 μM; cocoa mass only: 9.3 μM; cocoa mass + 2% polydextrose: 20.5 μM], propionate [four-stage treatment mean; baseline: 7.8 μM; cocoa mass only: 12.8 μM; cocoa mass + 2% polydextrose: 16.8 μM], and total SCFA [four-stage treatment mean; baseline: 49.8 μM; cocoa mass only: 49.3 μM; cocoa mass + 2% polydextrose: 94.5 μM] concentrations increased ($P<0.01$) in the vessel containing cocoa mass + 2% polydextrose compared with baseline and vessels not containing polydextrose, with the greatest difference observed in butyrate production. Those researchers also concluded that both digested cocoa mass and polydextrose-supplemented cocoa mass tended to decrease the total concentration of biogenic amines in all vessels as compared to baseline vessels, but the decrease in cadaverine concentrations was the only biogenic amine to reach statistical significance ($P<0.04$; V1: 25 μM; V2: 20 μM; V3: 20 μM; V4: 15 μM). These *in vitro* studies displayed the prebiotic potential of polydextrose, highlighted

by increased SCFA concentrations and numbers of beneficial bacteria (Probert et al., 2004; Mäkivuokko et al., 2007).

Jie et al. (2000) evaluated the effects of feeding 0, 4, 8, or 12 g polydextrose/d on GI tolerance and fecal pH, SCFA and carcinogenic metabolite concentrations, and microbiota of healthy adult humans (mean age = 31.2 yr; n=100). That study consisted of a 28-d feeding phase and fresh fecal collection (within 1 h of defecation) on d 1 and d 28. The subjects were required to consume the provided meals on the dietary control phase days (d -4 to -1 and 26 to 28). They were also asked to limit fruit consumption to one piece per day. Those researchers concluded that dietary intake of polydextrose increased ($P<0.01$) the ease of defecation (scale of -3 to 3; 0 g polydextrose/d: before = -0.21, after = 0.41; 4 g polydextrose/d: before = -0.18, after = 1.36; 8 g polydextrose/d: before = 0.20, after = 1.88; 12 g polydextrose/d: before = -0.14, after = 2.35) and fecal output (as-is g/d; 0 g polydextrose/d: 103 to 106; 4 g polydextrose/d: 106 to 115; 8 g polydextrose/d: 101 to 128; and 12 g polydextrose/d: 98 to 142), likely due to its water-holding capacity. Researchers reported that fecal pH was lower ($P<0.05$) in subjects consuming 8 or 12 g polydextrose/d after 28 d of supplementation (6.71 and 6.37, respectively) compared to control subjects (7.04). Also, in subjects consuming 8 or 12 g polydextrose/d, fecal butyrate (8 g/d: 1.31 mg/g; 12 g/d: 1.41 mg/g) and acetate (8 g/d: 4.70 mg/g; 12g/d: 5.12 mg/g) were greater ($P<0.05$) after 28 d of supplementation than control subjects (0.94 mg/g and 4.12 mg/g, respectively). Jie et al. (2000) also concluded that concentrations of beneficial bacteria, as assessed using a culture-based technique, were increased ($P<0.05$) with polydextrose consumption. Of the specific bacteria tested (data reflect means of individuals consuming all polydextrose treatments), *Bacteroides fragilis* (d1: 1.42 x 10^9/g stool; d28: 0.36 x 10^9/g stool), *Bacteroides vulgatus* (d1: 0.77 x 10^9/g stool; d28: 0.25 x 10^9/g stool), and *Bacteroides*

intermedius (d1: 0.39 x 10^9/g stool; d28: 0.12 x 10^9/g stool) were decreased (P<0.05) from baseline and were lower than control individuals. However, *Lactobacillus* (d1: 0.29 x 10^9/g stool; d28: 1.46 x 10^9/g stool) and *Bifidobacterium* (d1: 0.46 x 10^9/g stool; d28: 3.29 x 10^9/g stool) increased (P<0.05) from baseline and were higher than control individuals (Jie et al., 2000).

Vester Boler et al. (2011) evaluated the digestive physiological outcomes (e.g., burping, cramping, distension, flatulence, nausea, reflux, and vomiting) and fecal fermentative end-product concentrations of polydextrose and soluble corn fiber supplementation in healthy adult male humans (mean age = 27.5 yr; mean BMI = 27 kg/m^2; mean BW = 86.2 kg; n=21). The participants consumed the fibers in the form of snack bars for a total of ~0 g (no supplemental fiber control; NFC) or 21 g (polydextrose or soluble corn fiber) per day. Gastrointestinal tolerance was scoreded using a 4-point scale, where 1 = none, 2 = mild, 3 = moderate, and 4 = severe. Flatulence (P<0.001) was greater with the consumption of polydextrose (2.23/4) or soluble corn fiber (2.08/4) when compared to NFC (1.83/4); distension (P =0.07) was greater with the consumption of polydextrose (1.52/4) or soluble corn fiber (1.45/4) when compared to NFC (1.33/4); reflux was greater (P<0.04) when subjects consumed soluble corn fiber (1.08/4) when compared to the NFC (1.03/4). Those researchers observed lower (P<0.01) fecal ammonia (NFC: 137.5 µmol/g DM feces; polydextrose: 97.5 µmol/g DM feces; soluble corn fiber: 117 µmol/g DM feces), phenol (NFC: 1.5 µmol/g DM feces; polydextrose: 0.6 µmol/g DM feces; soluble corn fiber: 0.9 µmol/g DM feces), indole (NFC: 1.0 µmol/g DM feces; polydextrose: 0.3 µmol/g DM feces; soluble corn fiber: 0.5 µmol/g DM feces), and total BCFA (NFC: 30.3 µmol/g DM feces; polydextrose: 18.6 µmol/g DM feces; soluble corn fiber: 22.4 µmol/g DM feces) concentrations in participants consuming polydextrose. They also observed lower (P<0.01) fecal

pH (NFC: 6.4; polydextrose: 6.3; soluble corn fiber: 6.2) in participants consuming soluble corn fiber, following similar trends as in previous studies. Hooda et al. (2012) used 16S rRNA gene amplicon-based 454 pyrosequencing to evaluate the effects of polydextrose and soluble corn fiber on the microbiota from the fecal samples collected in the study performed by Vester Boler et al. (2011). Those researchers reported greater ($P<0.05$) *Faecalibacterium* (control = 20.7% of sequences; polydextrose = 24.1% of sequences) and lower ($P<0.05$) *Ruminococcus* (control = 13.1% of sequences; polydextrose = 9.2% of sequences), *Eubacterium* (control = 11.8% of sequences; polydextrose = 7.6% of sequences), and *Dorea* (control = 1.4% of sequences; polydextrose = 0.7% sequences) in individuals consuming polydextrose compared to the no-fiber controls (Hooda et al., 2012). Fecal *Lactobacillus*, *Bifidobacterium*, and *Bacteroides* spp., all altered in the study of Jie et al. (2000) using traditional culture methods, were not observed to be different using pyrosequencing

The prebiotic potential of polydextrose has not been studied in pets, but Knapp et al. (2008) used dogs to test GI tolerance (mean age = 13 mo; mean BW = 17.5 kg; n = 9) and glycemic/insulinemic response. In the GI tolerance portion of that study, adult hound dogs were fed dry extruded diets containing 0, 14, or 28 g polydextrose/1000 kcal. In that study, dogs were used as a model for humans, with diets containing test carbohydrates at 0, 100, or 200% of adequate intake (AI) of dietary fiber for adult humans (IMNA, 2005). Dogs consumed between 19 and 39 g polydextrose/d. Those researchers reported that fecal scores (5-point scale, where 1 = dry, hard pellets and 5 = watery liquid that can be poured) increased ($P<0.05$) as intake of polydextrose increased (control: 2.9; 100% AI: 4.2; 200% AI: 4.6). The doses used by Knapp et al. (2008) were higher than would be present in pet foods, and tolerance may be better at lower

doses. Additionally, GI microbial populations were not studied by Knapp et al. (2008); therefore, the prebiotic potential in dogs has not been adequately tested.

RESISTANT STARCH (RS)

A starch molecule is a polysaccharide composed of multiple glucose units, which are linked together with α-1,4 and/or α-1,6 glycosidic bonds. The two main forms of starch are amylose and amylopectin. Amylose is a linear polymer of α-1,4 glycosidic linkages with a DP range of 324 to 4920 and molecular weight of approximately 250 kDa, comprising about 20-30% of starch (Morrison and Karkalas, 1990; Mua and Jackson, 1997; Buléon et al., 1998; Tester et al., 2004). Amylopectin is a much larger polymer composed of α-1,4 and α-1,6 glycosidic linkages with a DP typically within the range of 9600 to 15900, but some have DP up to 26000, and molecular weight of approximately $1 \times 10^7 – 1 \times 10^9$ kDa, and comprises about 70-80% of starch (Morrison and Karkalas, 1990; Mua and Jackson, 1997; Buléon et al., 1998; Tester et al., 2004). A starch granule is considered to be approximately 70% amorphous, composed mainly of amylose with some amylopectin, and 30% crystalline, composed primarily of amylopectin (Sajilata et al., 2006). Starches can be classified into three main categories: rapidly digestible starch (RDS), slowly digestible starch (SDS), or resistant starch (RS). Rapidly and slowly digestible starches are completely digested in the small intestine, but at varying rates (Englyst et al., 1992; Sajilata et al., 2006; Zhang and Hamaker, 2009). By definition, RS is starch that escapes digestion in the small intestine and ends up in the large bowel, part of which may be fermented in the colon (Sajilata et al., 2006).

There are four main types of resistant starch: RS1 is made up of starch that is physically entrapped within a cellular or multi-cellular structure that prevents contact with digestive

enzymes. An example of RS1 is a whole grain; RS2 is made up of raw starch granules with a crystal structure resistant to digestive enzymes. Examples of RS2 include raw potato or green banana starch; RS3 is made up of retrograded starch that forms from repeated cooking and cooling. Most RS3 is composed of amylose that has recrystallized into a configuration highly resistant to digestive enzymes; and RS4 is made up of chemically modified starch, including starches that have been treated with chemicals to form ether and ester linkages with starch moieties, thus inhibiting their digestion by digestive enzymes (Englyst et al., 1992).

Effects of RS on fecal fermentative end-products and microbiota

Previous research has demonstrated that RS is highly fermentable and may affect fecal characteristics (e.g., fecal SCFA concentrations and gut microbial populations; Bird et al., 2007; McOrist et al., 2011). Bird et al. (2007) compared the effects of experimental diets containing one of four starches [(1) control, 0% amylose; (2) high-amylose maize starch (HAMS); (3) hydrothermally treated high-amylose maize starch (HTHAMS); (4) combination of 2 and 3 (HAMS + HTHAMS)] in the large bowel of pigs (age = 4 wk; n = 24). The diets contained approximately 15.5% RS/kg diet (HAMS), 30.9% RS/kg diet (HTHAMS), or 22.8% RS/kg diet (HAMS + HTHAMS). Those researchers concluded that fecal pH was lower ($P<0.001$) in pigs consuming the HAMS (pH = 5.27), HTHAMS (pH = 5.14), or HAMS + HTHAMS (pH = 5.20) compared to the control-fed pigs (pH = 6.40). Additionally, Bird et al. (2007) observed higher ($P<0.001$) distal colonic digesta total SCFA (96.7 mmol/L), acetate (43.3 mmol/L), propionate (35.7 mmol/L), and butyrate (7.2 mmol/L) concentrations in pigs fed RS-containing diets compared to the control-fed pigs (total SCFA = 35 mmol/L; acetate = 20 mmol/L; propionate = 10 mmol/L; butyrate = 4 mmol/L). These researchers also noted that the HAMS + HTHAMS

treatment was the most effective at increasing fecal SCFA (data not provided) when compared to control-fed pigs (Bird et al., 2007).

Rideout et al. (2008) investigated the nutrient utilization and intestinal fermentation of various RS varieties and conventional fiber sources, including granular high amylose corn starch, granular potato starch, retrograded high amylose corn starch, guar gum, or cellulose, when fed to Yorkshire pigs (BW = 30 kg; n = 36). At 10% RS inclusion, those authors observed higher ($P<0.05$) cecal butyrate concentrations (4.98 mg/g DM digesta vs. 11.89 mg/g DM digesta) and lower ($P<0.05$) cecal indole (0.12 mg/g DM digesta vs. 0.04 mg/g DM digesta), isobutyrate (4.03 mg/g DM digesta vs. 1.98 mg/g DM digesta), and isovalerate (4.20 mg/g DM digesta vs. 1.73 mg/g DM digesta) concentrations in pigs. Tachon et al. (2013) evaluated the structure of cecal microbial communities of male C57BL/6J mice (age = 18-20 mo old; n = 6 per diet) fed one of three diets: Control (Amioca corn starch; 100% amylopectin, 0% RS), 18% RS, or 36% RS (type 2 RS from high-amylose maize; Hi-maize 260®). Because Hi-maize 260® is composed of 60% RS and 40% digestible starch, these diets contained approximately 10.8% and 21.6% RS, respectively. Those researchers observed higher ($P<0.05$) proportions of cecal *Lactobacillus* and *Bifidobacterium* spp. in mice fed diets containing 18% RS from Hi-maize 260® when compared to the control fed mice.

WHOLE GRAINS

According to the American Association of Cereal Chemists (AACC), whole grains are defined as the intact, ground, cracked, or flaked caryopsis, that which consists of a starchy endosperm, germ, and bran portions that are similar to the intact caryopsis, and may be malted or sprouted, as long as labeled as such (AACC, 2009). Whole cereal grains belong to the *Poaceae*

or *Gramineae* families, better known as grasses. The endosperm is the largest portion of the grain and contains carbohydrates, proteins, vitamins, and minerals. The germ is also known as the embryo and contains vitamins, some protein, minerals, and fats. The bran portion is the outer covering of the grain, which protects the germ and endosperm. It contains phenolic compounds, vitamins, and minerals (Okarter and Liu, 2010). Whole grains vary greatly in their macronutrient and micronutrient composition and contain many bioactive compounds such as fiber, folate, phenolic compounds, lignan, and sterols (De Moura et al., 2009). The benefits of consuming whole grains have been well studied in humans (Anderson, 2003; Slavin, 2004; Okarter and Liu, 2010), but little research exists on their effects in dogs.

Bioactive compounds present in whole grains

Cereal grains may contain many bioactive compounds, such as γ-oryzanol in rice, avenanthramides and saponins in oats, and β-glucans in oats and barley. The antioxidant activity of γ-oryzanol has been shown to be effective in lowering the risk of cardiovascular disease and hyperlipidemia, by means of cholesterol-lowering, lipid peroxidation reduction and anti-atherogenic effects (Seetharamaiah and Chandrasekhara, 1993; Rong et al., 1997; Cicero and Gaddi, 2001; Suh et al., 2005; Wilson et al., 2007). The antioxidant properties of barley, pearl millet, rye, and sorghum are thought to be comparable to that of butylated hydroxytoluene, a commonly used synthetic antioxidant (Ragaee et al., 2006). Avenanthramides are involved in the prevention of cardiovascular disease because of their anti-inflammatory and anti-atherogenic effects (Liu et al., 2004). Saponins have a variety of biological activities, being both anti-carcinogenic and hypocholesterolemic, and may stimulate the immune system (Matsuura, 2001; Güçlü-Üstündag and Mazza, 2007). The health effects of β-glucans include glycemia- and

cholesterol-lowering properties, which have relevance to type 2 diabetes and cardiovascular disease (Sadiq Butt et al., 2008; Kalra and Joad, 2000; Kim et al., 2009). Even though cardiovascular disease is not that common in dogs, these components may provide benefits for those that are obese or have diabetes.

The potential health benefits of whole grains have not been identified in dogs. Because grains are not always included in the diet in whole form, analysis of the various grain fractions used in the pet food industry is also justified. Although some research has been performed on grain fractions, more research is needed to determine what components differ among whole grains and individual fractions, and how they may act differently in the body (Okarter and Liu, 2010; USDA, 2013).

Effects of processing on whole grains

In addition to the phytochemicals they contain, whole grains may provide RS to pets, but likely depends on processing conditions. Murray et al. (2001), who evaluated the effects of extrusion [low temperatures = 79-93°C; high temperatures (HT) = 124-140°C] on cereal grains (barley, corn, rice, sorghum, wheat) and potato, concluded that extrusion at HT decreased the RS concentration in all 6 native substrates (RS of native substrates: barley = 17%, corn = 23.6%, potato starch = 60%, rice = 26.9%, sorghum = 33.8%, wheat =13.0%; RS of HT extruded substrates: HT barley = 6.0%, HT corn = 1.4%, HT potato starch = not determined, HT rice = 4.0%, HT sorghum = 2.1%, HT wheat = 0.6%). Dust et al. (2004) evaluated the effects of extrusion on select pet food ingredients, including barley grits, cornmeal, oat bran, soybean flour, soybean hulls, and wheat bran. Extrusion conditions were (1) mild, (2) medium, or (3) extreme, in which ingredients were subjected to 80-90°C + screw profile of 1 reverse lobe, 100-110°C +

screw profile of 3 reverse lobes, or 120-130°C + screw profile of 5 reverse lobes, respectively. They also evaluated the ingredients in their native state (unprocessed). Those researchers concluded that because of extrusion, the insoluble dietary fiber concentrations decreased and the soluble fiber concentrations increased, decreasing the insoluble:soluble fiber ratio in all ingredients tested, except soybean hulls subjected to mild and moderate extrusion conditions. Furthermore, RS concentrations were decreased for barley grits, cornmeal, and wheat bran due to extrusion conditions (Dust et al., 2004). Hernot et al. (2008), who compared common whole grains (barley, corn, oat, rice, and wheat) and their fractions, concluded that the RS concentrations of barley (unprocessed: 12.2%; processed: 8.7%), corn (unprocessed: 16.3%; processed: 5.3), oat (unprocessed: 8.7%; processed: 4.4%), and wheat (unprocessed: 16.4%; processed: 6.7%) were decreased after processing for 1 h at 100°C and expanded with hot air.

Effects of whole grains on fecal fermentative end-products and microbiota

Previous research has identified the importance of whole grain consumption in human health. In humans, increased consumption of whole grains has been associated with improved weight management, decreased risk of cardiovascular diseases, decreased risk of type II diabetes, and improved digestive health (Okarter and Liu, 2010; Jonnalagadda et al., 2011). In regards to GI health, Connolly et al. (2010) investigated the effects of two different sized whole oat grain flakes on fecal microbial populations and SCFA production in an *in vitro* system using human fecal inoculum. The evaluated substrates included whole oat flakes of varying thickness derived from the same crop (Oat 23's = 0.53-0.63 mm; Oat 25's/26's = 0.85-1.0 mm) and 2 controls (oligofructose and cellulose). These researchers assessed changes in fecal bacterial populations using FISH and concluded that bifidobacteria were higher ($P<0.05$) for cultures including Oat

25’s/26’s (8.42 $\log_{10}$ cells/mL batch culture fluid) vs. Oat 23’s (7.93 $\log_{10}$ cells/mL batch culture fluid) after 24 h of fermentation. Additionally, these researchers observed an increase ($P<0.05$) in total SCFA concentrations for Oat 25’s/26’s after 10 h (16.3 mmol/mL) and 24 h of fermentation (42.7 mmol/mL) compared to baseline (1.0 mmol/mL). Each individual SCFA was also increased ($P<0.05$) after 24 h fermentation compared to baseline (acetate: 0.7 vs. 30.7 mmol/mL; propionate: 0.1 vs. 10.1 mmol/mL; butyrate: -0.2 vs. 1.9 mmol/mL) for Oat 25’s/26’s. Those researchers concluded that the thicker oat flakes resulted in a more beneficial modulation of bacterial populations and SCFA (Connolly et al., 2010). Connolly et al. (2012) performed a similar study investigating the effects of raw and toasted whole grain wheat flakes on microbiota and SCFA in an *in vitro* system using human fecal inoculum. The substrates tested included raw wheat flakes, partially toasted wheat flakes (removed from oven before fully toasted), toasted wheat flakes (baked up to 200°C until fully toasted), and two controls (oligofructose and cellulose). Fecal bacterial populations were assessed using FISH. Those researchers concluded that after 24 h of fermentation, *Bifidobacterium* genus was increased ($P<0.05$) in raw wheat (0 h: 7.7 $\log_{10}$ cells/mL vs. 24 h: 8.5 $\log_{10}$ cells/mL), partially toasted wheat (0 h: 7.8 $\log_{10}$ cells/mL; 24 h: 8.4 $\log_{10}$ cells/mL), toasted wheat (0 h: 7.8 $\log_{10}$ cells/mL; 24 h: 8.5 $\log_{10}$ cells/mL), and oligofructose (0 h: 7.8 $\log_{10}$ cells/mL; 24 h: 8.4 $\log_{10}$ cells/mL) when compared to the baseline values. Lactobacilli populations were only increased ($P<0.05$) in the raw wheat flakes (0 h: 7.4 $\log_{10}$ cells/mL; 24 h: 7.9 $\log_{10}$ cells/mL). Furthermore, SCFA concentrations were increased ($P<0.05$) for all substrates, in which acetate was the dominant SCFA (raw wheat: 0 h: 0.5 mmol/L 24 h: 35.0 mmol/L; partially toasted: 0 h: 0.7 mmol/L, 24 h: 36.7 mmol/L; toasted: 0 h: 0.6 mmol/L, 24 h: 33.0 mmol/L; oligofructose: 0 h: 0.8 mmol/L, 24 h: 32.3 mmol/L; cellulose: 0 h: 0.3 mmol/L, 24 h: 3.1 mmol/L).

Bird et al. (2004) investigated the effects of a diet containing a unique barley cultivar (*Himalaya 292*; lower starch content and a substantial increase in the relative proportion of amylose) compared with traditional cereal grains on the large bowel of young adult male Sprague-Dawley rats (n= 6 rats per treatment). Rats were randomly assigned to one of five diets containing the following grains: (1) *Himalaya 292*; (2) hull-less Namoi Australian barley; (3) hull-less Waxiro Australian barley; (4) oat bran; and (5) wheat bran. The diets were balanced for macronutrients and contained the following: 180 g protein/kg, 630 g carbohydrate/kg (530 g starch and 100 g sucrose), 70 g fat/kg, and 50 g NSP/kg. Rats had unrestricted access to food, but intake did not differ among treatment groups. However, fecal output was higher ($P<0.05$) in rats fed the *Himalaya 292*–containing diet (3.8 g/d) compared to rats fed Namoi-containing (2.5 g/d) or oat bran-containing (2.3 g/d) diets. Cecal and fecal pH were lower ($P<0.01$) in rats consuming the *Himalaya 292*-containing diet compared to all other groups (fecal pH: wheat bran: 6.09; *Himalaya 292*: 5.61; Namoi: 6.08; Waxiro: 6.00; oat bran: 5.86; cecal pH: wheat bran: 6.63; *Himalaya 292*: 5.76; Namoi: 6.04; Waxiro: 6.21; oat bran: 6.00). Finally, total and individual fecal SCFA concentrations were all higher ($P<0.05$) in rats fed the *Himalaya 292* diet (total SCFA: wheat bran: 335 µmol, *Himalaya 292*: 471 µmol, Namoi: 310 µmol, Waxiro: 321 µmol, oat bran: 250 µmol; fecal acetate: wheat bran: 306 µmol, *Himalaya 292*: 394 µmol, Namoi: 252 µmol, Waxiro: 250 µmol, oat bran: 209 µmol; fecal propionate: wheat bran: 15 µmol, *Himalaya 292*: 52 µmol, Namoi: 43 µmol, Waxiro: 40 µmol, oat bran: 26 µmol; fecal butyrate: wheat bran: 9 µmol, *Himalaya 292*: 24 µmol, Namoi: 12 µmol, Waxiro: 25 µmol, oat bran: 14 µmol; Bird et al., 2004).

Costabile et al. (2008) evaluated the effects of whole grain wheat breakfast cereal on fecal microbiota, as assessed by FISH, and GI tolerance in adult humans. Thirty-one healthy

men and women (mean age = 25 yr; BMI range = 20-30 kg/m^2) were recruited and randomized to one of two treatment groups in a crossover design: (1) whole grain wheat breakfast cereal (48 g/d), or (2) whole bran breakfast cereal (48 g/d). For each period, subjects remained on the treatment for 3 wk and underwent a 2-wk wash-out phase. Feces, urine, and blood were collected before and after each treatment phase as well as 14 d after the second treatment phase (washout). Those researchers observed an increase ($P<0.001$) in fecal bifidobacteria after consumption of whole grain wheat (9.3 $\log_{10}$ cells/g feces) compared to whole bran (8.8 $\log_{10}$ cells/g feces). *Bifidobacterium* spp. were also increased ($P<0.05$) after consumption of whole grain wheat (9.3 $\log_{10}$ cells/g feces) compared to baseline (8.5 $\log_{10}$ cells/g feces). Fecal lactobacilli/enterococci were increased ($P<0.01$) with either treatment when compared to baseline (whole bran: d 0: 8.0 $\log_{10}$ cells/g feces; d 21: 8.4 $\log_{10}$ cells/g feces; whole grain: d 0: 8.1 $\log_{10}$ cells/g feces; d 21: 8.7 $\log_{10}$ cells/g feces). The participants reported a greater ($P<0.05$) frequency of defecation, incidence of softer stools, and flatulence with the consumption of whole bran compared to whole grain wheat (Costabile et al., 2008).

Limited research exists in regards to pure RS fed to companion animals (Spears and Fahey, 2004). Most of the research that exists has evaluated RS in the form of whole grains or whole grain fractions using canine fecal inoculum *in vitro* and *in vivo*. Because some varieties of high-amylose corn have been shown to escape digestion in the small intestine and are available for fermentation in the large bowel, Gajda et al. (2005) evaluated select corn hybrids both *in vitro* and *in vivo*. In Experiment 1, three experimental corns and two conventional corns were chemically evaluated and then studied *in vitro* using canine fecal inoculum: (1) high-protein corn (HP); (2) high-protein, low-phytate corn (HPLP); (3) high-amylose corn (HA; composed of >70% amylopectin); (4) conventional No. 2 yellow dent corn (CONV); and (5) amylomaize

(AM; nearly 100% amylose). The composition of both the raw substrates and extruded substrates (speed = 360 rpm, steam flow = 23 kg/h, water flow = 5 kg/h, temperature = 91°C). In that experiment, RS concentrations for all substrates, except AM, were decreased as a result of extrusion cooking (raw: CONV: 34.9%, HP: 48.5%, HPLP: 49.7%, HA: 50.4%, AM: 57.0%; extruded: CONV: 16.0%, HP: 20.4%, HPLP: 23.2%, HA: 34.7%, AM: 63.9%). The AM and HA diets had higher ($P<0.05$) organic matter disappearance compared to the other three substrates (fermentation at 8 h: CONV: 3.1%, HP: 3.1%, HPLP: 1.1%, HA: 7.0%, AM: 7.6%). Additionally, acetate production was higher ($P<0.05$) for AM (5.1 μmol/g) vs. HA (3.8 μmol/g), but in conjunction with the organic matter disappearance data, it appeared that the majority of the hybrids were hydrolytically digested, leaving little to be fermented by colonic microbes. In Experiment 2, five ileal-cannulated hound dogs were randomly assigned to diets containing the 5 experimental treatments described in Experiment 1 (HA =33.18% of diet, HP = 31.97% of diet, HPLP = 32.35% of diet, CONV = 31.87% of diet, and AM = 26.00% of diet). Those researchers concluded that dogs fed HA had the highest ($P<0.05$) as-is fecal output (HA: 218.0 g/d; HP: 158.0 g/d; HPLP: 123.4 g/d; CONV: 164.1 g/d; AM: 200.2 g/d) and HA and AM fed dogs had the highest ($P<0.05$) fecal output on a DM basis (HA: 72.6 g DM/d; AM: 68.6 g DM/d; HP: 47.4 g DM/d; HPLP: 46.8 g DM/d; CONV: 53.6 g DM/d). High-amylose corn- and AM-fed dogs also had the highest ($P<0.05$) as-is fecal output: DM intake ratio (HA: 0.71 g/g DMI; AM: 0.66 g/g DMI; HP: 0.53 g/g DMI; HPLP: 0.40 g/g DMI; CONV: 0.54 g/g DMI). No statistical differences were observed in fecal microbial populations (bifidobacteria, lactobacilli, or *Clostridium perfringens*) were observed (Gajda et al., 2005). Goudez et al. (2011) evaluated different concentrations (0, 2.5, 4.3, and 7.4% of diet) and sources (high-amylose maize starch and potato starch flour) of RS fed to dogs of various body sizes and breeds (6 miniature poodles,

6 miniature schnauzers, 3 giant schnauzers, and 6 German shepherds). The aim of that study was to evaluate how the amount of RS, type of RS, or body size affected stool consistency. Those researchers concluded that regardless of dietary RS concentration and source, fecal scores of miniature schnauzers were always optimal, whereas the fecal scores of German shepherds increased (i.e., looser stools; $P < 0.0001$) with increased RS consumption, and therefore the percentage of optimal fecal scores decreased ($P<0.0001$; Goudez et al., 2011).

CONCLUSIONS

In conclusion, non-digestible carbohydrates are functional ingredients that are incorporated into a variety of foods, including pet food. Gut microbiota play an integral role in maintaining GI health and overall host health and can be modulated with the consumption of non-digestible carbohydrates. Canine GI health may be improved by incorporating ingredients such as polydextrose, RS, whole grains, or grain fractions, but more research is needed to explore these fermentable non-digestible carbohydrates for inclusion into canine diets.

TABLE

Table 2.1. Molecular methods used for microbiome analysis[1]

Method[2]	Description	Characteristics	Primary advantages	Primary disadvantages
qPCR	Amplifies and quantifies a targeted DNA molecule	Dye or probe used to bind double stranded DNA, which causes intensity of fluorescent emissions to increase	Low cost; high sensitivity allows for detection of sequences at low concentrations	Limited in scope
FISH	Sensitive detection of specific nucleic acid sequences in metaphase or interphase cells	Manual procedure of biological samples; fluorescence intensities measured using FLEX (a quantitative fluorescence microscope system)	Allows for localization and study of spatial organization of cells as they occur in their natural habitat	Costly; not easily scalable for disease screenings
RFLP	High-throughput fingerprinting technique used to explore changes in structure and composition of microbial communities	DNA sample digested by restriction enzymes to characterize microbiota of specific regions. Fragments then separated according to length by gel electrophoresis.	Provides a broad view of microbial systems	Primers not specific
DGGE	PCR-amplified 16S rRNA fragments separated on polyacrylamide gel containing gradient of denaturant (e.g., urea, formamide)	Gel-based method of fingerprinting	Provides a broad view of microbial systems	Only semiquantitative and insensitive

Table 2.1 (cont.)

TGGE	PCR-amplified 16S rRNA fragments separated on polyacrylamide gel containing gradient of temperatures	Gel-based method of fingerprinting	Generate qualitative differences in microbial ecology	Only semiquantitative and insensitive
454-sequencing	Pyrosequencing light emission	500-700 base reads	16S coverage is good	Cost limits shotgun coverage
Illumina sequencing	Fluorescent, stepwise sequencing	100-250 base reads	Very high coverage owing to high instrument output and very low cost	Increased bioinformatics costs and time
Sanger sequencing	Fluorescent, dideoxy terminator	750 base reads or greater	High read length and accuracy	Compared with next-generation sequencing, is costly and has low throughput

[1]Summarized from Pinkel et al. (1986), Muyzer et al. (1993), Liu et al. (1997), Zoetendal et al. (1998), Rigottier-Gois et al. (2003), Richards et al. (2005), Schütte et al. (2008), Petrosino et al. (2009), and Weinstock (2012).

[2]qPCR = quantitative PCR; FISH = fluorescent in situ hybridization; DGGE = denaturing gradient gel electrophoresis; TGGE = temperature gradient gel electrophoresis.

CHAPTER 3: COMPOSITIONAL ANALYSIS OF WHOLE GRAINS, PROCESSED GRAINS, AND GRAIN COPRODUCTS

ABSTRACT

Whole grains may be valuable components of canine diets due to the functional ingredients, such as dietary fiber and β-glucans, or the amino acids (AA) and minerals they provide. The use of whole grains in pet food has not been thoroughly evaluated, however, to adequately evaluate their effects *in vivo*, a detailed compositional analysis must first be performed. Our objective was to measure the chemical, starch, amino acid, and mineral composition of various whole grains, processed grains, and grain coproducts that may be incorporated into dog foods and treats. Thirty-two samples, including barley samples (barley flake, cut barley, ground pearled barley, malted barley, whole pearled barley, pearled barley flakes, and steamed rolled barley); oat samples (groats, ground oatmeal, ground steamed groats, instant oats, oat bran #1, oat bran #2, oat fiber, oat flour, quick oats, regular rolled oats, steamed rolled oat groats, and steel cut groats); rice samples (brown rice, polished rice, defatted rice bran, and rice flour); and miscellaneous cereals and pseudocereals (canary grass seed, conventional hulled millet, conventional whole millet, conventional quinoa, organic spelt hull pellets, potato flake, sorghum, whole wheat, and whole yellow corn) were analyzed. The chemical composition of grains and grain components varied greatly: crude protein [(1.71-17.04% dry matter basis (DMB)], fat (1.28-8.49% DMB), total dietary fiber (2.16-85.16% DMB), and resistant starch (RS; 0.0-9.38% DMB). Total essential (0.65 – 7.51% DMB) and individual AA concentrations were highly variable among ingredients tested: arginine (0.01 – 1.38% DMB), histidine (0.02 – 0.47% DMB), isoleucine (0.07 – 0.62% DMB), leucine (0.15 – 1.49% DMB), lysine (0.06 –

0.88% DMB), methionine (0.03 – 0.36% DMB), phenylalanine (0.08 – 0.77% DMB), threonine (0.05 – 0.66% DMB), tryptophan (<0.04 – 0.20% DMB), and valine (0.09 – 0.96% DMB). Of the ingredients tested, oat fiber had the lowest concentrations of most essential AA and defatted rice bran had the highest concentrations of most essential AA. Calcium, phosphorus, magnesium, and potassium concentrations ranged from 0.0 – 2.22% DMB, 0.04 – 2.03% DMB, 0.03 – 0.88%, 0.10 – 1.70% DMB, respectively, with defatted rice bran having the highest concentration of all four of these minerals. Sodium, chloride, and sulfur concentrations were low (≤0.10% DMB) in all tested ingredients except for potato flakes (Cl = 0.20% DMB) and canary grass seed (S = 0.20% DMB). Based on our compositional analysis, whole grains vary greatly in nutritive value. Some may provide a generous amount of functional nutrients (i.e., total dietary fiber and RS) to dogs, but more research is needed to test the effects of whole grain-containing dog foods and treats *in vivo*.

INTRODUCTION

According to the American Association of Cereal Chemists (AACC), whole grains are defined as the intact, ground, cracked, or flaked caryopsis, which consists of a starchy endosperm, germ, and bran portions that are similar to the intact caryopsis, and may be malted or sprouted, as long as labeled as such (AACC, 2009). Whole cereal grains belong to the *Poaceae* or *Gramineae* families, better known as grasses. The endosperm is the largest portion of the grain and contains carbohydrates, proteins, vitamins, and minerals. The germ is also known as the embryo and contains vitamins, some protein, minerals, and fats. The bran portion is the outer covering of the grain, which protects the germ and endosperm. It contains phenolic compounds, vitamins, and minerals (Okarter and Liu, 2010). Whole grains vary greatly in their

macronutrient and micronutrient composition and include a variety of bioactive compounds such as fiber, folate, phenolic compounds, lignan, and sterols (De Moura et al., 2009). Whole grains have been well studied for their application to human health (Anderson, 2003; Slavin, 2004; Okarter and Liu, 2010); however, the use of whole grains in pet food has not been thoroughly evaluated.

The largest portion of a whole grain is comprised of starch, a polysaccharide composed of many glucose units, which is linked together with α-1,4 and/or α-1,6 glycosidic linkages (Englyst et al., 1992). Some of this starch may escape digestion in the small intestine, allowing for fermentation in the colon (Sajilata et al., 2006), and is commonly known as resistant starch (RS). There are four main types of RS: RS1 is made up of starch that is physically entrapped within a cellular or multi-cellular structure that prevents contact with digestive enzymes. An example of RS1 is a whole grain; RS2 is made up of raw starch granules with a crystal structure resistant to digestive enzymes. Examples of RS2 include raw potato or green banana starch; RS3 is made up of retrograded starch that forms from repeated cooking and cooling. Most RS3 is composed of amylose that has recrystallized into a configuration highly resistant to digestive enzymes; and RS4 is made up of chemically modified starch, including starches that have been treated with chemicals to form ether and ester linkages with starch moieties, thus inhibiting their digestion by digestive enzymes (Englyst et al., 1992).

Extrusion or heat-treatment processing may alter the RS content or bioactive compound concentration of a whole grain. Hernot et al. (2008), who compared common whole grains (barley, corn, oat, rice, and wheat) and their fractions before and after processing, concluded that the RS concentrations of barley (unprocessed: 12.2%; processed: 8.7%), corn (unprocessed: 16.3%; processed: 5.3), oat (unprocessed: 8.7%; processed: 4.4%), and wheat (unprocessed:

16.4%; processed: 6.7%) were decreased after processing for 1 h at 100°C and expanded with hot air. While the cooking conditions used in that study were extreme to simulate extrusion, some whole grains are mildly processed prior to use (i.e., steamed, rolled, or kilned). Therefore, the objective of this study was to measure the chemical, starch, amino acid (AA), and mineral composition of various whole grains, processed grains, and grain components that may be incorporated into dog foods and treats.

MATERIALS AND METHODS

Grain samples

Thirty-two grain samples were analyzed: barley samples (barley flake, cut barley, ground pearled barley, malted barley, whole pearled barley, pearled barley flakes, and steamed rolled barley; Figure 3.1); oat samples (groats, ground oatmeal, ground steamed groats, instant oats, oat bran #1, oat bran #2, oat fiber, oat flour, quick oats, regular rolled oats, steamed rolled oat groats, and steel cut groats; Figure 3.2); rice samples (brown rice, polished rice, defatted rice bran, and rice flour; Figure 3.3); and miscellaneous cereals and pseudocereals (canary grass seed, conventional hulled millet, conventional whole millet, conventional quinoa, organic spelt hull pellets, potato flake, sorghum, whole wheat, and whole yellow corn; Figure 3.4).

Chemical analyses

Grain samples were ground through a 1-mm screen in a Wiley mill (intermediate, Thomas Scientific, Swedesboro, NJ). Samples were analyzed according to procedures by the Association of Official Analytical Chemists (AOAC) for dry matter (DM; 105°C), organic matter (OM), and ash (AOAC, 2006; methods 934.01, 942.05). Crude protein (CP) content was

calculated from Leco total N values (model FP-2000, Leco Corporation, St. Joseph, MI, USA; AOAC, 2006; method 992.15). Total lipid content (acid hydrolyzed fat; AHF) of the samples was determined according to the methods of the American Association of Cereal Chemists (AACC, 1983) and Budde (1952). Gross energy (GE) of the samples was measured using an oxygen bomb calorimeter (model 1261, Parr Instruments, Moline, IL). Dietary fiber concentrations [total dietary fiber (TDF), soluble dietary fiber (SDF), and insoluble dietary fiber (IDF)] were determined according to Prosky et al. (1992). All samples were sent to the University of Missouri Experiment Station Chemical Laboratories for AA (AOAC, 2006; method 982.30E) and mineral analyses, including calcium (Ca; AOAC, 2006; method 985.01), chloride (Cl; AOAC, 2006; method 943.01), magnesium (Mg; AOAC, 2006; method 985.01), phosphorus (P; AOAC, 2006; method 985.01), potassium (K; AOAC, 2006; method 956.01), sodium (Na; AOAC, 2006; method 956.01), and sulfur (S; AOAC, 2006; method 956.01). Compositional data were not analyzed using statistical methods because accuracy was ensured by adequate replication, with acceptance of mean values that were within 5% of each other.

Starch analyses

Grain subsamples were ground through a 0.5-mm screen in a Wiley mill (intermediate, Thomas Scientific, Swedesboro, NJ). Values were determined by duplication. The method of Muir and O'Dea (1992; 1993) was used to determine the amount of starch digested in the stomach and small intestine by measuring glucose in the supernatant resulting from acid-enzyme digestion of the substrate. Briefly, 0.2 g of each substrate was weighed in duplicate and exposed to pepsin/HCl, amyloglucosidase, and α-amylase. Tubes containing reagents but no substrate were run as blanks. All tubes were incubated for 15 h at 37 °C and then centrifuged for 15 min.

Glucose concentrations in the supernatant were determined by reading the absorbance of individual samples at 450 nm on a DU 640 spectrophotometer (Beckman Instruments, Schaumburg, IL) and comparing those values against a glucose standard curve. Digestible starch (DS) was determined by subtracting (free glucose x 0.9) from (total glucose/original sample weight) present in the supernatant after 15 h of digestion. The 0.9 value used in the calculation of DS is a correction factor for the difference in weight between a free glucose (FG) unit and a glucose residue in starch. Because the measurement of glucose was used to determine starch content, the correction factor was needed. Total starch (TS) content of samples was determined using the method of Thivend et al. (1972) with amyloglucosidase. Resistant starch was calculated by subtracting [DS + (FG x 0.9)] from TS. The released glucose value corresponds to the amount of glucose resulting from hydrolytic starch digestion that is available for absorption *in vivo*. Compositional data were not analyzed using statistical methods because accuracy was ensured by adequate replication, with acceptance of mean values that were within 5% of each other.

RESULTS

The chemical composition of all samples is listed in Table 3.1. The starch composition of all samples is listed in Table 3.2. The complete amino acid profile of all samples is listed in Tables 3.3 and 3.4. The mineral composition of all samples is listed in Table 3.5.

Barley category (Hordeum vulgare)

Samples in the barley category had DM that ranged from 89.20% (whole pearled barley) to 95.63% (malted barley). Organic matter ranged from 97.27% DMB (malted barley) to

98.07% DMB (cut barley). Crude protein ranged from 10.87% DMB (pearled barley flakes) to 14.45% DMB (barley flake). Total lipids ranged from 2.74% DMB (cut barley) to 8.49% DMB (barley flake). Total dietary fiber ranged from 10.78% DMB (barley flake) to 42.14% DMB (malted barley), with IDF ranging from 6.53% DMB (barley flake) to 22.90% DMB (malted barley) and SDF ranging from 3.88% DMB (steamed rolled barley) to 19.24% DMB (malted barley). Gross energy ranged from 4.42 kcal/g DMB (ground pearled barley) to 4.83 kcal/g DMB (malted barley). Free glucose was low for all samples, ranging from 0.08% DMB (barley flake) to 0.13% DMB (cut barley). Total starch ranged from 16.17% DMB (malted barley) to 74.23% DMB (cut barley). Digestible starch ranged from 11.41% DMB (malted barley) to 67.01% DMB (cut barley). Resistant starch ranged from 3.77% DMB (barley flake) to 9.38% DMB (ground pearled barley).

Total essential amino acids (EAA) ranged from 2.24% DMB (malted barley) to 6.06% DMB (barley flake) for the samples in the barley category. Malted barley contained the lowest concentration of all EAA, while barley flake contained the highest concentration of all EAA. For the minerals tested, the Ca concentration ranged from 0.03% DMB (cut barley, ground pearled barley, pearled barley flakes, and whole pearled barley) to 0.05% DMB (malted barley); Mg ranged from 0.10% DMB (cut barley, ground pearled barley, and pearled barley flakes) to 0.15% DMB (malted barley); P ranged from 0.27% DMB (cut barley) to 0.33% DMB (barley flake); and K ranged from 0.30% DMB (barley flake, cut barley, ground pearled barley, pearled barley flakes, whole pearled barley) to 0.40% DMB (malted barley, steamed rolled barley). Sodium, chloride, and sulfur concentrations were low (≤0.10% DMB) in all tested ingredients.

Oat category (Avena sativa)

Samples in the oat category had DM that ranged from 90.35% (steel cut groats) to 95.91% (oat fiber). Organic matter ranged from 94.31% DMB (oat fiber) to 97.99% DMB (ground steamed groats). Crude protein ranged from 1.71% DMB (oat fiber) to 13.90% DMB (oat bran #2). Total lipids ranged from 1.60% DMB (oat fiber) to 8.82% DMB (ground oatmeal). Total dietary fiber ranged from 6.57% DMB (steamed rolled oat groats) to 85.16% DMB (oat fiber), with IDF ranging from 3.35% DMB (steamed rolled oat groats) to 80.89% DMB (oat fiber) and SDF ranging from 1.00% DMB (instant oats) to 4.40% DMB (oat flour). Gross energy ranged from 4.44 kcal/g DMB (oat fiber) to 4.91 kcal/g DMB (steel cut groats). Free glucose was low in all samples ranging from 0.05% DMB (regular rolled oats and instant oats) to 0.10% DMB (groats). Total starch ranged from 8.52% DMB (oat fiber) to 73.49% DMB (quick oats). Digestible starch ranged from 6.85% DMB (oat fiber) to 69.52% DMB (groats). Resistant starch ranged from 0.12% DMB (steel cut groats) to 6.34% DMB (quick oats).

Total EAA ranged from 0.65% DMB (oat flour) to 5.45% DMB (oat fiber) for the samples in the oat category. Oat flour contained the lowest concentration of all EAA and oat fiber contained the highest concentration of most EAA. For the minerals tested, the concentration of Ca ranged from 0.03% DMB (ground steamed groats, quick oats, steamed rolled oat groats, steel cut groats, instant oats, regular rolled oats, oat bran) to 0.08% DMB (oat fiber); Mg ranged from 0.06% DMB (quick oats and oat fiber) to 0.11% DMB (oat flour, ground oatmeal, and oat bran); P ranged from 0.04% DMB (oat fiber) to 0.36% DMB (oat bran); and K ranged from 0.30% DMB (ground steamed groats, quick oats, steamed rolled oat groats, steel cut groats, instant oats, regular rolled oats, oat bran #1, oat flour, groats, and oatmeal) to 0.50% DMB (oat fiber). Sodium, chloride, and sulfur concentrations were low (≤0.10% DMB) in all tested ingredients.

Rice category (Oryza sativa)

Samples in the rice category had DM that ranged from 88.71% (polished rice) to 92.37% (rice flour). Organic matter ranged from 84.29% DMB (defatted rice bran) to 98.70% DMB (polished rice). Crude protein ranged from 8.30% DMB (polished rice) to 17.04% DMB (defatted rice bran). Total lipids ranged from 1.53% DMB (rice flour) to 4.59% DMB (brown rice). Total dietary fiber ranged from 2.16% DMB (polished rice) to 24.20% DMB (defatted rice bran), with IDF ranging from 1.93% DMB (polished rice) to 22.47% DMB (defatted rice bran) and SDF ranging from 0.23% DMB (polished rice) to 2.99% DMB (rice flour). Gross energy ranged from 4.11 kcal/g DMB (rice flour) to 4.44 kcal/g DMB (brown rice). Free glucose ranged from 0.06% DMB (defatted rice bran and polished rice) to 0.48% DMB (rice flour). Total starch ranged from 34.21% DMB (defatted rice bran) to 87.94% DMB (polished rice). Digestible starch ranged from 29.79% DMB (defatted rice bran) to 74.73% DMB (polished rice). Resistant starch ranged from 4.42% DMB (defatted rice bran) to 13.21% DMB (polished rice).

Total EAA ranged from 3.25% DMB (rice flour) to 7.51% DMB (defatted rice bran). Rice flour contained the lowest concentration of arginine, histidine, isoleucine, leucine, methionine, phenylalanine, and valine. Polished rice contained the lowest concentration of lysine and threonine and brown rice contained the lowest concentration of tryptophan. Defatted rice bran contained the highest concentration of all EAA. Polished rice contained the lowest concentration of Ca, Mg, P, and K (0% DMB, 0.03% DMB, 0.14% DMB, and 0.10% DMB, respectively). Defatted rice bran contained the highest concentration of Ca, Mg, P, and K (2.22% DMB, 0.88% DMB, 2.03% DMB, and 1.50% DMB, respectively). Sodium, chloride, and sulfur concentrations were low (≤0.10% DMB) in all tested ingredients.

Miscellaneous cereal grains and pseudocereals

Samples in the miscellaneous category had DM that ranged from 88.58% (sorghum) to 93.42% (canary grass seed). Organic matter ranged from 93.82% DMB (canary grass seed) to 98.59% DMB (whole yellow corn). Crude protein ranged from 7.10% DMB (whole yellow corn) to 19.66% DMB (canary grass seed). Total lipids ranged from 1.28% DMB (potato flake) to 7.27% DMB (canary grass seed). Total dietary fiber content ranged from 6.96% DMB (conventional hulled millet) to 44.00% DMB (organic spelt hull pellets), with IDF ranging from 2.82% DMB (potato flake) to 39.03% DMB (organic spelt hull pellets) and SDF ranging from 0.61% DMB (whole wheat) to 4.97% DMB (organic spelt hull pellets). Gross energy content ranged from 4.26 kcal/g DMB (potato flake) to 4.77 kcal/g DMB (conventional hulled millet). Free glucose ranged from 0.08% DMB (conventional hulled millet) to 1.31% DMB (conventional quinoa). Total starch ranged from 42.0% DMB (organic spelt hull pellets) to 73.53% DMB (conventional hulled millet). Digestible starch ranged from 38.31% DMB (organic spelt hull pellets) to 66.89% DMB (potato flake). Resistant starch ranged from 0.0% DMB (whole yellow corn) to 7.24% DMB (sorghum).

Total EAA ranged from 3.06% DMB (whole yellow corn) to 7.17% DMB (canary grass seed). Whole yellow corn contained the lowest concentration of isoleucine, phenylalanine, threonine, tryptophan, and valine (0.26% DMB, 0.35% DMB, 0.25% DMB, 0.06% DMB, and 0.35% DMB, respectively). Conventional quinoa contained the lowest concentration of histidine, leucine, and methionine (0.17% DMB, 0.49% DMB, and 0.14% DMB, respectively). Sorghum contained the lowest concentration of arginine (0.33% DMB) and lysine (0.18% DMB). Conventional hulled millet contained the highest concentration of leucine (1.49% DMB)

and methionine (0.36% DMB). Conventional whole millet contained the highest concentration of arginine, histidine, and lysine (1.07% DMB, 0.38% DMB, and 0.79% DMB, respectively). Canary grass seed contained the highest concentration of isoleucine, phenylalanine, threonine, tryptophan, and valine (0.80% DMB, 1.18% DMB, 0.46% DMB, 0.32% DMB, and 0.86% DMB, respectively). Of the minerals tested, Ca ranged from 0.0% DMB (whole yellow corn) to 0.07% DMB (organic spelt hull pellets); Mg ranged from 0.09% DMB (potato flake, whole yellow corn, and whole wheat) to 0.20% DMB (conventional quinoa); P ranged from 0.21% DMB (potato flake) to 0.49% DMB (canary grass seed); and K ranged from 0.20% DMB (conventional whole millet) to 1.70% DMB (potato flake). Sodium, chloride, and sulfur concentrations were all low (≤0.10% DMB) in all tested ingredients, except for potato flake (Cl = 0.20% DMB) and canary grass seed (S = 0.20% DMB).

DISCUSSION

Grains are a staple carbohydrate source in both human and pet diets. In comparison to animal proteins or purified fiber sources, the low cost and high nutritive value of many cereal grains make them ideal to incorporate into extruded pet foods. In general, worldwide cereal grain production ranks corn (maize) as the most widely grown and highest production, followed by rice, which is a staple to over half of the world's population. Wheat has the third highest production, followed by barley, sorghum, and oats (Stevens et al., 2004; FAO, 2014). Cereal grains are widely used in the pet food industry, but poorly studied. Furthermore, many pet owners are apprehensive to feed their pets cereal grains (Beaton, 2014). Therefore, a detailed compositional analysis of commonly used cereal grains and *in vivo* effects of feeding novel cereal grains to pets is greatly needed.

Barley (*Hordeum vulgare*) is rich in fermentable and soluble fibers and is gaining interest from the pet food industry as a novel carbohydrate source. Barley is harvested with the hull attached and contains a high level of β-glucans. Steamed rolled barley has steam applied above the roller mill to decrease the production of fine particles and allows for a more uniform particle size (Hironaka et al., 1979; Bamforth and Barclay, 1993; Mathison, 1996; Dehghan-Banadaky et al., 2007). Cut barley, also known as barley grits, is produced when barley kernels are cut into several small pieces. If cut barley comes from hulled or hulless barley, they are considered to be a whole grain, but cut barley from pearl barley is not (Bamforth and Barclay, 1993; Mathison, 1996; Dehghan-Banadaky et al., 2007). Barley flake is produced when steam is applied to whole grain barley kernels, then they are rolled and dried. Barley flakes have decreased cooking time because of the steam that had been applied and increased surface area (Bamforth and Barclay, 1993; Zinn, 1993; Mathison, 1996; Dehghan-Banadaky et al., 2007). Whole pearled barley has the hull removed and has been polished to remove some or the entire outer bran layer. Pearled barley can be tan or white and is not technically considered a whole grain, but is more nutritious than other refined grains (i.e., polished rice) due to the high levels of β-glucans and fiber distribution throughout the kernel. Pearled barley cooks more rapidly than whole grain barley because both the tough outer bran layer and hull have been polished off, and is the common type of barley found in supermarkets (Engstrom et al., 1992; Bamforth and Barclay, 1993; Dehghan-Banadaky et al., 2007). Pearled barley flakes are produced the same way as barley flakes, but from pearled barley kernels (Bamforth and Barclay, 1993; Zinn, 1993; Mathison, 1996; Dehghan-Banadaky et al., 2007). Ground pearled barley is pearled barley kernels that have been ground to a meal form for use as flour (Bamforth and Barclay, 1993; Mathison, 1996; Dehghan-Banadaky et al., 2007). Malted barley requires several more processing steps. Three main steps

are involved in the malting process: (1) initial steeping, where whole kernels are soaked to achieve a moisture content of 42-46% (approximately 48-52 h); (2) germination, where hydrolytic enzymes are synthesized by the aleurone cells and scutellum, and are eventually secreted into the starchy endosperm of the soaked barley kernel, promoting endosperm modification (typically performed at 13-16°C for 8-10 d); and (3) kilning, drying process to cease germination and preserve the malt (typically dried to an approximate final moisture of 2-3%; Bamforth and Barclay, 1993). Malted barley can then be used for brewing, distilling, or malt vinegar production (Bamforth and Barclay, 1993; Haraldsson et al., 2004).

Oats (*Avena sativa*) are not the most common cereal grain used in pet foods, but the use of oats is growing in popularity due to the nutrient profile and lack of pet exposure, which is especially important in hypersensitivity or elimination diets (Aldrich, 2006). Oats are harvested with the hull attached, which is often removed prior to consumption. Oat bran and germ are rarely removed, however. Like barley, oats are high in β-glucans and other constituents, such as carbohydrates, proteins, avenanthramides, tocols, lipids, alkaloids, flavonoids, saponins, and sterols (Singh et al., 2013). Oat groats are cleaned oat kernels that have had their inedible hull removed and take the longest to cook (Welch et al., 2000). Steel cut groats are groats that have been cut into 2-3 pieces with a sharp metal blade. They are often referred to as Irish oatmeal and cook faster than whole oat groats (Owens et al., 1997; Winfield et al., 2007). Steamed rolled oat groats are produced by applying steam to whole oat groats while being rolled into flakes (Owens et al., 1997; Winfield et al., 2007). Ground steamed groats are rolled oat groats that have been ground to a meal form (Owens et al., 1997). Regular rolled oats are often referred to as old fashioned oats. They are created when oat groats are steamed and rolled into flakes. Regular rolled oat groats are thinner than steamed rolled oat groats, but thicker than quick or instant oats.

This process also helps to preserve the healthy lipids in the oat (Owens et al., 1997; Winfield et al., 2007). Oatmeal is ground rolled oats (Owens et al., 1997; Winfield et al., 2007). Quick and instant oats are oat flakes that have been cut, then rolled thinner and steamed longer, ultimately resulting in a change in texture and decreased cooking time (i.e., instant oats are mushy; Owens et al., 1997; Winfield et al., 2007). Oat bran is produced when whole oat groats are passed through several rollers that flatten the kernels. The bran is separated from the flour and sifted to be further separated, resulting in oat bran and debranned oat flour (Sadiq Butt et al., 2008). Oat fiber is produced from finely ground oat hulls (Owens et al., 1997; Winfield et al., 2007). Oat flour is produced from whole groats sent to a stone or hammer mill that can then be used for baking or thickening of soups and stews (Owens et al., 1997; Winfield et al., 2007).

Rice (*Oryza sativa*) is commonly used in pet foods due to the relative low-cost and ease of obtaining the grain. Rice is commonly consumed worldwide and is easily digested. Rice is harvested with the hull attached and is known as paddy rice. Brown rice is a rice kernel that has had the hull removed and is rich in B vitamins and minerals (Shams-Ud-Din and Bhattacharaya, 1978). Polished rice has had both the hull and bran removed, therefore making it less nutritious than brown rice (Shams-Ud-Din and Bhattacharaya, 1978). Rice bran is produced from the outer layers of the harvested kernel and is rich in fiber, vitamins, and minerals (Shams-Ud-Din and Bhattacharaya, 1978; Saunders, 1990). Rice flour is produced from broken white rice kernels that have been finely milled (Shams-Ud-Din and Bhattacharaya, 1978; Hasjim et al., 2013).

Many cereal grains and pseudocereals were also evaluated, many of which are not currently used in the pet food industry. Canary grass seed (or annual canarygrass; *Phalaris canariensis*) is a grain crop that is produced similarly to oat and wheat and is most commonly used as birdfeed (Putnam et al., 1990). Whole yellow corn (*Zea mays*) is the largest size cereal

grain produced and is the most produced grain worldwide, for both food and non-edible products (Owens et al., 1997). Millet (proso variety; *Panicum miliaceum*) is a group of several small seeded grains. In the U.S., it is most typically used as birdfeed and livestock feed, but millet is consumed by humans in India, Africa, China, Japan, and parts of Europe (Oelke et al., 1990). Whole millet is cleaned and sized with the hull still attached, whereas hulled millet has had the hull removed. Potato flakes were first introduced as a means to increase shelf-life of potatoes. To produce potato flakes, potatoes are first peeled, trimmed, and sliced. Potato slices are then cooked at a low temperature (150-160°C) for 20 min, known as a pre-cook step. Next, they are cooled to halt the cooking process and as a means to decrease the stickiness of the starchy vegetable. The final cooking step occurs in a steam cooker for anywhere from 15-60 min. Finally, the potatoes are dried in a single-drum drier and broken into flakes (Ginsberg, 2007). Quinoa (*Chenopodium quinoa*) is a tiny, round, highly nutritious pseudocereal. Quinoa is often light-colored, but can also be red, purple, or black (Oelke et al., 1992). Sorghum grain (milo; *Sorghum bicolor*) is a hearty grain that can survive under conditions that other grains would not (i.e., drought). Some sorghum varieties contain polyphenols or other pigments/tannins that contain antinutritional factors. In the U.S., it is commonly used for livestock feed, but is a great source of fiber and easily incorporated into human and pet food (Carter et al., 1989; Twomey et al., 2002). Spelt (*Triticum aestivum spelta*) is an ancient subspecies of wheat. Spelt is often used as an alternative feed grain, but can also be used as a food grain once the hulls are removed. Spelt hull pellets are comprised of spelt hulls that have been ground and formed into pellets (Oplinger et al., 1990; Neeson, 2011). Wheat (*Triticum aestivum*, or bread wheat) is the most common grain used in breads, pastas (Durum wheat), and other grain foods. Wheat contains a protein known as gluten, which contributes to the elasticity of bread dough (Shewry et al., 2013).

The samples evaluated in this compositional study include a variety of carbohydrate and fiber sources including: whole grains, processed grains, grain fractions, and pseudocereal grains. A potential limitation of this study was the possibility of RS to be decreased through the methodology performed to obtain the compositional data (i.e., if total starch samples were allowed to cool below 55°C, then reheated to 55°C for 24 h, resulting in altered content of RS). This may make application or formulation using these ingredients difficult because raw ingredients were tested and it has been well documented that RS concentration and the concentrations of other nutrients could be impacted by the processing (e.g., extrusion; retort; baking) of pet foods (Murray et al., 2001; Dust et al., 2004; Hernot et al., 2008). This database of compositional information generated from this study provides a framework for many grains, some of which do not have much data available. In conclusion, the whole grains studied in this compositional analysis vary when compared to their respective fractions (i.e., barley flake vs. malted barley vs. steamed rolled barley) as well as compared to other grains (i.e., barley flake vs. brown rice vs. canary grass seed).

Based on our analyses, we believe the most interesting ingredients for future research include oat fiber, malted barley, rice bran, canary grass seed, and barley flake. We feel that these ingredients are valuable because of their total dietary fiber content, insoluble:soluble fiber ratio, and AA profile. It would be beneficial to further investigate these 5 ingredients for their effects *in vivo*. It is expected that they would have the greatest impact when incorporated into pet foods, given the total dietary fiber, insoluble dietary fiber, and soluble dietary fiber concentrations of these ingredients. Because of the high fiber content of these 5 ingredients, they may beneficially alter indices of gastrointestinal health. Furthermore, they would impact pet food formulations by potentially decreasing the amount of animal proteins needed, which can be costly from a

formulation standpoint. Although grains are often called “fillers” and grain-free diets are increasingly popular with pet owners, our data demonstrate that these ingredients may contribute both as a readily available energy source and source of dietary fiber, RS, EAA, and macrominerals for pet diets. Many of these may be beneficial to incorporate into pet food, but more research is needed to test the efficacy of the grains, mainly RS and bioactive compounds contained therein, once incorporated into an extruded diet or baked treat matrix for pets.

TABLES

Table 3.1 Chemical composition of whole grain, processed grain, and grain coproduct samples

		% DM[1] basis						
					Total dietary fiber			
Item	DM, %	OM	CP	AHF	TDF	IDF	SDF	GE, kcal/g DM
Barley category (Hordeum vulgare)								
Barley flake	91.78	97.80	14.45	8.49	10.78	6.53	4.25	4.81
Cut barley	89.81	98.07	11.59	2.74	15.53	10.72	4.81	4.58
Ground pearled barley	90.49	97.74	11.44	2.97	15.92	11.45	4.47	4.42
Malted barley	95.63	97.27	13.53	3.20	42.14	22.90	19.24	4.83
Pearled barley flakes	90.51	97.97	10.87	2.75	15.86	10.44	5.42	4.57
Steamed rolled barley	90.29	97.43	11.27	3.06	23.14	19.26	3.88	4.52
Whole pearled barley	89.20	97.89	11.33	2.90	13.41	8.62	4.79	4.65
Oat category (Avena sativa)								
Groats	90.52	97.62	12.07	7.44	9.91	7.88	2.03	4.72
Ground steamed groats	91.62	97.99	12.37	8.01	10.94	9.88	1.06	4.81
Instant oats	91.85	97.66	13.36	8.17	10.66	9.67	1.00	4.81
Oat bran #1	91.19	97.42	13.90	8.35	11.01	7.29	3.72	4.87
Oat bran #2	91.85	97.56	13.80	8.39	10.97	8.82	2.14	4.89
Oat fiber	95.91	94.31	1.71	1.60	85.16	80.89	4.27	4.44
Oat flour	91.86	97.97	13.62	8.09	10.42	6.03	4.40	4.82
Oatmeal (ground)	92.21	97.58	13.63	8.82	10.18	7.79	2.39	4.85
Quick oats	92.34	97.05	12.44	7.50	8.80	7.73	1.07	4.76
Regular rolled oats	91.79	97.61	13.05	7.96	7.45	6.34	1.11	4.84

Table 3.1 (cont.)

Item	DM, %	% DM[1] basis						GE, kcal/g DM
					Total dietary fiber			
		OM	CP	AHF	TDF	IDF	SDF	
Steamed rolled oat groats	90.68	97.59	13.80	7.51	6.57	3.35	3.23	4.83
Steel cut groats	90.35	97.57	13.17	7.90	11.11	8.54	2.57	4.91
Rice category (Oryza sativa)								
Brown rice	89.33	97.37	10.30	4.59	9.74	8.83	0.91	4.44
Defatted rice bran	90.59	84.29	17.04	4.40	24.2	22.47	1.72	4.31
Polished rice	88.71	98.70	8.30	1.75	2.16	1.93	0.23	4.38
Rice flour	92.37	96.12	8.86	1.53	8.57	5.58	2.99	4.11
Miscellaneous cereal grains and pseudocereals								
Canary grass seed	93.42	93.82	19.66	7.27	20.75	17.61	3.14	4.50
Conventional hulled millet	89.00	97.89	12.34	5.73	6.96	3.04	3.92	4.77
Conventional quinoa	92.83	97.10	13.62	6.22	19.94	18.89	1.04	4.58
Conventional whole millet	92.93	96.07	10.39	4.95	15.31	13.89	1.43	4.34
Organic spelt hull pellets	89.24	95.44	10.16	3.24	44.00	39.03	4.97	4.53
Potato flake	91.98	95.51	9.68	1.28	7.02	2.82	4.19	4.26
Sorghum	88.58	98.30	10.43	4.30	12.54	9.86	2.68	4.54
Whole wheat	89.27	97.74	12.20	2.63	13.79	13.19	0.61	4.51
Whole yellow corn	88.72	98.59	7.10	5.09	13.49	12.10	1.40	4.63

[1]DM = dry matter; OM = organic matter; CP = crude protein; AHF = acid hydrolyzed fat; TDF = total dietary fiber; IDF = insoluble dietary fiber; SDF = soluble dietary fiber; GE = gross energy.

Table 3.2. Total starch and starch fractions of whole grain, processed grain, and grain coproduct samples

Item	% DM basis					
	FG[1]	TS	TS (w/o FG)	DS	DS (w/o FG)	RS
Barley category (Hordeum vulgare)						
Barley flake	0.08	67.67	67.60	63.90	63.83	3.77
Cut barley	0.13	74.23	74.11	67.01	66.90	7.21
Ground pearled barley	0.09	73.08	73.00	63.70	63.63	9.38
Malted barley	0.09	16.17	16.09	11.41	11.33	4.77
Pearled barley flakes	0.08	73.83	73.76	65.72	65.64	8.12
Steamed rolled barley	0.08	67.65	67.58	61.94	61.87	5.72
Whole pearled barley	0.11	72.30	72.20	64.90	64.80	7.40
Oat category (Avena sativa)						
Groats	0.10	73.43	73.34	69.52	69.43	3.90
Ground steamed groats	0.07	71.90	71.84	67.63	67.57	4.27
Instant oats	0.05	69.47	69.43	64.41	64.37	5.06
Oat bran #1	0.07	65.28	65.21	64.33	64.27	0.94
Oat bran #2	0.06	67.43	67.37	61.66	61.60	5.77
Oat fiber	0.08	8.52	8.45	6.85	6.78	1.67
Oat flour	0.06	69.24	69.18	65.56	65.51	3.67
Oatmeal (ground)	0.07	66.22	66.15	61.81	61.74	4.41
Quick oats	0.06	73.49	73.44	67.15	67.10	6.34
Regular rolled oats	0.05	68.72	68.67	65.04	64.99	3.68
Steamed rolled oat groats	0.06	71.16	71.10	66.08	66.03	5.08
Steel cut groats	0.06	68.35	68.30	68.24	68.18	0.12
Rice category (Oryza sativa)						
Brown rice	0.16	77.38	77.24	66.81	66.67	10.57

Table 3.2 (cont.)

Item	% DM basis					
	FG[1]	TS	TS (w/o FG)	DS	DS (w/o FG)	RS
Defatted rice bran	0.06	34.21	34.16	29.79	29.74	4.42
Polished rice	0.06	87.94	87.89	74.73	74.68	13.21
Rice flour	0.48	73.64	73.21	66.81	66.38	6.30
Miscellaneous cereal grains and pseudocereals						
Canary grass seed	0.17	49.66	49.51	47.28	47.13	2.38
Conventional hulled millet	0.08	73.53	73.45	66.55	66.47	6.98
Conventional quinoa	1.31	55.72	54.54	53.34	52.16	2.39
Conventional whole millet	0.14	64.86	64.74	61.09	60.97	3.77
Organic spelt hull pellets	0.16	42.00	41.85	38.31	38.16	3.69
Potato flake	0.49	73.22	72.78	66.89	66.45	6.34
Sorghum	0.20	70.53	70.35	63.29	63.11	7.24
Whole wheat	0.19	68.72	68.55	62.17	62.00	6.36
Whole yellow corn	0.24	64.99	64.78	65.55	65.33	0.00

[1]FG = free glucose; TS = total starch; DS = digestible starch; RS = resistant starch.

Table 3.3. Essential amino acid (EAA) composition[1] of whole grain, processed grain, and grain coproduct samples

	Essential Amino Acids (% DM basis)										
Item	Arg[2]	His	Ile	Leu	Lys	Met	Phe	Thr	Trp	Val	Total EAA
Barley category (Hordeum vulgare)											
Barley flake	0.94	0.32	0.57	1.11	0.66	0.27	0.77	0.52	0.14	0.76	6.06
Cut barley	0.51	0.23	0.41	0.80	0.42	0.19	0.61	0.39	0.11	0.56	4.23
Ground pearled barley	0.53	0.24	0.42	0.82	0.42	0.20	0.63	0.40	0.11	0.56	4.33
Malted barley	0.01	0.05	0.30	0.67	0.06	0.10	0.49	0.05	0.04	0.47	2.24
Pearled barley flakes	0.55	0.25	0.41	0.80	0.45	0.18	0.62	0.40	0.10	0.56	4.32
Steamed rolled barley	0.49	0.23	0.42	0.80	0.41	0.20	0.61	0.39	0.11	0.55	4.21
Whole pearled parley	0.58	0.25	0.42	0.82	0.48	0.20	0.62	0.42	0.10	0.59	4.48
Oat category (Avena sativa)											
Groats	0.83	0.27	0.49	0.96	0.57	0.23	0.67	0.48	0.14	0.65	5.29
Ground steamed groats	0.79	0.27	0.48	0.93	0.55	0.20	0.65	0.42	0.15	0.64	5.08
Instant oats	0.72	0.24	0.45	0.86	0.50	0.21	0.61	0.40	0.14	0.58	4.71
Oat bran #1	0.74	0.26	0.47	0.90	0.53	0.22	0.63	0.41	0.17	0.61	4.94
Oat bran #2	0.83	0.27	0.48	0.97	0.61	0.21	0.67	0.47	0.16	0.66	5.33
Oat fiber	0.86	0.28	0.49	0.97	0.64	0.22	0.68	0.48	0.17	0.66	5.45
Oat flour	0.07	0.02	0.07	0.15	0.07	0.03	0.08	0.07	< 0.04	0.09	0.65
Oatmeal (ground)	0.81	0.27	0.49	0.96	0.57	0.21	0.67	0.45	0.16	0.65	5.24
Quick oats	0.69	0.24	0.43	0.84	0.51	0.18	0.60	0.39	0.15	0.56	4.59
Regular rolled oats	0.71	0.25	0.45	0.88	0.51	0.22	0.62	0.40	0.16	0.59	4.79
Steamed rolled oat groats	0.76	0.25	0.49	0.95	0.50	0.22	0.67	0.43	0.14	0.63	5.04
Steel cut groats	0.74	0.25	0.45	0.90	0.53	0.19	0.63	0.42	0.15	0.60	4.86
Rice category (Oryza sativa)											
Brown rice	0.79	0.26	0.43	0.84	0.44	0.24	0.54	0.38	0.07	0.60	4.59
Defatted rice bran	1.38	0.47	0.62	1.23	0.88	0.34	0.77	0.66	0.20	0.96	7.51
Polished rice	0.62	0.20	0.36	0.70	0.35	0.20	0.45	0.28	0.08	0.50	3.74

Table 3.3 (cont.)

Item	Essential Amino Acids (% DM basis)										
	Arg[2]	His	Ile	Leu	Lys	Met	Phe	Thr	Trp	Val	Total EAA
Rice flour	0.44	0.15	0.31	0.45	0.49	0.15	0.38	0.30	0.10	0.48	3.25
Miscellaneous cereal grains and pseudocereals											
Canary grass seed	1.05	0.37	0.80	1.45	0.42	0.26	1.18	0.46	0.32	0.86	7.17
Conventional hulled millet	0.40	0.24	0.51	1.49	0.20	0.36	0.69	0.39	0.08	0.61	4.97
Conventional quinoa	0.51	0.17	0.33	0.49	0.51	0.14	0.38	0.32	0.11	0.49	3.45
Conventional whole millet	1.07	0.38	0.54	0.87	0.79	0.26	0.56	0.45	0.13	0.62	5.67
Organic spelt hull pellets	0.40	0.23	0.41	1.23	0.29	0.19	0.52	0.36	0.06	0.52	4.21
Potato flake	0.46	0.24	0.40	0.76	0.36	0.18	0.52	0.35	0.10	0.49	3.86
Sorghum	0.33	0.22	0.44	1.32	0.18	0.27	0.60	0.30	0.08	0.54	4.28
Whole wheat	0.55	0.27	0.44	0.84	0.40	0.20	0.57	0.39	0.18	0.54	4.38
Whole yellow corn	0.36	0.21	0.26	0.78	0.29	0.15	0.35	0.25	0.06	0.35	3.06

[1]AOAC, 2006; method 982.30E.

[2]Arg = arginine; His = histidine; Ile = isoleucine; Leu = leucine; Lys = lysine; Met = methionine; Phe = phenylalanine; Thr = threonine; Trp = tryptophan; Val = valine.

Table 3.4. Nonessential amino acid (NEAA) composition[1] of whole grain, processed grain, and grain coproduct samples

Item	Ala[2]	Asp	Cys	Glu	Gly	Hyl	Hyp	Orn	Pro	Ser	Tau	Tyr	Total NEAA
	Nonessential Amino Acids (% DM basis)												
Barley category (Hordeum vulgare)													
Barley flake	0.69	1.20	0.42	3.01	0.73	0.02	0.03	0.01	0.78	0.66	0.15	0.42	8.12
Cut barley	0.45	0.68	0.23	2.71	0.43	0.03	0.01	0.00	1.24	0.45	0.17	0.26	6.66
Ground pearled Barley	0.43	0.67	0.24	2.85	0.44	0.02	0.01	0.00	1.28	0.46	0.17	0.29	6.86
Malted barley	0.42	0.46	0.03	1.97	0.33	0.05	0.02	0.06	0.86	0.02	0.14	0.24	4.60
Pearled barley flakes	0.45	0.70	0.24	2.70	0.45	0.02	0.01	0.00	1.21	0.46	0.16	0.28	6.68
Steamed rolled barley	0.42	0.64	0.24	2.81	0.43	0.02	0.00	0.00	1.28	0.45	0.17	0.24	6.70
Whole pearled barley	0.48	0.73	0.23	2.68	0.49	0.02	0.01	0.00	1.21	0.47	0.16	0.29	6.77
Oat category (Avena sativa)													
Groats	0.61	1.05	0.42	2.63	0.66	0.02	0.00	0.01	0.67	0.62	0.17	0.41	7.27
Ground steamed groats	0.57	0.95	0.38	2.57	0.63	0.01	0.01	0.01	0.64	0.56	0.17	0.37	6.87
Instant oats	0.51	0.92	0.35	2.47	0.55	0.02	0.00	0.01	0.61	0.57	0.14	0.35	6.50
Oat bran #1	0.53	0.96	0.35	2.56	0.58	0.02	0.02	0.01	0.63	0.58	0.15	0.32	6.71
Oat bran #2	0.60	1.03	0.37	2.60	0.64	0.03	0.01	0.01	0.65	0.61	0.19	0.43	7.17
Oat fiber	0.64	1.06	0.40	2.60	0.70	0.02	0.01	0.01	0.65	0.62	0.19	0.41	7.31
Oat flour	0.10	0.16	0.04	0.29	0.10	0.01	0.02	0.00	0.10	0.08	0.08	0.03	1.01
Oatmeal (ground)	0.58	1.01	0.40	2.69	0.62	0.01	0.01	0.01	0.65	0.61	0.17	0.38	7.14
Quick oats	0.50	0.90	0.36	2.41	0.54	0.01	0.01	0.01	0.60	0.55	0.15	0.29	6.33
Regular rolled oats	0.51	0.92	0.35	2.51	0.56	0.01	0.00	0.01	0.61	0.56	0.19	0.32	6.55
Steamed rolled oat groats	0.56	0.97	0.36	2.83	0.63	0.02	0.00	0.01	0.75	0.60	0.17	0.39	7.29
Steel cut groats	0.55	0.95	0.39	2.52	0.60	0.02	0.01	0.01	0.62	0.58	0.15	0.37	6.77
Rice category (Oryza sativa)													

Table 3.4 (cont.)

Item	Nonessential Amino Acids (% DM basis)												Total NEAA
	Ala[2]	Asp	Cys	Glu	Gly	Hyl	Hyp	Orn	Pro	Ser	Tau	Tyr	
Brown rice	0.58	0.93	0.21	1.75	0.48	0.01	0.01	0.01	0.45	0.48	0.17	0.28	5.36
Defatted rice bran	1.06	1.53	0.33	2.42	0.94	0.04	0.06	0.01	0.76	0.74	0.14	0.44	8.47
Polished rice	0.46	0.76	0.18	1.48	0.38	0.01	0.00	0.01	0.37	0.39	0.12	0.20	4.36
Rice flour	0.32	1.86	0.12	1.67	0.26	0.31	0.02	0.01	0.29	0.29	0.17	0.26	5.58
Miscellaneous cereal grains and pseudocereals													
Canary grass seed	0.82	0.88	0.45	5.07	0.59	0.02	0.01	0.01	1.13	0.72	0.14	0.46	10.30
Conventional hulled millet	1.26	0.74	0.20	2.54	0.33	0.01	0.01	0.00	0.82	0.70	0.16	0.27	7.04
Conventional quinoa	0.30	2.30	0.12	1.61	0.27	0.33	0.02	0.01	0.28	0.30	0.17	0.28	5.99
Conventional whole millet	0.58	1.07	0.20	1.85	0.74	0.02	0.03	0.01	0.48	0.52	0.15	0.34	5.99
Organic spelt hull pellets	0.85	0.71	0.18	1.93	0.37	0.02	0.01	0.00	0.77	0.44	0.17	0.23	5.68
Potato flake	0.41	0.58	0.25	3.00	0.45	0.02	0.02	0.01	1.05	0.46	0.13	0.24	6.62
Sorghum	1.12	0.62	0.15	2.23	0.28	0.01	0.01	0.00	0.73	0.58	0.00	0.26	5.99
Whole wheat	0.45	0.66	0.27	3.50	0.50	0.01	0.01	0.01	1.20	0.57	0.17	0.27	7.62
Whole yellow corn	0.52	0.50	0.16	1.24	0.34	0.02	0.03	0.00	0.62	0.33	0.10	0.17	4.03

[1]AOAC, 2006; method 982.30E.

[2]Ala = alanine; Asp = aspartic acid; Cys = cysteine; Glu = glutamic acid; Gly = glycine; Hyl = hydroxylysine; Hyp = hydroxyproline; Orn = ornithine; Pro = proline; Ser = serine; Tau = taurine; Tyr = tyrosine.

Table 3.5. Mineral composition of whole grain, processed grain, and grain coproduct samples

Item	Calcium[1]	Chloride[2]	Magnesium[1]	Phosphorus[1]	Potassium[3]	Sodium[3]	Sulfur[3]
	---------------------------------- % dry matter basis ------------------------------						
Barley category (Hordeum vulgare)							
Barley flake	0.04	< 0.10	0.11	0.33	0.30	< 0.10	0.10
Cut barley	0.03	< 0.10	0.10	0.27	0.30	< 0.10	< 0.10
Ground pearled barley	0.03	< 0.10	0.10	0.28	0.30	< 0.10	0.10
Malted barley	0.05	< 0.10	0.15	0.40	0.40	< 0.10	0.10
Pearled barley flakes	0.03	< 0.10	0.10	0.30	0.30	< 0.10	0.10
Steamed rolled barley	0.04	< 0.10	0.11	0.31	0.40	< 0.10	0.10
Whole pearled barley	0.03	< 0.10	0.11	0.31	0.30	< 0.10	< 0.10
Oat category (Avena sativa)							
Groats	0.04	< 0.10	0.10	0.33	0.30	< 0.10	0.10
Ground steamed groats	0.03	< 0.10	0.07	0.24	0.30	< 0.10	0.10
Instant oats	0.03	< 0.10	0.09	0.27	0.30	< 0.10	< 0.10
Oat bran #1	0.03	< 0.10	0.10	0.32	0.30	< 0.10	0.10
Oat bran #2	0.04	< 0.10	0.11	0.36	0.40	< 0.10	0.10
Oat fiber	0.08	< 0.10	0.06	0.04	0.50	< 0.10	< 0.10
Oat flour	0.04	< 0.10	0.11	0.32	0.30	< 0.10	0.10
Oatmeal (ground)	0.04	< 0.10	0.11	0.35	0.30	< 0.10	< 0.10
Quick oats	0.03	< 0.10	0.06	0.25	0.30	< 0.10	< 0.10
Regular rolled oats	0.03	< 0.10	0.09	0.27	0.30	< 0.10	< 0.10
Steamed rolled oat groats	0.03	< 0.10	0.08	0.25	0.30	< 0.10	0.10
Steel cut groats	0.03	< 0.10	0.08	0.25	0.30	< 0.10	< 0.10
Rice category (Oryza sativa)							
Brown rice	0.01	< 0.10	0.13	0.37	0.30	< 0.10	< 0.10
Defatted rice bran	2.22	< 0.10	0.88	2.03	1.50	< 0.10	< 0.10

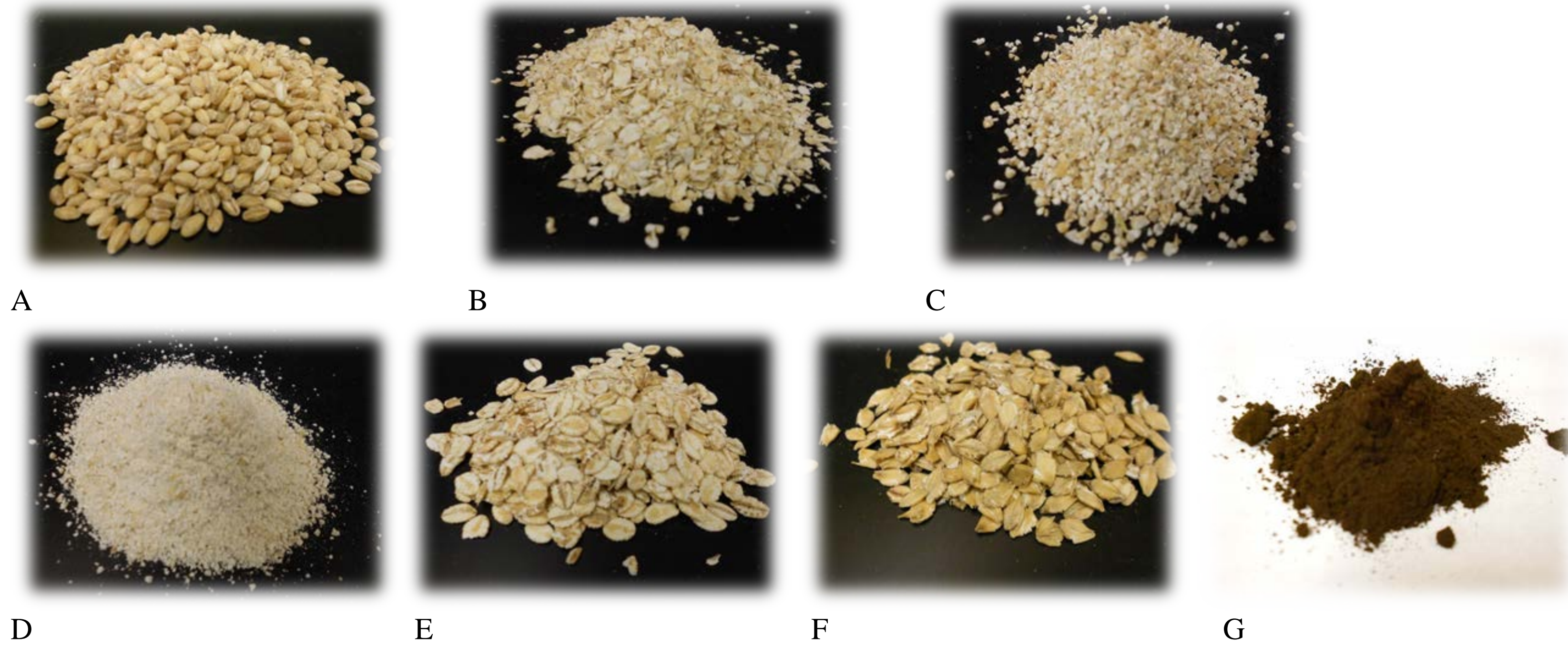
Table 3.5 (cont.)

Item	Calcium[1]	Chloride[2]	Magnesium[1]	Phosphorus[1]	Potassium[3]	Sodium[3]	Sulfur[3]
	---------------------------------- % dry matter basis ------------------------------						
Polished rice	0.00	< 0.10	0.03	0.14	0.10	< 0.10	< 0.10
Rice flour	0.05	< 0.10	0.09	0.23	1.30	< 0.10	< 0.10
Miscellaneous cereal grains and pseudocereals							
Canary grass seed	0.03	< 0.10	0.17	0.49	0.40	< 0.10	0.20
Conventional hulled millet	0.01	< 0.10	0.17	0.37	0.30	< 0.10	0.10
Conventional quinoa	0.05	< 0.10	0.20	0.45	0.80	< 0.10	0.10
Conventional whole millet	0.01	< 0.10	0.12	0.27	0.20	< 0.10	< 0.10
Organic spelt hull pellets	0.07	< 0.10	0.15	0.40	0.40	< 0.10	0.10
Potato flake	0.03	0.20	0.09	0.21	1.70	< 0.10	< 0.10
Sorghum	0.02	< 0.10	0.18	0.36	0.40	< 0.10	0.10
Whole wheat	0.03	< 0.10	0.09	0.30	0.30	< 0.10	< 0.10
Whole yellow corn	0.00	< 0.10	0.09	0.26	0.30	< 0.10	< 0.10

[1]AOAC, 2006; method 985.01.
[2]AOAC, 2006; method 943.01.
[3]AOAC, 2006; method 956.01.

FIGURES

Figure 3.1. Photographs of barley samples before analysis



A = whole pearled barley; B = barley flake; C = cut barley; D = ground pearled barley; E = pearled barley flakes; F = steamed rolled barley; G = malted barley.

Figure 3.2. Photographs of oat samples before analysis

A

B

C

D

E

F

G

H

I

Figure 3.2 (cont.)

J

K

L

A = groats; B = steel cut groats; C = ground steamed groats; D = steamed rolled oat groats; E = instant oats; F = quick oats; G = regular rolled oats; H = oat fiber; I = oat bran #1; J = oat bran #2; K = oat flour; L = oatmeal (ground).

Figure 3.3. Photographs of rice samples before analysis

A B C D

A = brown rice; B = rice (white); C = rice flour; D = rice bran.

Figure 3.4. Photographs of miscellaneous cereals and pseudocereals before analysis

A

B

C

D

E

F

G

H

I

Figure 3.4 (cont.)
A = canary grass seed; B = whole yellow corn; C = quinoa; D = organic spelt hull pellets; E = whole millet; F = hulled millet; G = sorghum; H = whole wheat; I = potato flake.

CHAPTER 4: EFFECTS OF FEEDING POLYDEXTROSE ON FECAL CHARACTERISTICS, MICROBIOTA, AND FERMENTATIVE END-PRODUCTS IN HEALTHY ADULT DOGS

ABSTRACT

Polydextrose is a potential prebiotic, but has not been well tested in dogs. Thus, the objective of the present study was to determine the effects of polydextrose on fecal characteristics, microbial populations, and fermentative end-products in healthy adult dogs. A total of eight hound dogs (3.5 ± 0.5 years; 20 ± 0.5 kg) were randomly allotted to one of four test diets containing the following concentrations of polydextrose: (1) 0% (control); (2) 0.5%; (3) 1.0%; or (4) 1.5%. A Latin square design was used, with each treatment period lasting 14 d (days 0-10 adaptation; days 11-14 fresh and total fecal collection). All dogs were fed to maintain body weight. Data were evaluated for linear and quadratic effects using SAS software. Although apparent total tract DM digestibility was unaffected, total tract crude protein digestibility tended to decrease ($P<0.10$) linearly with increasing dietary polydextrose concentrations. Fresh fecal DM percentage tended to decrease ($P<0.10$) linearly, while fecal scores increased ($P<0.05$; looser stools) with increasing dietary concentrations of polydextrose. Fecal pH decreased ($P<0.05$) linearly with increasing polydextrose. Fecal indole tended to decrease ($P<0.10$) linearly with increasing polydextrose, but other fecal protein catabolites were not changed. Fecal *Clostridium perfringens* linearly decreased ($P<0.05$) with increasing dietary polydextrose concentrations, but *Escherichia coli*, *Lactobacillus* spp., and *Bifidobacterium* spp.

were not affected. Based on the present results, polydextrose appears to act as a highly fermentable fiber, but requires further research to test its potential as a prebiotic in dogs.

INTRODUCTION

A stable and balanced gut microbiota is important for overall gastrointestinal (GI) health (Simpson et al., 2006; Janeczko et al., 2008; Suchodolski et al., 2010). Indices of GI health include fermentative end-product concentrations, fecal scores, and gut microbial populations. The composition and activity of gut microbial populations can be manipulated by one's diet. As defined, prebiotics are non-digestible food ingredients that are 1) resistant to gastric acidity, hydrolysis by mammalian enzymes, and GI absorption; 2) fermented by intestinal microbiota; and 3) able to selectively stimulate the growth and/or activity of those intestinal bacteria that contribute to the host's health and well-being (Gibson and Roberfroid, 1995; Gibson et al., 2004; Roberfroid et al., 2010; Gibson et al., 2010). Currently, there are three established classes of prebiotics (e.g., fructans, galactooligosaccharides, and lactulose), but others may exist.

Prebiotics are widely used in human and pet nutrition products because of their many functional and nutritional properties. Fructans, for example, serve as a highly fermentable substrate in the hindgut, leading to decreased fecal pH, increased fecal SCFA concentrations, and increased *Bifidobacterium* spp. and *Lactobacilli* spp. in healthy adult dogs (Willard et al., 2000; Swanson et al., 2002a; Flickinger et al., 2003; Propst et al., 2003). The majority of prebiotic research in dogs has focused on fructans, galactooligosaccharides, and lactulose. More research, however, is needed to test the potential of other carbohydrate sources with prebiotic potential for use in pet nutrition.

Polydextrose is a polysaccharide synthesized by random polymerization of glucose, sorbitol, and a suitable acid catalyst at a high temperature and partial vacuum. It is composed of many glycosidic bonds, but the 1,6-glycosidic bond is predominant in this polymer (Allingham, 1982). Polydextrose has an average degree of polymerization of 12 and an average molecular weight of 2,000, ranging anywhere from 162 to 20,000 (Craig, 2008). It is a water-soluble, low-calorie bulking agent that is currently used in a variety of foods, including baked goods, functional beverages, and diabetic products (Jie et al., 2000; Probert et al., 2004; Fava et al., 2007; Mäkivuokko et al., 2007). Because polydextrose is a randomly bonded polysaccharide, it is resistant to mammalian enzymes, which allows for most of the substrate to pass through the body unabsorbed (Figdor and Bianchine, 1983). Previous human research has shown polydextrose to be partially fermented in the large intestine, leading to increased fecal bulk, softening of the feces, decreased fecal pH, increased fecal short-chain fatty acid (SCFA) concentrations, increased fecal *Lactobacillus* and *Bifidobacterium*, and decreased fecal *Bacteroides* (Jie et al., 2000). That study, which used traditional culture methods, is the only *in vivo* evidence that polydextrose possesses prebiotic activity in humans. Moreover, the prebiotic potential of polydextrose has not yet been tested for use in pet food.

The purpose of this study was to evaluate the effects of graded concentrations of polydextrose on fecal characteristics, microbial populations, and fermentative end-products in healthy adult dogs. The ultimate aim of this study was to test whether polydextrose has prebiotic potential in dogs when fed at doses that are practical in terms of cost and gastrointestinal tolerance. Increased inclusion of polydextrose was hypothesized to decrease fecal pH, increase fecal SCFA concentrations, and decrease fecal phenol and indole concentrations. Based on human literature, the inclusion of polydextrose was also hypothesized to alter the gut microbial

populations by increasing *Lactobacillus* spp. and *Bifidobacterium* spp. and decreasing *Clostridium perfringens* and *Escherichia coli*.

MATERIALS AND METHODS

Animals and Diets

All animal care and study procedures were approved by the University of Illinois Institutional Animal Care and Use Committee prior to animal experimentation. Eight healthy adult intact female hound-mix dogs (3.5 ± 0.5 yr; 20 ± 0.5 kg) were used. Dogs were housed individually in runs (2.4 x 1.2 m) in a temperature-controlled room (22°C; 23% relative humidity) with a 16-h light: 8-h dark cycle. Dogs were weighed and assessed for body condition score (BCS; 9 point scale) prior to the AM feeding on every Friday of the study.

Four diets were formulated to contain approximately 30% protein and 20% fat, with low-ash poultry by-product meal, brewer's rice, poultry fat, and corn constituting the main ingredients of the dry, extruded kibble diets (Table 1). Diets were formulated to meet or exceed the National Research Council (NRC, 2006) recommended allowances for adult dogs at maintenance. Diets were extruded at Kansas State University's Bioprocessing and Industrial Value-Added Program facility (Manhattan, KS, USA) under the supervision of Pet Food and Ingredient Technology Inc. (Topeka, KS, USA). Each diet contained a specified concentration of polydextrose (Sta-Lite® Polydextrose; Tate and Lyle; Decatur, IL, USA; 0, 0.5, 1.0, or 1.5%), in place of cellulose (Solka-Floc; International Fiber Corporation, North Tonawanda, NY, USA). All polydextrose concentrations were incorporated into the diets before extrusion. All dogs were fed to maintain BW throughout the duration of the study. Fresh water was offered ad libitum.

Sample Collection

A replicated 4 X 4 Latin square design with 14-d periods was conducted. Each period consisted of a diet adaptation phase (d 0-10) and a total and fresh fecal collection phase (d 11-14). Total feces excreted during the collection phase of each period were taken from the pen floor, weighed, and frozen at -20°C until further analyses. All fecal samples during the collection phase were subjected to a consistency score according to the following scale: 1 = hard, dry pellets, and small hard mass; 2 = hard formed, dry stool, and remains firm and soft; 3 = soft, formed and moist stool, and retains shape; 4 = soft, unformed stool, and assumes shape of container; and 5 = watery, liquid that can be poured.

One fresh fecal sample per period was collected within 15 min of defecation on d 1 of the 4-d collection phase. Fresh fecal samples were prepared immediately to minimize loss of volatile components. Samples were weighed and pH determined using a Denver Instrument AP10 pH meter (Denver Instrument, Bohemia, NY, USA) equipped with a Beckman electrode (Beckman Instruments, Inc., Fullerton, CA, USA). Fresh fecal dry matter (DM) was determined. Aliquots for analysis of phenols and indoles were frozen at -20°C immediately after collection. An aliquot (2 g) of feces was mixed with 5 mL 2N hydrochloric acid (HCl) for ammonia, SCFA and branched-chain fatty acid (BCFA) determination and stored at -20°C until analyzed. Aliquots of fresh feces were transferred to sterile cryogenic vials (Nalgene, Rochester, NY, USA) and frozen at -80°C until DNA extraction for microbial analysis.

Chemical Analyses

Diet samples were subsampled and ground through a 2-mm screen in a Wiley Mill (model 4, Thomas Scientific, Swedesboro, NJ, USA). Composited fecal samples (1 per dog per period) were dried at 55°C for 1 wk and ground through a 2-mm screen in a Wiley Mill. Diet and fecal samples were analyzed according to procedures by the Association of Official

Analytical Chemists (AOAC) for DM (105°C), organic matter (OM) and ash (AOAC, 2006). Diet and fecal crude protein (CP) was calculated from Leco total N values (model FP-2000, Leco Corporation, St. Joseph, MI, USA; AOAC, 2006). Total lipid content (acid hydrolyzed fat; AHF) of the diets and feces was determined according to the methods of the American Association of Cereal Chemists (1983) and Budde (1952). Gross energy (GE) of diet and fecal samples was measured using an oxygen bomb calorimeter (model 1261, Parr Instruments, Moline, IL, USA). Dietary fiber concentrations [total dietary fiber (TDF)] were determined according to Prosky et al. (1985). All diet and fecal samples were analyzed in duplicate, with a 5% error allowed between duplicates; otherwise, the analyses were repeated.

Short-chain fatty acid and BCFA concentrations were determined by gas chromatography according to Erwin et al. (1961) using Hewlett-Packard 5890A series II gas chromatograph (Palo Alto, CA, USA) and a glass column (180 cm x 4 mm i.d.) packed with 10% SP™-1200/1% H_3PO_4 on 80/100+ mesh Chromosorb WAW (Supelco Inc., Bellefonte, PA, USA). Phenol and indole concentrations were determined using gas chromatography according to the methods of Flickinger et al. (2003). Ammonia concentrations were determined according to the method of Chaney and Marbach (1962).

Microbial Analyses

Fecal microbial populations were analyzed using methods described by Middelbos et al. (2007b) with minor adaptations. Briefly, fecal DNA was extracted from freshly collected samples that had been stored at -80°C until analysis, using the repeated bead beater method described by Yu and Morrison (2004) with a DNA extraction kit (QIAamp DNA Stool Mini Kit, Qiagen, Valencia, CA, USA) according to the manufacturer's instructions. Extracted DNA was quantified using a spectrophotometer (NanoDrop ND-1000, Nano-Drop Technologies,

Wilmington, DE, USA). Quantitative PCR (qPCR) was performed using specific primers for *Bifidobacterium* spp. (Matsuki et al., 2002), *Lactobacillus* spp. (Collier et al., 2003), *E. coli* (Malinen et al., 2003), and *C. perfringens* (Wang et al., 1994). While *Bifidobacterium* and *Lactobacillus* are generally considered to be "beneficial" microbes, *E. coli* and *C. perfringens* represent potential pathogens, and are commonly measured in prebiotic studies. Amplification was performed according to DePlancke et al. (2002). Briefly, a 10-µL final volume contained 5 µL of 2 x SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA, USA), 15 pmol of the forward and reverse primers for the bacterium of interest, and 10 ng of extracted fecal DNA. Standard curves were obtained by harvesting pure cultures of the bacterium of interest in the log growth phase in triplicate, followed by serial dilution. Bacterial DNA was extracted from each dilution using a DNA extraction kit (Qiagen) and amplified with the fecal DNA to create triplicate standard curves (ABI PRISM 7900HT Sequence Detection System, Applied Biosystems, Foster City, CA, USA). Colony-forming units in each dilution were determined by plating on specific agars; lactobacilli MRS (Difco, BD, Franklin Lackes, NJ, USA) for *Lactobacillus*, reinforced clostridial medium (*Bifidobacterium*, *C. perfringens*), and Luria Bertani medium (*E. coli*). The calculated log cfu per ml of each serial dilution was plotted against the cycle threshold to create a linear equation to calculate cfu per gram of dry feces. Although the standard curves are meant to represent a group of bacteria, our qPCR assays were based on a single bacterial strain within each group. Because operon copy number is different among strains, a potential bias in our assay exists. Because our design used each dog as its own control, however, dietary effects that are truly occurring should be identified using these assays.

Calculations and Statistical Analysis

Apparent total tract apparent macronutrient digestibility values were calculated using the following equation: nutrient intake (g DM/d) - nutrient output (g DM/d)/ nutrient intake (g DM/d) x 100. Data were analyzed using the MIXED procedure of SAS (version 9.2, SAS Institute Inc., Cary, NC, USA). Fecal score data were compared using the GLIMMIX procedure of SAS. The statistical model included period and dog as random effects, whereas treatment was a fixed effect. Data were analyzed using the type 3 test of the MIXED procedure. All treatment least squares means were compared using preplanned contrasts that tested for linear and quadratic effects of polydextrose supplementation. Means were separated using a protected least squares difference with a Tukey adjustment. Outlier data were removed from analysis after analyzing data using the UNIVARIATE procedure to produce a normal probability plot based on residual data and visual inspection of the raw data. Outlier data were defined as data points 3 or more standard deviations from the mean. A probability of $P < 0.05$ was accepted as being statistically significant and $P \leq 0.10$ accepted as trends.

RESULTS

Dietary ingredient and chemical composition data are presented in Table 4.1. Dry matter, OM, CP, AHF, and GE concentrations were consistent among diets. Total dietary fiber content decreased with increasing polydextrose concentrations because polydextrose is soluble and not detected by the TDF assay.

Nutrient intakes, apparent total tract macronutrient digestibilities, and fecal characteristics are presented in Table 4.2. Food refusals were minimal. Dogs consumed 0.0, 1.3, 2.7, and 3.9 grams of polydextrose per day for the 0.0, 0.5, 1.0, and 1.5% polydextrose treatments, respectively. All data are reflective of the 4-d collection period. Polydextrose did

not alter food intake, fecal output, or apparent total tract DM and OM digestibility. Apparent total tract CP digestibility, however, tended to decrease ($P<0.10$) linearly with increasing dietary polydextrose concentrations. There was a trend for a linear decrease ($P<0.10$) in fresh fecal DM % and increased ($P<0.05$) fecal scores (looser stools) with increasing dietary concentrations of polydextrose. However, no diarrhea was observed.

Fecal pH, ammonia, SCFA, BCFA, phenol, and indole concentrations are presented in Table 4.3. Fecal pH decreased ($P<0.05$) linearly with increasing polydextrose. Fecal acetate, propionate, and total SCFA concentrations increased ($P<0.05$) linearly with increasing dietary polydextrose. Polydextrose had a curvilinear effect on fecal indole concentrations, in which concentrations tended to decrease ($P<0.10$) linearly with increasing polydextrose. Polydextrose had a quadratic effect ($P=0.05$) on fecal isobutyrate concentrations, in which concentrations increased with 0.5% and 1.0%, but decreased with 1.5% in comparison to the control. However, other fecal protein catabolites were not changed.

Fecal microbial concentrations are presented in Table 4.4. Fecal *C. perfringens* decreased ($P<0.05$) linearly with increasing dietary polydextrose concentrations, but *E. coli*, *Lactobacillus* spp., and *Bifidobacterium* spp. were not affected by the inclusion of polydextrose in the diet.

DISCUSSION

The consumption of prebiotics has been shown to improve gastrointestinal health by selectively stimulating the growth and activity of “beneficial” intestinal bacteria, decreasing fecal pH, increasing fecal bulk, and relieving constipation (Mussato and Mancilha, 2006; Roberfroid, 2007). Previously, polydextrose has been studied *in vitro*, in humans, and in dogs used as a

model for humans and shown to possess prebiotic properties. Probert et al. (2004) evaluated the prebiotic potential of adding polydextrose at 1% (wt/vol) and 2% (wt/vol) of an anaerobic medium using human fecal inoculum. Six substrates were tested in that study including: (1) polydextrose (Litesse Ultra, a high grade form of polydextrose), (2) lactitol monohydrate, (3) a lactitol monohydrate:polydextrose (50:50) blend, (4) short-chain fructooligosaccharides, (5) polydextrose (using a pooled inoculum), and (6) oligofructose (using a pooled inoculum) for a period of 48 hr. For test substrates 1-4, the same healthy human donated a sample for inoculation; test substrates 5 and 6 were inoculated using a pooled sample from four healthy human donors. Those researchers concluded that SCFA concentrations, namely acetate and butyrate, were numerically increased with the addition of polydextrose. Those researchers also concluded that the addition of polydextrose led to increased (P=0.01) bifidobacteria during all four fermentations. In another *in vitro* study, Mäkivuokko et al. (2007) examined the effects of adding polydextrose to dark chocolate using two *in vitro* simulation techniques, including gastric and small intestinal digestion simulation, adapted from Fuller (1991), and an automated four-stage colon simulator (Mäkivuokko et al., 2005). Those researchers concluded that SCFA production was highest for acetate, butyrate, propionate, and total SCFA in each vessel of the cocoa mass + 2% polydextrose vs. baseline and no added polydextrose. The *in vitro* studies displayed the prebiotic potential of polydextrose, with increased SCFA concentrations and increased numbers of beneficial bacteria (Probert et al., 2004; Mäkivuokko et al., 2007).

Jie et al. (2000) evaluated the effects of feeding 0, 4, 8, or 12 g polydextrose/d to healthy adult humans. That study consisted of a 28-d feeding phase and fresh fecal collection (within 1 h of defecation) on d 1 and d 28. Those researchers concluded that dietary intake of polydextrose increased (P<0.01) the ease of defecation (scale of -3 to 3; 0 g polydextrose/d: -0.21 to 0.41; 4 g

polydextrose/d: -0.18 to 1.36; 8 g polydextrose/d: 0.20 to 1.88; 12 g polydextrose/d: -0.14 to 2.35) and fecal output (as is g/d; 0 g polydextrose/d: 103 to 106; 4 g polydextrose/d: 106 to 115; 8 g polydextrose/d: 101 to 128; and 12 g polydextrose/d: 98 to 142), likely due to its water-holding capacity. Subjects also reported softer stools and improved ease of defecation after a couple days of ingestion. Researchers reported that fecal pH was decreased ($P<0.05$) in subjects consuming 8 or 12 g polydextrose/d (6.71 and 6.37, respectively) after 28 d of supplementation vs. control subjects (7.04). Also, in subjects consuming 8 or 12 g polydextrose/d, fecal butyrate (8 g/d: 1.31 mg/g; 12 g/d: 1.41 mg/g) and acetate (8 g/d: 4.70 mg/g; 12g/d: 5.12 mg/g) were increased ($P<0.05$) after 28 d of supplementation vs. the control subjects (0.94 mg/g and 4.12 mg/g, respectively). That human study displayed the prebiotic potential of polydextrose, with decreased fecal pH, increased SCFA concentrations, decreased concentrations of carcinogenic metabolites, and increased numbers of beneficial bacteria in feces following consumption (Jie et al., 2000).

Its prebiotic potential has not been well studied in pets, but Knapp et al. (2008) used the dog as a model for humans to test tolerance and glycemic/insulinemic responses of polydextrose. In that study, dogs were fed extruded diets containing 0, 14, or 28 g polydextrose/1000 kcal ME. Those diets were formulated so that dogs consumed test carbohydrates at 0%, 100%, or 200% the adequate intake (AI) of dietary fiber for humans (IMNA, 2005). Dogs consumed between 19 and 39 g polydextrose/d. Those researchers reported that a portion of the non-digested polydextrose was highly fermentable and that fecal scores (scale of 1 to 5, where 1 = dry, hard pellets and 5 = watery liquid that can be poured) increased as intake of polydextrose increased (control: 2.9; 100% AI: 4.2; 200% AI: 4.6). Because the dogs in that study were fed to imitate what adult humans should consume, it is not surprising that the high dosage of polydextrose led

to increased fecal score. The results of the Knapp et al. (2008) study were used to establish the polydextrose inclusion levels in the current study that were expected to avoid tolerance problems, yet were practical from a commercial stand point. In the current study, the dogs were consuming about 3.5 g polydextrose/d for the diet with the highest concentration of polydextrose (1.5%). To maintain a desirable fecal score quality, our results suggest that polydextrose not exceed 1.5% in canine diets.

The results of the current study demonstrate that polydextrose beneficially alters fecal pH and fermentative end-products, with little effect on food intake, nutrient digestibility, and fecal microbiota, at dietary concentrations up to 1.5%. Apparent total tract macronutrient digestibility values for all diets were consistent with what is expected with extruded diets in which high-quality ingredients are used. Apparent total tract CP digestibility tended to decrease as the concentration of polydextrose increased in the diet. This response is common in diets containing fermentable fibers and was likely due to the increased fermentable substrate and formation of bacterial biomass as compared to the control diet (0% polydextrose), which has been observed previously (Middelbos et al., 2007a). In addition to the potential for decreased CP digestibility, increasing the concentration of dietary fiber may lead to a decrease in fecal quality (i.e, looser stools); high fiber inclusion can have a laxative effect and cause cramping, bloating, and flatulence. The fermentable nature of polydextrose is evident in the current dog study due to the decreased fecal pH, increased fecal SCFA concentrations, and decreased fecal protein catabolites that were observed. The dogs in the current study, however, did not have changes in *Bifidobacterium* spp. or *Lactobacillus* spp. Further research is needed to determine which bacterial groups in the intestinal tract of dogs are capable of fermenting polydextrose, causing the increased fecal SCFA concentrations and decreased fecal pH observed in this study.

In conclusion, the results of the current study demonstrate the beneficial fermentable properties of polydextrose. In this study, polydextrose appeared to be fermentable, which is evident by the increased concentrations of fecal SCFA, primarily acetate and propionate, and by the decrease in fecal pH, without affecting food intake or fecal output. The inclusion of polydextrose also decreased some protein catabolites, in particular fecal indole concentrations. Fecal *C. perfringens* concentrations were decreased by including polydextrose in the diet, but other bacteria measured were unaffected. While many beneficial effects were observed by the inclusion of polydextrose, based on the current and previous dog studies, we would recommend feeding 1.5% polydextrose or less to adult dogs to avoid any adverse effects. For example, fecal scores were increased (softer stools) when dietary polydextrose was included at 1.5%. Polydextrose appears to act as a highly fermentable fiber, providing benefits through fermentation and laxation, but requires further research to test its potential as a prebiotic in dogs.

TABLES

Table 4.1. Ingredient and chemical composition of canine diets containing varying levels of polydextrose

	Diet, % Polydextrose			
Ingredient	0.0	0.5	1.0	1.5
Poultry by-product meal, low ash	39.00	39.00	39.00	39.00
Brewer's rice	32.09	32.09	32.09	32.09
Poultry fat	12.00	12.00	12.00	12.00
Corn (yellow, ground)	11.00	11.00	11.00	11.00
Solka-Floc	4.00	3.50	3.00	2.50
Salt	0.65	0.65	0.65	0.65
Potassium chloride	0.90	0.90	0.90	0.90
Vitamin mix[1]	0.18	0.18	0.18	0.18
Mineral mix[2]	0.18	0.18	0.18	0.18
Polydextrose	0.00	0.50	1.00	1.50
Chemical Composition				
Dry matter (DM), %	92.45	92.26	92.16	91.73
	---------------------------% DM basis-----------------------			
Organic matter	93.01	93.08	93.17	93.13
Crude protein (CP)	35.19	34.61	34.50	34.78
Acid hydrolyzed fat	20.23	20.09	19.96	20.64
Total dietary fiber	8.55	7.16	6.69	6.17
Gross energy (GE), kcal/g	5.39	5.39	5.40	5.41
ME_{AAFCO}[3], kcal/g	3.97	4.01	4.02	4.08
ME_C[4], kcal/g	4.31	4.34	4.31	4.36

[1]Provided per kilogram of diet: vitamin A, 5.28 mg; vitamin D_3, 0.04 mg; vitamin E, 120 mg; vitamin K, 0.88 mg; thiamine, 4.40 mg; riboflavin, 5.72 mg; pantothenic acid, 22.00 mg; niacin, 39.60 mg; pyridoxine, 3.52 mg; biotin, 0.13 mg; folic acid, 0.44 mg; vitamin B_{12}, 0.11 mg.
[2]Provided per kilogram of diet: Mn (as $MnSO_4$), 66.00 mg; Fe (as $FeSO_4$), 120 mg; Cu (as $CuSO_4$), 18 mg; Co (as $CoSO_4$), 1.20 mg; Zn (as $ZnSO_4$), 240 mg; I (as KI), 1.8 mg; Se (as Na_2SeO_3), 0.24 mg.
[3]Metabolizable Energy $(ME)_{AAFCO}$ = 8.5 kcal ME/g fat + 3.5 kcal ME/g CP + 3.5 kcal ME/g nitrogen-free extract.
[4]ME_C = [GE intake (kcal/d) - fecal GE (kcal/d) - [(CP intake/100) - (fecal CP/100)]*1.25]/ DM intake (g/d).

Table 4.2. Mean food intake, fecal characteristics, and apparent total tract macronutrient digestibility of adult dogs fed diets containing polydextrose

	Diet, % Polydextrose					P value	
Item	0.0	0.5	1.0	1.5	SEM	Linear	Quadratic
Food intake							
g dry matter (DM)/d	259.4	266.5	271.3	261.6	12.00	0.81	0.44
g organic matter (OM)/d	241.3	248.1	252.8	243.7	11.17	0.79	0.43
g crude protein (CP)/d	91.3	92.2	93.6	91.0	4.20	0.98	0.64
g acid hydrolyzed fat (AHF)/d	52.5	53.5	54.2	54.0	2.44	0.60	0.79
kcal/d	1397.9	1437.2	1464.4	1416.6	64.78	0.75	0.46
Fecal output, g/d (as is)	126.3	133.7	144.3	137.6	8.55	0.24	0.39
Fecal output, g/d (DMB)	45.7	45.1	48.9	44.9	2.53	0.90	0.49
Fecal output (as is)/food intake (DMB)	0.48	0.50	0.53	0.52	0.018	0.12	0.51
Digestibility							
DM, %	82.4	83.0	82.0	82.9	0.61	0.78	0.81
OM, %	85.6	86.1	85.4	86.1	0.50	0.73	0.81
CP, %	84.8	84.8	83.0	83.8	0.61	0.06	0.50
AHF, %	95.2	95.3	94.8	95.1	0.19	0.25	0.63
Energy, %	86.9	87.3	86.5	87.2	0.44	0.94	0.75
Fecal Scores[1]	3.11	3.22	3.35	3.40	0.111	<0.01	0.66
Fecal DM %	34.7	34.8	34.0	32.2	1.70	0.10	0.38

[1]Fecal score scale: 1 = hard, dry pellets; 2 = dry, well-formed stool; 3 = soft, moist, formed stool; 4 = soft, unformed stool; 5 = watery, liquid that can be poured.

Table 4.3. Mean fecal pH, ammonia, short-chain fatty acid (SCFA), branched-chain fatty acid (BCFA), phenol, and indole concentrations of adult dogs fed diets containing polydextrose

	Diet, % Polydextrose					P value	
Item	0.0	0.5	1.0	1.5	SEM	Linear	Quadratic
Fecal pH	6.7	6.6	6.3	6.3	0.11	<0.01	0.96
Ammonia, µmol/g DM	904.1	1294.1	1110.9	1283.8	694.32	0.32	0.61
Short-chain fatty acids, µmol/g DM							
Acetate	279.5	283.8	330.0	345.9	22.86	0.01	0.77
Propionate	93.8	97.9	113.1	118.0	8.30	0.01	0.95
Butyrate	50.0	46.9	58.8	52.4	6.39	0.49	0.79
Total SCFA	423.3	428.6	501.9	516.3	36.23	0.02	0.89
Branched-chain fatty acids, µmol/g DM							
Valerate	0.8	0.6	0.8	0.8	0.08	0.89	0.26
Isovalerate	16.7	16.8	18.8	17.3	1.77	0.51	0.52
Isobutyrate	12.1	14.2	14.0	11.7	1.57	0.78	0.05
Total BCFA	29.6	31.6	33.5	29.6	3.14	0.85	0.19
Phenols and indoles, µmol/g DM							
Phenol	1.6	1.5	1.7	1.2	0.46	0.42	0.54
Indole	3.2	2.9	2.2	2.7	0.36	0.06	0.10
Total phenols and indoles	4.8	4.3	3.9	3.9	0.66	0.14	0.59

Table 4.4. Mean fecal microbial populations of adult dogs fed diets containing polydextrose

	Diet, % Polydextrose					P value	
Item	0.0	0.5	1.0	1.5	SEM	Linear	Quadratic
	CFU[1], log 10/g fecal DM						
Escherichia coli	11.7	11.2	11.9	11.6	0.21	0.56	0.60
Lactobacillus spp.	11.5	11.4	11.5	11.3	0.14	0.24	0.48
Bifidobacterium spp.	7.7	7.7	7.9	7.6	0.21	0.81	0.53
Clostridium perfringens	11.4	11.1	11.0	10.6	0.30	0.02	0.91

[1]CFU = colony-forming units.

CHAPTER 5: EFFECTS OF FEEDING A WHOLE GRAIN-CONTAINING DOG TREAT ON APPARENT TOTAL TRACT MACRONUTRIENT DIGESTIBILITY, FECAL CHARACTERISTICS, AND FECAL FERMENTATIVE END-PRODUCTS IN HEALTHY ADULT DOGS

ABSTRACT

The benefits of whole grain consumption have been studied in humans, but little research exists on their effects in dogs. The objective of this study was to test the effects of RS in the diet of healthy adult dogs. Twelve adult Miniature Schnauzer dogs (8 males, 4 females; mean age: 3.3 ± 1.6 yr; mean BW: 8.4 ± 1.2 kg; mean BCS: D/ideal) were randomly allotted to one of 3 treatment groups, which consisted of different amounts of RS supplied in a biscuit format. Dogs received either 0, 10, or 20 g biscuits/d (estimated to be 0, 2.5, or 5 g RS/d) that were fed within their daily caloric allowance. A replicated Latin square design was used, with each treatment period lasting 21 days (d 0-17 adaptation; d 18-21 fresh and total fecal collection). All dogs were fed the same diet to maintain BW throughout the study. Dogs fed 5 g RS/d had lower (P = 0.03) fat digestibility than dogs fed 0 g RS/d, but dry matter, organic matter, and crude protein digestibilities were not affected. Fecal fermentative end-products, including short- and branched-chain fatty acids, ammonia, phenols, and indoles, and microbial populations were not affected. The minor changes observed in this study suggest the RS doses provided to the dogs were too low. Further work is required to assess the dose of RS required to affect gut health.

INTRODUCTION

A healthy gastrointestinal (GI) tract is important for the overall health of dogs. It is well known that diet can alter GI health. The benefits of consuming whole grains have been greatly studied in humans (Anderson, 2003; Slavin, 2004; Okarter and Liu, 2010), but little research exists on their effects in dogs. Previous research has demonstrated the benefits of feeding dietary fiber and prebiotics to dogs (Vickers et al., 2001; Swanson et al., 2002a; 2002b; Flickinger et al., 2003). Similarly, resistant starch (RS) has been shown to be highly fermentable and can modulate the gut microbial composition and fecal short-chain fatty acid concentrations (Haenen et al., 2013). Feeding whole grains, which contain RS when minimally processed (Dust et al., 2004; Hernot et al., 2008), may have similar beneficial effects on the canine gut.

Because extrusion conditions can alter the chemical composition of ingredients (Dust et al., 2004), the use of a treat matrix produced by baking may be a more effective way to deliver the RS portion of whole grains to the colon of a dog. A treat matrix will allow for more whole grains to be included, without the need for fine grinding and complete gelatinization that occurs with extrusion. The objective of this study was to test the effects of RS in healthy adult dogs by feeding a biscuit treat, containing whole brown rice, whole wheat, and whole grain oatmeal. The aim of this trial was to determine a RS dose (0, 2.5, or 5 g/RS per day) that manipulates fecal bifidobacteria, lactobacilli, and fermentative end-products, but that does not negatively affect stool quality in healthy dogs fed treats.

MATERIALS AND METHODS

Animals and diets

The study was performed at the WALTHAM® Centre for Pet Nutrition and all animal care and study procedures were approved by the Animal Welfare and Ethics Review Body. A

total of twelve healthy adult Miniature Schnauzer dogs (8 males; 4 females; mean age: 3.3 ± 1.6 yr; mean BW: 8.4 ± 1.2 kg; mean BCS: D/ideal) were included in the study. A replicated 3 x 3 Latin square design with 21d trial periods was conducted. Each period consisted of a diet adaptation phase (d 0-17) and a total and fresh (within 15 min of defecation) fecal collection phase (d 18-21). During the adaptation phase, dogs were pair-housed overnight, with free access to indoor and outdoor paddock areas, where they were also group-housed during the day. During the collection phase, dogs were individually housed overnight and group-housed during the day.

All dogs were fed a complete and balanced canned diet twice daily (08:30 and 15:00) to maintain BW throughout the study. The diet consisted of 92.4 g protein/1000 kcal metabolizable energy and 65.0 g fat/1000 kcal metabolizable energy. Intake for each dog was recorded at each meal. Prior to receiving their meals, dogs were given 0, 10 or 20 g of biscuit treats per day (split into 2 doses) that provided approximately 0, 2.5 or 5 g of RS/d from the biscuits. The biscuits consisted of 43.2 g protein/1000 kcal metabolizable energy and 33.5 g fat/1000 kcal metabolizable energy. Dogs were weighed and assessed for body condition score (German et al., 2006) weekly. The chemical composition of the maintenance diet and biscuits can be found in Table 5.1.

Sample collection

During the 4-d collection phase, all fecal output was collected, weighed, scored, and frozen at -20ºC for further analysis; one fresh fecal sample from each dog per period was also collected. Fecal quality was assessed on each sample according to visual appearance as described by Rolfe et al. (2002) with grade 1 representing 'hard, dry, crumbly feces' and grade 5

‘watery diarrhea’. To account for all the discernible points in between, half-scores are used, giving a total of 9 possible categories, with a score of 1.5-2.5 considered ideal.

The fresh fecal sample was collected within 15 min of defecation on d 1 of the 4-d collection phase and prepared immediately to minimize loss of volatile components. Samples were weighed and pH measured using a Mettler Toledo FG2/EL2 pH meter equipped with a Mettler Toledo LE 438 probe (Mettler Toledo, Switzerland) after mixing 2 g of fresh feces into 18 mL of sterile water. Fresh fecal dry matter (DM) was determined (105°C). Aliquots of feces for analysis of phenols and indoles were frozen at -20°C immediately after collection. An aliquot (5 g) of feces was mixed with 5 mL 2N hydrochloric acid (HCl) for ammonia, short-chain fatty acid (SCFA) and branched-chain fatty acid (BCFA) determination and stored at -20ºC until analyzed. Aliquots of fresh feces were transferred to sterile cryogenic vials (Nalgene, Rochester, NY, USA) and frozen at -80°C until DNA extraction for microbial analysis.

Chemical analyses

Dried diet and fecal samples were ground through a 2-mm screen in a Wiley mill (intermediate, Thomas Scientific, Swedesboro, NJ, USA). Samples were analyzed according to procedures by the Association of Official Analytical Chemists (AOAC) for DM (105°C), organic matter (OM), and ash (AOAC, 2006). Crude protein (CP) content was calculated from Leco total N values (TruMac® N, Leco Corporation, St. Joseph, MI, USA; AOAC, 2006). Total lipid content (acid hydrolyzed fat) of the samples was determined according to the methods of the American Association of Cereal Chemists (AACC; 1983) and Budde (1952). Gross energy (GE) of the samples was measured using an oxygen bomb calorimeter (model 1261, Parr Instruments, Moline, IL). Dietary fiber concentrations [total dietary fiber (TDF), soluble dietary fiber (SDF),

and insoluble dietary fiber (IDF)] were determined according to Prosky et al. (1992). The diet, treat, and all fecal samples were analyzed in duplicate, with a 5% error allowed between duplicates; otherwise, the analyses were repeated.

Short-chain fatty acid and BCFA concentrations were determined by gas chromatography according to Erwin et al. (1961) using a Hewlett-Packard 5890A series II gas chromatograph (Palo Alto, CA, USA) and a glass column (180 cm x 4 mm i.d.) packed with 10% SP^{TM}-1200/1% H_3PO_4 on 80/100+ mesh Chromosorb WAW (Supelco Inc., Bellefonte, PA, USA). Phenol and indole concentrations were determined using gas chromatography according to the methods of Flickinger et al. (2003). Ammonia concentrations were determined according to the method of Chaney and Marbach (1962).

Starch

Biscuit and diet subsamples were ground through a 0.5-mm screen in a Wiley Mill (intermediate, Thomas Scientific, Swedesboro, NJ, USA). The method of Muir and O'Dea (1992; 1993) was used to determine the amount of starch digested in the stomach and small intestine by measuring glucose in the supernatant resulting from acid-enzyme digestion of the substrate. Briefly, 0.2 g of each substrate was weighed in duplicate and exposed to pepsin/HCl, amyloglucosidase, and α-amylase digestion. Tubes containing reagents but no substrate were run as blanks. All tubes were incubated for 15 h at 37 °C and then centrifuged for 15 min. Glucose concentrations in the supernatant were determined by reading the absorbance of individual samples at 450 nm on a DU 640 spectrophotometer (Beckman Instruments, Schaumburg, IL, USA) and comparing those values against a glucose standard curve. Digestible starch (DS) was determined by subtracting [free glucose (FG) x 0.9] from [total glucose/original

sample weight] present in the supernatant after 15 h of digestion. Because the measurement of glucose was used to determine starch content, a correction factor of 0.9 for the difference in weight between a FG unit and a glucose residue in starch was used. Total starch (TS) content of samples was determined using the method of Thivend et al. (1972) with amyloglucosidase. Resistant starch was calculated by subtracting [DS + (FG x 0.9)] from TS. The released glucose value corresponds to the amount of glucose resulting from hydrolytic starch digestion that is available for absorption *in vivo*. Compositional data were not analyzed using statistical methods because accuracy was ensured by adequate replication, with acceptance of mean values that were within 5% of each other.

Microbial analyses

Fecal DNA was extracted from freshly collected samples that had been stored at -80°C until analysis, using the PowerLyzer™ PowerSoil® DNA Isolation Kit (MO BIO Laboratories, Inc., Carlsbad, CA, USA) according to the manufacturer's instructions. Extracted DNA was quantified using a Qubit® 2.0 Fluorometer (Life Technologies™, Invitrogen™, Grand Island, NY, USA). Quantitative PCR (qPCR) was performed using specific primers for *Bifidobacterium* spp. (Matsuki et al., 2002), *Lactobacillus* spp. (Collier et al., 2003), *E. coli* (Malinen et al., 2003), and *C. perfringens* (Wang et al., 1994). While *Bifidobacterium* and *Lactobacillus* are generally considered to be "beneficial" microbes, *E. coli* and *C. perfringens* represent potential pathogens, and are commonly measured in prebiotic studies, thus, were also analyzed for this study. Amplification was performed according to DePlancke et al. (2002). Briefly, a 10-µL final volume contained 5 µL of 2 x SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA, USA), 0.05 µL of bovine serum albumin, 15 pmol of the forward and reverse primers for the

bacterium of interest, and 10 ng of extracted fecal DNA. Standard curves were obtained by harvesting pure cultures of the bacterium of interest in the log growth phase in triplicate, followed by serial dilution. Bacterial DNA was extracted from each dilution using a DNA extraction kit (Qiagen) and amplified with the fecal DNA to create triplicate standard curves (ABI PRISM 7900HT Sequence Detection System, Applied Biosystems, Foster City, CA, USA). Colony-forming units in each dilution were determined by plating on specific agars; lactobacilli MRS (Difco, BD, Franklin Lackes, NJ, USA) for *Lactobacillus*, reinforced clostridial medium (*Bifidobacterium*, *C. perfringens*), and Luria Bertani medium (*E. coli*). The calculated log colony-forming units (CFU) per ml of each serial dilution were plotted against the cycle threshold to create a linear equation to calculate CFU per gram of dry feces. Although the standard curves are meant to represent a group of bacteria, our qPCR assays were based on a single bacterial strain within each group. As operon copy number is different among strains, a potential bias does exist in our assay, to minimize this each dog acted as its own control.

Calculations and statistical analysis

Apparent total tract apparent macronutrient digestibility values were calculated using the following equation: nutrient intake (g DM/d) - nutrient output (g DM/d)/ nutrient intake (g DM/d) x 100. Data were analyzed using the MIXED procedure of SAS (version 9.3, SAS Institute Inc., Cary, NC, USA). Fecal score data were compared using the GLIMMIX procedure of SAS. The statistical model included period and dog as random effects, whereas treatment was a fixed effect. Data were analyzed using the type 3 test of the MIXED procedure. All treatment least squares means were compared using preplanned contrasts that tested for linear effects of RS supplementation. Means were separated using a protected least squares difference with a Tukey

adjustment. Data were analyzed using the UNIVARIATE procedure to produce a normal probability plot based on residual data and visual inspection of the raw data. A probability of $P \leq 0.05$ was accepted as being statistically significant and $P \leq 0.10$ accepted as trends.

RESULTS

All results can be found in Table 5.2. Dietary OM intake was greater ($P=0.05$) in dogs fed 20 g biscuits/d compared to those fed 0 g biscuits/d (Table 5.2). Dogs consumed an average of 5.5 g of RS/d when fed the canned diet alone. Therefore, dogs fed 0, 10, or 20 g of biscuits/d consumed approximately 5.5, 8.0, or 10.5 g of RS/d, respectively. Dogs fed 20 g biscuits/d had lower ($P = 0.03$) fat digestibility than dogs fed 0 g biscuits/d. Dry matter, OM, and CP digestibilities; fecal output, fecal DM%, and fecal scores; fecal SCFA, BCFA, and ammonia concentrations; and fecal microbiota were not affected (Tables 5.3 and 5.4).

DISCUSSION

Even though RS has been demonstrated to affect fecal characteristics (e.g., fecal SCFA concentrations and gut microbial populations) in previous studies (Bird et al., 2007; McOrist et al., 2011), very little change was observed in this study. While fat digestibility decreased from 95.0% to 94.3% and reached statistical significance, this change is of little biological relevance from an energetics standpoint. It is speculated that the limitation of treat intake (10% of daily ME) and the minor changes observed in this study suggest the RS/fermentable fiber doses provided by the whole grain treats were too low. Additionally, the fermentable fiber included in the base diet may have interfered with the possible benefits of consuming RS from whole grains. At 10% RS inclusion, Rideout et al. (2008) observed higher ($P<0.05$) cecal butyrate

concentrations and lower (P<0.05) cecal indole, isobutyrate, and isovalerate concentrations in pigs than control fed pigs. Additionally, Tachon et al. (2013) observed higher proportions of *Lactobacillus* and *Bifidobacterium* in mice fed diets containing 18% RS from Hi-maize 260® than control fed mice. Because Hi-maize 260® is composed of 60% RS and 40% digestible starch, these diets contained approximately 10.8% RS. Based on our food intake data of the dogs in this study, they consumed approximately 1.4% and 2.7% RS from the treats. Further studies are required to assess the effectiveness of higher doses of RS/fermentable fiber on macronutrient, fecal characteristics and fermentative end-products in the dog.

TABLES

Table 5.1. Ingredient and chemical composition of Pedigree Loaf Original Canned Diet for dogs and NUTRO® NATURAL CHOICE® Skin and Coat All Natural Biscuits

	Diet	
Chemical Composition	Pedigree Loaf[1]	Nutro Natural Choice Skin & Coat Biscuits[2]
Dry matter, %	20.3	95.06
	------------% DM basis--------------	
Organic matter	84.39	96.95
Crude protein	36.13	15.74
Acid hydrolyzed fat	25.41	12.21
Total dietary fiber	8.86	10.47
Insoluble	4.40	7.44
Soluble	4.46	3.02
Gross energy, kcal/g	5.37	4.95
ME_{AAFCO}[3], kcal/g	3.91	3.64
Free glucose (FG)	0.18	0.17
Total starch	16.11	58.2
Total starch (without FG)	15.95	58.05
Digestible starch	12.77	46.46
Digestible starch (without FG)	12.61	46.31
Resistant starch	3.34	11.73

[1]Composition: meat and animal derivatives (42%, including 4% beef), cereals, fish and fish derivatives, minerals, derivatives of vegetable origin (0.5% dried sugar beet pulp), oils and fats (0.5% sunflower oil).
[2]Ingredients: Chicken, whole brown rice, whole wheat, oatmeal, flaxseed, fish oil (preserved with mixed tocopherols), natural flavors, coconut meal, sunflower oil (preserved with mixed tocopherols), dried chicory pulp, brewers dried yeast, preserved with mixed tocopherols.
[3]ME_{AAFCO} = 8.5 kcal ME/g fat + 3.5 kcal ME/g CP + 3.5 kcal ME/g nitrogen-free extract.

Table 5.2. Food intake, fecal characteristics, and total tract apparent macronutrient digestibility of adult dogs fed Pedigree Loaf Original Canned Diet for dogs and 0, 10, or 20 g NUTRO® NATURAL CHOICE® Skin and Coat All Natural Biscuits

	Treatment, g biscuits per day				P value
Item	0.0	10.0	20.0	SEM	Treatment
Food intake					
g DM/d	174.29^{x}	181.35^{xy}	193.33^{y}	20.166	0.09
g OM/d	147.09^{a}	154.31^{ab}	165.60^{b}	17.019	0.05
g CP/d	62.97	63.47	65.87	7.286	0.59
g fat/d	44.29	44.75	46.55	5.124	0.53
GE, kcal/d	935.96	969.64	1029.99	108.29	0.12
ME_C[1], kcal/g	4.27	4.29	4.23	0.042	0.30
Fecal output, g/d (as is)	142.19	135.66	151.02	21.214	0.41
Fecal output, g/d (DMB)	35.47	34.81	38.17	4.330	0.35
Fecal output (as is)/food intake (DMB)	0.81	0.73	0.77	0.047	0.13
Digestibility					
Dry matter (DM), %	79.49	80.91	80.20	1.013	0.26
Organic matter (OM), %	84.31	85.38	84.59	0.808	0.29
Crude protein (CP), %	81.82	82.28	80.74	1.045	0.14
Acid hydrolyzed fat (AHF), %	95.02^{b}	94.83^{ab}	94.31^{a}	0.506	0.03
Energy, %	86.32	87.01	85.85	0.868	0.38
Fecal scores[2]	2.54	2.53	2.60	0.072	0.22
Fecal DM %	26.89	26.18	26.60	0.926	0.67

^{a,b}Means not sharing a common superscript differ ($P \leq 0.05$).

^{x,y}Means not sharing a common superscript differ ($P \leq 0.10$).

[1]ME_C = [GE intake (kcal/d) - fecal GE (kcal/d) - [(CP intake/100) - (fecal CP/100)]*1.25]/ DM intake (g/d).

[2]Fecal score scale: 1-5; 1= hard, dry crumbly feces; 5= watery diarrhea (Rolfe et al., 2002).

Table 5.3. Fecal pH, ammonia, short-chain fatty acid (SCFA), branched-chain fatty acid (BCFA), phenol, and indole concentrations of adult dogs Pedigree Loaf Original Canned Diet for dogs and 0, 10, or 20 g NUTRO® NATURAL CHOICE® Skin and Coat All Natural Biscuits

	Treatment, g biscuits per day				P value
Item	0.0	10.0	20.0	SEM	Treatment
Fecal pH	6.81	6.72	6.41	0.196	0.25
Ammonia, umol/g DM	1035.41	1063.28	1157.85	735.530	0.66
Short-chain fatty acids, µmol/g DM					
Acetate	247.44	270.66	274.73	23.695	0.38
Propionate	164.85	120.37	132.62	27.658	0.50
Butyrate	41.43	48.86	45.73	4.805	0.11
Total SCFA	453.17	440.45	453.07	41.809	0.96
Acetate/total SCFA	0.58	0.62	0.61	0.023	0.22
Propionate/total SCFA	0.33	0.27	0.29	0.024	0.13
Butyrate/total SCFA	0.10	0.11	0.10	0.006	0.21
Branched-chain fatty acids, µmol/g DM					
Valerate	0.70	0.80	0.92	0.101	0.15
Isovalerate	14.67	16.04	15.36	1.394	0.27
Isobutyrate	9.73	10.59	10.19	0.925	0.27
Total BCFA	25.10	27.43	26.47	2.392	0.26
Phenols and indoles, µmol/g DM					
Phenol	1.07	1.23	1.45	0.412	0.42
Indole	2.78	2.86	2.93	0.411	0.91
Total phenols and indoles	3.85	4.09	4.35	0.717	0.63

Table 5.4. Fecal microbial populations of adult dogs fed Pedigree Loaf Original Canned Diet for dogs and 0, 10, or 20 g NUTRO® NATURAL CHOICE® Skin and Coat All Natural Biscuits

	Diet, g biscuits per day				P value
Item	0.0	10.0	20.0	SEM	Treatment
	CFU[1], log 10/g fecal DM				
Escherichia coli	9.42	9.11	9.69	0.539	0.67
Lactobacillus spp.	9.87	9.98	9.66	0.563	0.76
Bifidobacterium spp.	7.01	6.88	6.74	0.269	0.39
Clostridium perfringens	9.64	9.74	9.65	0.653	0.97

[1]CFU = colony-forming units.

CHAPTER 6: EFFECTS OF GRADED DIETARY RESISTANT STARCH CONCENTRATIONS ON APPARENT TOTAL TRACT MACRONUTRIENT DIGESTIBILITY, FECAL FERMENTATIVE END-PRODUCTS, AND FECAL MICROBIAL POPULATIONS OF HEALTHY ADULT DOGS

ABSTRACT

Resistant starch (RS) is fermentable by gut microbiota and effectively modulates fecal short-chain fatty acid (SCFA) concentrations in pigs, mice, and humans. Resistant starch may have similar beneficial effects on the canine gut; however, the dose of a single source of RS that is effective in manipulating fecal fermentative end-products, but does not negatively affect stool quality, has yet to be determined. Thus, the objective of this study was to evaluate the effects of 0, 1, 2, 3, and 4% dietary high-amylose maize cornstarch (Hi-maize® 260; RS) on apparent total tract macronutrient digestibility, fecal characteristics, and fecal fermentative end-product concentrations and fecal microbial populations in healthy adult dogs. An incomplete Latin square design was used, with each treatment period lasting 21 d (d 0-17 adaptation; d 18-21 fresh and total fecal collection) and each dog serving as its own control. Seven dogs (mean age = 5.3 yr; mean BW = 20 kg) were randomly allotted to one of five treatments formulated to be iso-energetic and consisting of graded amounts of 100% amylopectin cornstarch, RS, and cellulose, and fed as a top dressing on the food each day. All dogs were fed the same amount of a basal diet throughout the study and fresh water was offered ad libitum. Data were evaluated for linear and quadratic effects using SAS. Because the RS used in this study is approximately 40%

digestible and 60% indigestible, the dogs received the following amounts of indigestible starch daily: 0% = 0 g; 1% = 1.8 g; 2% = 3.6 g; 3% = 5.4 g; and 4% = 7.2 g. Apparent total tract dry matter, organic matter, crude protein, fat, and gross energy digestibilities and fecal pH were linearly decreased ($P < 0.05$) with increased RS consumption. Fecal output was linearly increased ($P < 0.05$) with increased RS consumption. Fecal scores and fecal fermentative end-product concentrations, including ammonia, short-chain fatty acids, branched-chain fatty acids, phenols, and indoles were not affected by RS consumption. Predominant bacterial phyla present in all dogs included Firmicutes, Bacteroidetes, Fusobacteria, Proteobacteria, Actinobacteria, and Tenericutes. Although most fecal microbial taxa were not altered, *Faecalibacterium* were increased ($P < 0.05$) with increased RS consumption. Compared to previous studies performed in other animal models and humans, our results seem to indicate that RS is poorly and/or slowly fermentable in dogs and may not greatly impact large bowel health in this species.

INTRODUCTION

Dietary fibers and prebiotics have been a consistent area of interest in the pet food industry. Previous dietary fiber research in dogs has focused on a few key indices of gastrointestinal (GI) health, usually leading to reduced fecal pH and concentrations of putrefactive compounds, increased concentrations of fecal short-chain fatty acids (SCFA; acetate, propionate, and butyrate), and alterations in gut bacterial populations. Dietary fibers often increase concentrations of beneficial or commensal bacteria (*Bifidobacterium*, *Lactobacilli*, *Blautia*, *Lachnospira*, *Veillonella*, *Megasphaera*, and *Faecalibacterium)* and decrease concentrations of potentially pathogenic bacteria (*Clostridium, Escherichia coli, C. hiranonis*, and *Fusobacterium)*, improving gut health (Vickers et al., 2001; Swanson et al., 2002a; 2002b;

Flickinger et al., 2003; Propst et al., 2003; Zentek et al., 2003; Grieshop et al., 2004; Middelbos 2007a; 2007b; Beloshapka et al., 2012; 2013). Many of today's dry extruded diets contain 20-50% carbohydrate (Bradshaw, 2006), with the majority in the form of digestible starch. It may be possible to improve GI health and maintain a healthy body weight by incorporating dietary fiber and other non-digestible carbohydrates.

Starches can be classified into three main categories: rapidly digestible starch (RDS), slowly digestible starch (SDS), or resistant starch (RS). Rapidly and slowly digestible starches are completely digested in the small intestine, but at varying rates (Englyst et al., 1992; Sajilata et al., 2006; Zhang and Hamaker, 2009). By definition, RS is starch that escapes digestion in the small intestine and ends up in the large bowel, part of which may be fermented in the colon (Sajilata et al., 2006). In humans and pigs, RS has been shown to be highly fermentable and effective in modulating the gut microbial composition and fecal SCFA when compared to digestible starch (Haenen et al., 2013). Additionally, mice fed approximately 10.8% pure RS (Hi-maize 260®) had higher proportions of cecal *Lactobacillus* and *Bifidobacterium* spp. (Tachon et al., 2013). Resistant starch may provide similar beneficial effects on the canine gut, but a specific dose of RS that is effective in manipulating fecal microbial populations and fermentative end-products, but does not negatively affect stool quality, has yet to be determined.

Hi-maize 260® (Ingredion; Bridgewater, N.J., USA) is a pure source of RS isolated from high amylose corn hybrids and processed to be approximately 60% RS and 40% digestible starch. It is a low-calorie (~2.8 kcal/g), low-glycemic index product that may be substituted in place of flour into a variety of foods, including baked goods, pasta, snack foods, cereals, and nutrition bars. Because it is a relatively pure source of digestible starch and RS, it allows for accurate doses to be tested. Therefore, the objective of this study was to evaluate the effects of

0, 1, 2, 3, and 4% dietary high-amylose maize cornstarch on apparent total tract macronutrient digestibility, fecal characteristics (e.g., pH, fecal scores, dry matter percentage), microbial populations, and fermentative end-product concentrations (e.g., SCFA, branched-chain fatty acids (BCFA), ammonia, phenols, and indoles) in healthy adult dogs.

MATERIALS AND METHODS

Animals and diets

All animal care and study procedures were approved by the University of Illinois Institutional Animal Care and Use Committee prior to experimentation. A total of seven healthy adult female hound-cross dogs (average age: 5.3 yr; average BW: 20-25 kg) were studied. Dogs were housed individually in runs (2.4 x 1.2 m), which allowed for nose-nose contact between dogs in adjacent runs and visual contact with all dogs in the room, in two temperature-controlled rooms (22°C; 23% relative humidity) with 12-h light: 12-h dark cycles. Although animals were housed and fed individually, they were allowed exercise and play outside of their cages together (and with people and toy enrichment) within the animal room for several hours at least 3 times a week. Dogs were weighed and assessed for body condition score (BCS; 9 point scale) prior to the AM feeding on every Thursday of the study.

One experimental diet was formulated, with low-ash poultry by-product meal, brewer's rice, and poultry fat constituting the main ingredients of the dry, extruded kibble diet (Table 6.1). All dogs were fed the same experimental diet formulated to meet their nutritional requirements as provided by AAFCO (2012), but to contain little residual fiber to ensure that the effects of RS were not masked or biased by fiber in the diet. Diets were formulated by Lortscher Animal Nutrition, Inc. (Bern, KS, USA) and extruded by AFB International (St. Charles, MO, USA).

All dogs were fed the same amount of diet (300 g total) once daily (0800). To ensure the dogs consumed all of their dietary treatment, the starch blend was mixed with 50 mL of water and 30 g of their daily ration and offered prior to their daily meal. In order to keep treatments iso-energetic, graded amounts of the following were fed: (1) 100% amylopectin cornstarch (Amioca®; Ingredion, Bridgewater, N.J., USA), (2) high-amylose maize cornstarch (Hi-maize® 260; Ingredion), and (3) cellulose (Sigma-Aldrich α-cellulose; Table 6.2). Fresh water was offered ad libitum.

Sample collection

An incomplete 5 x 5 Latin square design with 7 dogs, 5 dietary treatments (Table 6.1), and five 21-d trial periods was conducted so that each animal served as its own control. Each period consisted of a diet adaptation phase (d 0-17) and a total and fresh fecal collection phase (d 18-21). Total feces excreted during the collection phase of each period were collected from the pen floor, weighed, and frozen at -20°C until further analyses. All fecal samples during the collection phase were subjected to a consistency score according to the following scale: 1 = hard, dry pellets, and small hard mass; 2 = hard formed, dry stool, and remains firm and soft; 3 = soft, formed and moist stool, and retains shape; 4 = soft, unformed stool, and assumes shape of container; and 5 = watery, liquid that can be poured.

One fresh fecal sample per period was collected within 15 min of defecation on d 1 of the 4-d collection phase. Fresh fecal samples were prepared immediately to minimize loss of volatile components. Samples were weighed and pH determined using a Denver Instrument AP10 pH meter (Denver Instrument, Bohemia, NY, USA) equipped with a Beckman electrode (Beckman Instruments, Inc., Fullerton, CA, USA). Fresh fecal dry matter (DM) was determined

at 105°C. Aliquots for analysis of phenols and indoles were frozen at -20°C immediately after collection. An aliquot (5 g) of feces was mixed with 5 mL 2N HCl for ammonia, SCFA and BCFA determination and stored at -20°C until analyzed. Aliquots of fresh feces were transferred to sterile cryogenic vials (Nalgene, Rochester, NY, USA) and frozen at -80°C until DNA extraction for microbial analysis.

Chemical analyses

The diet was subsampled and ground through a 2-mm screen in a Wiley mill (model 4, Thomas Scientific, Swedesboro, NJ, USA). Composited fecal samples (1 per dog per period) were dried at 55°C for 1 wk and ground through a 2-mm screen in a Wiley Mill. Samples were analyzed according to procedures by the Association of Official Analytical Chemists (AOAC) for dry matter (DM; 105°C), organic matter (OM), and ash (AOAC, 2006; methods 934.01, 942.05). Crude protein (CP) content was calculated from Leco total N values (TruMac® N, Leco Corporation, St. Joseph, MI, USA; AOAC, 2006). Total lipid content (acid hydrolyzed fat) of the samples was determined according to the methods of the American Association of Cereal Chemists (AACC, 1983) and Budde (1952). Gross energy (GE) of the samples was measured using an oxygen bomb calorimeter (model 1261, Parr Instruments, Moline, IL). Dietary fiber concentrations [total dietary fiber (TDF), soluble dietary fiber (SDF), and insoluble dietary fiber (IDF)] were determined according to Prosky et al. (1992). The diet and all fecal samples were analyzed in duplicate, with a 5% error allowed between duplicates; otherwise, the analyses were repeated.

Short-chain fatty acid and BCFA concentrations were determined by gas chromatography according to Erwin et al. (1961) using Hewlett-Packard 5890A series II gas chromatograph (Palo

Alto, CA, USA) and a glass column (180 cm x 4 mm i.d.) packed with 10% SP™-1200/1% H_3PO_4 on 80/100+ mesh Chromosorb WAW (Supelco Inc., Bellefonte, PA, USA). Phenol and indole concentrations were determined using gas chromatography according to the methods of Flickinger et al. (2003). Ammonia concentrations were determined according to the method of Chaney and Marbach (1962).

Microbial Analyses

Fecal DNA was extracted from freshly collected samples that had been stored at -80°C until analysis, using the PowerLyzer™ PowerSoil® DNA Isolation Kit (MO BIO Laboratories, Inc., Carlsbad, CA, USA) according to the manufacturer's instructions. Extracted DNA was quantified using a Qubit® 2.0 Fluorometer (Life Technologies™, Invitrogen™, Grand Island, NY, USA). PCR amplicons from the V4 region of the 16S rRNA gene were prepared for sequencing following a similar procedure as Cephas et al. (2011) and primers according to Kozich et al. (2013). PCR amplicons of all samples were further purified using AMPure XP beads (Beckman-Coulter, Inc.). Further DNA concentration and quality measured using Qubit® 2.0 Fluorometer. Finally, the amplicons were combined in equimolar ratios to create a DNA pool that was used for Illumina sequencing. DNA quality was assessed before sequencing using 2100 Bioanalyzer (Agilent). Dual-indexing Illumina sequencing was performed at the W. M. Keck Center for Biotechnology at the University of Illinois using a MiSeq 2x300nt v3 technology. After sequencing was completed, all reads were scored for quality and any poor quality reads and primer dimers were removed.

Bioinformatics of microbial data

Preprocessing of reads: Demultiplexed reads from each individual sample were trimmed to a length of 252 bases by trimmomatic program (Lohse et al., 2012; http://www.usadellab.org/cms/?page=trimmomatic) to ensure removal all adaptor sequences from the reads. Trimmed reads from each sample were given as input to PEAR 0.8.1 (Zhang et al., 2014; http://sco.h-its.org/exelixis/web/software/pear/index.html) to merge each read pair. PEAR was run with all default parameters.

OTU picking and taxonomy assignment: To perform quality filtering of the stitched reads, OTU picking and taxonomy assignment IM-Tornado pipeline (unpublished data) was performed. IM-Tornado was run with parameters MINIMUM_LENGTH=187, R1_TRIM =250 and R2_TRIM=250. Input files for IM-Tornado were fastq files for each sample and a mapping file that contained sample ID. Because IM-Tornado requires both R1 reads and R2 reads as input, stitched reads obtained from PEAR were considered as R1 reads and the reverse complement of R1 reads were treated as R2 reads for each sample. Output files related to R1 data only were used for downstream analysis.

Alpha and beta diversity measures: IM-Tornado generated output files, a BIOM file, a phylogenetic tree, and a mapping file were provided as input to alpha_rarefaction.py script of Qiime 1.8.0 (Caporaso et al., 2010). A rarefied OTU table was generated by multiple_rarefactions.py script invoked by alpha_rarefaction.py script. Alpha diversity measures, Shannon index, PD_whole_tree, chao1, and observed species were calculated on the rarefied OTU table using alpha_diversity.py script called by alpha_rarefaction.py. Rarefaction plots were also generated by the alpha_rarefaction.py script by invoking make_rarefaction_plots.py.

To compute beta diversity measures and generate Emperor PCoA plots, beta_diversity_through_plots.py script was run in QIIME with all default parameters and the

parameter –e was set to 74296 because all samples had at least 74,296 sequences. The script was run with a BIOM file, a phylogenetic tree, and a mapping file. Jackknifed beta diversity and hierarchical clustering was performed by running jackknifed_beta_diversity.py script using a BIOM file, a phylogenetic tree, a mapping file and the parameter –e, which ensures that a specified random subset of sequences selected from each sample. As per the recommendation of QIIME developers, –e was set to 55,722, which is 75% of sequences in the smallest sample.

Calculations and statistical analysis

Apparent total tract apparent macronutrient digestibility values were calculated using the following equation: nutrient intake (g DM/d) - nutrient output (g DM/d)/ nutrient intake (g DM/d) x 100. Data were analyzed using the MIXED procedure of SAS (version 9.2, SAS Institute Inc., Cary, NC, USA). Fecal score data were compared using the GLIMMIX procedure of SAS. The statistical model included period and dog as random effects, whereas treatment was a fixed effect. Data were analyzed using the type 3 test of the MIXED procedure. All treatment least squares means were compared using preplanned contrasts that tested for linear and quadratic effects of RS supplementation. Means were separated using a protected least squares difference with a Tukey adjustment. Data were analyzed using the UNIVARIATE procedure to produce a normal probability plot based on residual data and visual inspection of the raw data. A probability of $P < 0.05$ was accepted as being statistically significant and $P \leq 0.10$ accepted as trends.

RESULTS

Because the RS used in this study is approximately 40% digestible and 60% indigestible starch, the dogs received the following amounts of indigestible starch daily: 0% = 0 g; 1% = 1.8 g; 2% = 3.6 g; 3% = 5.4 g; and 4% = 7.2 g. Dry matter, OM, CP, fat, and GE digestibilities and fecal pH were linearly decreased ($P < 0.05$) with increased RS consumption (Table 6.3). Fecal output was linearly increased ($P < 0.05$) with increased RS consumption. Fecal scores and fecal fermentative end-product concentrations, including ammonia, SCFA, BCFA, phenols, and indoles, were not affected by RS consumption (Table 6.4).

A total of 9,109,434 reads were generated in the current dataset, with an average of 120,376 reads per sample used to provide diversity estimates. Predominant bacterial phyla present in all dogs included Firmicutes, Bacteroidetes, Fusobacteria, Proteobacteria, Actinobacteria, and Tenericutes (Table 6.5). The phyla, Deferribacteres and Synergistetes, were also detected, but at a much lesser degree than the other phyla (< 0.01% of sequences; data not shown). Together, Firmicutes and Bacteroidetes made up about 80% of all bacterial sequences, with Fusobacteria representing about 15%, Proteobacteria representing about 4%, and Actinobacteria and Tenericutes each representing <1% of all bacterial sequences. There was no clear separation of samples based on diet when principal coordinate analysis was performed (data not shown). Predominant bacterial genera included *Prevotella* (Bacteroidetes), *Clostridium* (Firmicutes), *Fusobacterium* (Firmicutes), *Bacteroides* (Bacteroidetes), and *Lactobacillus* (Firmicutes), which were present at an average of 25, 18, 15, 10, and 6% of bacterial sequences, respectively. *Faecalibacterium*, which belong to the phylum Firmicutes, were increased ($P \leq 0.05$) with increased RS consumption. *Roseburia*, which belongs to the phylum Firmicutes, tended to increase ($P<0.10$) with increased RS consumption. *Anaerotruncus*, which belongs to the phylum Firmicutes, tended to decrease ($P<0.10$) with increased RS consumption.

DISCUSSION

Increased fiber or RS consumption can lead to increased fecal output and ideal fecal scores, but decreased nutrient digestibility. Previous research has noted fat digestibility was lower in dogs fed high-amylose corn hybrid allegedly due to the formation of amylose-lipid complexes that may be produced during extrusion (Gajda et al., 2005). However, the results of the current study had lower fat digestibility as RS consumption increased and the high-amylose maize starch used in the current study was fed as a pre-meal and not incorporated into the extruded diet. While the apparent total tract macronutrient digestibility of the dogs in the current study was decreased with increased RS consumption, the changes observed were small and of little biological significance.

Previous research performed in other animal models and humans seem to indicate that RS is readily fermentable by GI microbiota, resulting in decreased fecal pH and fecal putrefactive compounds, and increased fecal SCFA. Bird et al. (2007) compared the effects of experimental diets containing one of four starches [(1) control, 0% amylose; (2) high-amylose maize starch (HAMS), (3) hydrothermally treated high-amylose maize starch (HTHAMS), (4) combination of 2 and 3 (HAMS + HTHAMS)] in the large bowel of pigs (age = 4 wk; n = 24). The diets contained approximately 15.5% RS/kg diet (HAMS), 30.9% RS/kg diet (HTHAMS), or 22.8% RS/kg diet (HAMS + HTHAMS). These researchers found that fecal pH was lower ($P<0.001$) in pigs consuming the HAMS (pH = 5.27), HTHAMS (pH = 5.14), or HAMS + HTHAMS (pH = 5.20) compared to the control-fed pigs (pH = 6.40). Additionally, Bird et al. (2007) observed higher ($P<0.001$) distal colonic digesta total SCFA (96.7 mmol/L), acetate (43.3 mmol/L), propionate (35.7 mmol/L), and butyrate (7.2 mmol/L) concentrations in pigs fed RS-containing

diets compared to the control-fed pigs (total SCFA = 35 mmol/L; acetate = 20 mmol/L; propionate = 10 mmol/L; butyrate = 4 mmol/L). These researchers also noted that the HAMS + HTHAMS treatment was the most effective at raising fecal SCFA (data not provided) when compared to control-fed pigs (Bird, et al., 2007). Rideout et al. (2008) investigated the nutrient utilization and intestinal fermentation of various RS varieties and conventional fiber sources, including granular high amylose corn starch, granular potato starch, retrograded high amylose corn starch, guar gum, or cellulose, when fed to Yorkshire pigs (BW = 30 kg; n = 36). At 10% RS inclusion, those authors observed higher (P<0.05) cecal butyrate concentrations (4.98 mg/g DM digesta vs. 11.89 mg/g DM digesta) and lower (P<0.05) cecal indole (0.12 mg/g DM digesta vs. 0.04 mg/g DM digesta), isobutyrate (4.03 mg/g DM digesta vs. 1.98 mg/g DM digesta), and isovalerate (4.20 mg/g DM digesta vs. 1.73 mg/g DM digesta) concentrations in pigs. Compared to previous studies performed in other animal models and humans, our results seem to indicate that RS is poorly and/or slowly fermentable in dogs and may not greatly impact large bowel health in this species.

The composition of Hi-maize 260® suggests that it would be fermented, and thus, beneficially manipulate gut microbial populations. Tachon et al. (2013) evaluated the structure of cecal microbial communities of male C57BL/6J mice (age = 18-20 mo old; n = 6 per diet/18 total) fed one of three diets: Control (Amioca corn starch; 100% amylopectin, 0% RS), 18% RS, or 36% RS (type 2 RS from high-amylose maize; Hi-maize 260®). Because Hi-maize 260® is composed of 60% RS and 40% digestible starch, these diets contained approximately 10.8 and 21.6% RS, respectively. They observed higher (P<0.05) proportions of cecal *Lactobacillus* and *Bifidobacterium* spp. in mice fed diets containing 18% RS from Hi-maize 260® when compared to the control fed mice. The substrate used in that study was the same as in the current study, but

fed at a higher dose. Furthermore, the fermentation site varies between the species (i.e., cecum vs. colon).

In the current study, few statistical changes were observed in the indices of GI health measured. However, many measurements had numerical differences that shifted in an expected manner. Predominant fecal bacterial phyla detected in the current study were similar to previous research, but different in distribution (Suchodolski, 2011a; Suchodolski et al., 2008, 2009; Middelbos et al., 2010; Handl et al., 2011; Swanson et al., 2011; Beloshapka et al., 2013). Based on the utilization of new primers, a more even distribution of bacterial groups was noted. Previous studies that used primers targeting V4-V6 regions and 454 pyrosequencing to evaluate fecal bacterial populations produced a composition that was low in Bacteroidetes, *Lactobacillus*, and *Bifidobacterium* spp. (Hooda et al., 2012; Beloshapka et al., 2013). Furthermore, the number of high quality sequences generated was much higher than those in previous studies at a lower cost (i.e., MiSeq sequencing costs vs. 454 pyrosequencing costs, and utilizing dual-indexing sequencing).

In conclusion, the doses of Hi-maize 260® used in the current study were chosen based on the purity of the substrate and expected amount at which GI tolerance was maintained, but greater concentrations of fecal SCFA and beneficial bacteria were achieved. Minor changes in these indices of GI health were observed, however. It is possible that a larger amount of this particular substrate is needed to manipulate typical indices of gut health in the dog. Finally, the results of the current study may indicate that canine gastrointestinal transit time or anatomy (i.e., lack of sacculations, colonic fermentor) does not support the use of pure sources of RS as a dietary fiber source.

TABLES

Table 6.1. Ingredient and chemical composition of the extruded experimental diet for canines

Ingredients	Basal extruded diet, %
Brewer's rice	45.22
Poultry by-product meal	37.00
Poultry fat	14.00
Dried egg	2.40
Salt	0.45
Potassium chloride	0.56
Choline chloride[1]	0.13
Vitamin mix[2]	0.12
Mineral mix[3]	0.12
Chemical composition	
Dry matter, %	93.76
	----% DM basis---
Organic matter	93.87
Crude protein	37.73
Acid hydrolyzed fat	13.21
Total dietary fiber	3.89
Insoluble	2.68
Soluble	1.21
Gross energy, kcal/g	5.12
ME_{AAFCO}[4], kcal/g	3.81
Free glucose	0.07
Total starch	40.86
Total starch (without FG)	40.8
Digestible starch	34.61
Digestible starch (without FG)	34.55
Resistant starch	6.25

ME_{AAFCO}, metabolizable energy by American Association of Feed Control Officials; ME_C, metabolizable energy calculated.

[1]Provided the following per kilogram of diet: choline, 2,284.2 mg.

[2]Provided the following per kilogram of diet: vitamin A, 11,000 IU; vitamin D_3, 900 IU; vitamin E, 57.5 IU; vitamin K, 0.6 mg; thiamin, 7.6 mg; riboflavin, 11.9 mg; pantothenic acid, 18.5 mg; niacin, 93.2 mg; pyridoxine, 6.6 mg; biotin, 12.4 mg; folic acid, 1,142.1 µg; and vitamin B_{12}, 164.9 µg.

[3]Provided the following per kilogram of diet: manganese ($MnSO_4$), 17.4 mg; iron ($FeSO_4$), 284.3 mg; copper ($CuSO_4$), 17.2 mg; cobalt ($CoSO_4$), 2.2 mg; zinc ($ZnSO_4$), 166.3 mg; iodine (KI), 7.5 mg; and selenium (Na_2SeO_3), 0.2 mg.

[4] ME_{AAFCO}, metabolizable energy by American Association of Feed Control Officials; ME_{AAFCO} = 8.5 kcal ME/g fat + 3.5 kcal ME/g CP + 3.5 kcal ME/g nitrogen-free extract.

Table 6.2. Five dietary starch blends used to determine the dose of resistant starch that is well-tolerated when fed to healthy adult dogs

	Control (0% RS)		**1% RS**		**2% RS**		**3% RS**		**4% RS**	
	g	kcal/g	g	kcal/g	g	kcal/g	g	kcal/g	g	kcal/g
Cornstarch[1]	8.4	33.6	6.3	25.2	4.2	16.8	2.1	8.4	0.0	0.0
Resistant starch (RS)[2]	0.0	0.0	3.0	8.4	6.0	16.8	9.0	25.2	12.0	33.6
Cellulose[3]	3.6	0.0	2.7	0.0	1.8	0.0	0.9	0.0	0.0	0.0
Total	**12.0**	**33.6**	**12.0**	**33.6**	**12.0**	**33.6**	**12.0**	**33.6**	**12.0**	**33.6**

[1]100% amylopectin cornstarch (Amioca®; Ingredion, Bridgewater, N.J., USA); 4 kcal/g.
[2]High-amylose maize cornstarch (Hi-maize® 260; Ingredion); 2.8 kcal/g.
[3]Sigma-Aldrich α-cellulose; 0 kcal/g.

Table 6.3. Mean food intake, fecal characteristics and apparent total tract macronutrient digestibility of adult dogs fed graded levels of resistant starch

	Diet, % Hi-maize 260						P value		
Item	0	1	2	3	4	Pooled SEM	TRT	Linear	Quadratic
Food intake									
g DM[1]/d	292.5	292.5	292.5	292.5	292.5	n/a[2]	n/a	n/a	n/a
g OM/d	275.3	275.3	275.2	275.2	275.2	n/a	n/a	n/a	n/a
g CP/d	110.4	110.4	110.4	110.4	110.4	n/a	n/a	n/a	n/a
g AHF/d	38.6	38.6	38.6	38.6	38.6	n/a	n/a	n/a	n/a
Gross energy, kcal/d	1496.7	1496.6	1496.5	1496.4	1496.3	n/a	n/a	n/a	n/a
ME_C[3], kcal/g	4.08	4.03	4.00	4.04	4.02	0.02	0.03	0.03	0.06
Fecal output (g/d, as-is)	138.6	149.3	156.3	156.0	155.0	7.01	0.06	0.01	0.10
Fecal output (g/d, DMB)	45.9	49.3	52.5	49.6	51.2	1.66	0.02	0.02	0.06
Fecal output (as-is)/food intake (DMB)	0.47	0.51	0.54	0.53	0.53	0.024	0.06	0.01	0.08
Digestibility									
DM, %	84.3	83.1	82.0	83.1	82.5	0.57	0.02	0.02	0.06
OM, %	87.8	86.8	85.9	86.7	86.2	0.49	0.02	0.01	0.06
CP, %	82.4	81.0	79.5	80.2	79.9	0.93	0.02	0.01	0.06
AHF, %	92.2	91.5	91.2	91.7	91.3	0.25	0.02	0.03	0.11
Energy, %	87.4	86.3	85.5	86.3	85.9	0.50	0.03	0.02	0.06
Fecal scores[4]	3.1	3.2	3.0	3.4	3.1	0.14	0.01	0.36	0.34
Fecal DM, %	33.5	31.8	33.5	33.6	32.3	1.27	0.69	0.73	0.86

[1]DM = dry matter; OM = organic matter; CP = crude protein; DMB = dry matter basis; AHF = acid hydrolysed fat; ME_C = metabolizable energy calculated.
[2]All dogs were fed the same amount of experimental diet throughout the duration of the study.
[3]ME_C = GE intake (kcal/d) - fecal GE (kcal/d) - urinary GE (kcal/d)/ DM intake (g/d).

Table 6.3 (cont.)
[4]Fecal score scale: 1= hard, dry pellets; 2= dry, well-formed stool; 3=soft, moist, formed stool; 4= soft, unformed stool; 5= watery, liquid that can be poured.

Table 6.4. Mean fecal pH, ammonia, short-chain fatty acid (SCFA), branched-chain fatty acid (BCFA), phenol and indole concentrations of adult dogs fed graded levels of dietary resistant starch

	Diet, % Hi-maize 260						P value		
Item	0	1	2	3	4	Pooled SEM	TRT	Linear	Quadratic
pH	6.7	6.4	6.5	6.4	6.4	0.12	0.10	0.03	0.20
	---------------------μmol/g DM---------------------								
Ammonia	155.6	151.1	140.4	136.2	149.6	8.07	0.33	0.32	0.13
Short-chain fatty acids (SCFA)									
Acetate	267.0	256.7	261.2	252.5	275.2	20.91	0.85	0.77	0.37
Propionate	105.1	112.1	107.5	96.0	102.9	6.02	0.21	0.28	0.69
Butyrate	69.1	63.9	62.5	63.7	67.5	5.05	0.75	0.79	0.19
Total SCFA	441.4	432.7	430.9	412.2	445.5	29.04	0.85	0.93	0.43
Acetate/total SCFA	0.60	0.59	0.61	0.61	0.61	0.01	0.49	0.25	0.42
Propionate/total SCFA	0.24	0.26	0.25	0.24	0.23	0.01	0.05	0.10	0.05
Butyrate/total SCFA	0.15	0.15	0.15	0.16	0.15	0.01	0.76	0.86	0.48
Branched-chain fatty acids (BCFA)									
Isobutryate	17	16.6	16.4	14.5	16.4	1.17	0.31	0.34	0.38
Isovalerate	23.3	22.7	21.9	20.8	22.4	1.57	0.75	0.44	0.44
Valerate	0.86	0.95	1.12	0.82	2.21	0.53	0.39	0.13	0.32
Total BCFA	41.2	40.2	39.3	36.3	40.9	2.71	0.53	0.64	0.28
Phenols and indoles									
Phenol	3.2	3.3	2.8	2.9	2.8	0.75	0.79	0.35	0.91
Indole	4.2	4.2	3.7	3.9	4.4	0.33	0.57	0.83	0.19
Total phenols/indoles	7.3	7.5	6.6	6.8	7.1	0.84	0.78	0.62	0.56

Table 6.5. Prominent bacterial phyla and genera (expressed as percentage of total sequences) in feces of dogs fed graded levels of dietary resistant starch

Item	Diet, % Hi-maize 260					Pooled SEM	P value		
	0	1	2	3	4		TRT	Linear	Quadratic
Actinobacteria	**0.91**	**1.04**	**0.91**	**0.94**	**0.78**	**0.21**	**0.87**	**0.52**	**0.52**
Collinsella	0.70	0.78	0.72	0.63	0.61	0.16	0.85	0.39	0.64
Slackia	0.08	0.08	0.07	0.07	0.07	0.02	0.92	0.43	0.83
Coriobacterium	0.06	0.13	0.09	0.12	0.07	0.04	0.64	0.89	0.24
Adlercreutzia	0.04	0.05	0.02	0.02	0.03	0.02	0.36	0.15	0.47
Bifidobacterium	0.02	<0.01	0.01	0.11	0.01	0.05	0.49	0.60	0.68
Bacteroidetes	**36.38**	**36.39**	**36.65**	**34.96**	**41.14**	**4.78**	**0.70**	**0.44**	**0.38**
Prevotella	25.48	24.88	24.79	23.58	28.55	3.64	0.72	0.59	0.29
Bacteroides	9.89	9.29	9.42	9.67	11.22	2.19	0.90	0.54	0.45
CF231	1.29	1.45	0.49	1.09	0.68	0.61	0.29	0.17	0.76
Parabacteroides	0.40	0.29	0.36	0.47	0.38	0.18	0.29	0.59	0.83
Unclassified	0.32	0.27	0.32	0.29	0.20	0.11	0.65	0.28	0.46
Parapedobacter	0.10	0.10	0.07	0.12	0.08	0.06	0.66	0.58	1.00
Firmicutes	**44.65**	**42.06**	**44.58**	**45.80**	**37.51**	**5.11**	**0.38**	**0.43**	**0.29**
Clostridium	16.28	18.67	17.16	19.31	16.91	2.72	0.89	0.81	0.51
Lactobacillus	10.23	5.32	4.12	7.41	3.76	2.90	0.35	0.21	0.46
Blautia	3.47	4.29	4.75	4.29	4.66	0.69	0.66	0.28	0.46
Phascolarctobacterium	3.13	3.75	4.20	3.58	3.73	0.74	0.73	0.56	0.33
Dorea	2.91	3.34	3.40	3.17	3.00	0.58	0.85	0.99	0.28
Ruminococcus	1.63	1.73	1.27	1.49	1.47	0.22	0.51	0.36	0.54
Megamonas	1.61	1.99	1.85	0.87	1.37	0.60	0.40	0.26	0.72
Faecalibacterium	1.23	0.96	1.49	1.46	1.80	0.30	0.20	0.05	0.46
unclassified	1.03	1.01	1.37	1.09	1.14	0.31	0.66	0.59	0.47

Table 6.5 (cont.)

SMB53	0.72	0.54	0.41	0.47	0.27	0.21	0.26	0.04	0.77
Turicibacter	0.52	0.42	0.52	0.40	0.27	0.19	0.54	0.18	0.51
Peptococcus	0.37	0.43	0.55	0.26	0.47	0.23	0.68	0.94	0.84
Allobaculum	0.21	0.23	0.29	0.39	0.32	0.08	0.41	0.11	0.55
Coprococcus	0.19	0.05	0.08	0.20	0.14	0.05	0.19	0.78	0.24
Anaerofilum	0.16	0.16	0.16	0.14	0.13	0.04	0.93	0.42	0.73
Oscillospira	0.11	0.09	0.11	0.10	0.10	0.03	0.95	0.77	0.80
Eubacterium	0.06	0.03	0.06	0.03	0.07	0.03	0.33	0.65	0.35
Catenibacterium	0.05	0.05	0.05	0.09	0.04	0.03	0.63	0.74	0.51
Lachnospira	0.05	0.02	0.03	0.04	0.04	0.01	0.58	0.73	0.17
Anaerostipes	0.04	0.05	0.05	0.04	0.06	0.01	0.60	0.28	0.77
Anaerotruncus	0.04	0.03	0.02	0.02	0.02	0.01	0.36	0.07	0.32
Roseburia	0.03	0.03	0.03	0.05	0.05	0.01	0.28	0.10	0.69
Coprobacillus	0.03	0.05	0.04	0.03	0.03	0.01	0.34	0.58	0.22
Pectinatus	0.02	0.03	0.04	0.02	0.03	0.01	0.26	0.84	0.16
Bulleidia	0.01	0.01	0.02	0.01	0.01	<0.01	0.83	0.39	0.54
Butyrivibrio	0.01	0.02	0.01	0.01	0.01	0.01	0.46	0.15	0.36
Epulopiscium	0.01	0.03	<0.01	0.05	<0.01	0.02	0.24	0.98	0.47
Fusobacteria	**15.08**	**15.97**	**15.54**	**15.38**	**14.23**	**2.05**	**0.91**	**0.59**	**0.45**
Fusobacterium	14.96	15.85	15.35	15.26	14.14	2.03	0.92	0.60	0.47
u114	0.10	0.13	0.21	0.12	0.09	0.04	0.04	0.65	0.01
Proteobacteria	**3.32**	**3.53**	**5.07**	**3.61**	**3.50**	**1.06**	**0.71**	**0.90**	**0.35**
Sutterella	2.51	3.24	2.33	2.42	2.22	0.55	0.38	0.25	0.56
Serratia	0.66	0.23	2.47	0.92	1.36	1.09	0.64	0.55	0.61
Anaerobiospirillum	0.09	0.17	0.08	0.10	0.08	0.08	0.64	0.53	0.68
Succinivibrio	0.04	0.07	0.02	0.02	0.02	0.03	0.35	0.15	0.92
Tenericutes	**0.01**	**0.01**	**0.03**	**0.01**	**0.01**	**<0.01**	**0.51**	**0.65**	**0.97**

Table 6.5 (cont.)

Anaeroplasma	0.01	0.01	0.01	0.01	0.01	<0.01	0.33	0.83	0.93

CHAPTER 7: CONCLUSIONS

Extruded diets remain the most common diet format fed to dogs. Even though dogs are classified as Carnivora, carbohydrates make up 20-50% of most extruded diets. The majority of carbohydrates are in the form of starch, providing a readily available energy source. Other carbohydrate forms include dietary fiber, oligosaccharides, and resistant starches (RS) that may elicit a multitude of health benefits and may enhance overall health and longevity. Previous dietary fiber research in dogs has focused on a few key indices of GI health, often resulting in decreased fecal pH and concentrations of putrefactive compounds [e.g., phenols, indoles, branched-chain fatty acids (BCFA)], increased concentrations of fecal short-chain fatty acids (SCFA; acetate, propionate, and butyrate), and alterations in gut bacterial populations. However, previous research has focused on the composition of traditional carbohydrate-based ingredients, but little has been done in regards to the composition of various whole ingredient, fractionated ingredient, or single-source carbohydrate sources.

The overall objective of this research was to evaluate glucose-based carbohydrate sources for use in canine diets, including ingredient compositional analyses, and effects on apparent total tract macronutrient digestibility and fecal characteristics, microbiota composition, and fermentative end-products.

Our first aim was to determine the chemical composition of carbohydrate sources commonly incorporated into pet foods. We evaluated various whole grains, processed grains, grain fractions, and pseudocereal grains. In this aim, we determined that whole grains vary compared to both their respective fractions (i.e., barley flake vs. malted barley vs. steamed rolled barley) as well as compared to other grains (i.e., barley flake vs. brown rice vs. canary grass

seed). Additionally, whole grains and grain fractions contain many beneficial nutrients including dietary fiber, amino acids, and minerals.

Our second aim was to evaluate the effects of graded concentrations of polydextrose, a novel fermentable fiber used extensively in the food industry, on apparent total tract macronutrient digestibility, fecal characteristics, fecal fermentative end-products, and fecal microbial populations in healthy adult dogs. Polydextrose was fed at 0, 0.5, 1, or 1.5% of the diet DM. In this aim, we determined that polydextrose appeared to be fermentable, which was evident by the increased concentrations of fecal SCFA, primarily acetate and propionate, and by the decrease in fecal pH, without affecting food intake or fecal output. The inclusion of polydextrose also decreased some protein catabolites, in particular fecal indole concentrations. Fecal *C. perfringens* concentrations were decreased by including polydextrose in the diet, but other bacteria measured were unaffected.

Our third aim was to determine the effects of the resistant starch (RS) component found naturally in whole grains on apparent total tract macronutrient digestibility, fecal characteristics, and fecal fermentative end-products in healthy adult dogs fed a baked whole grain-containing biscuit treat. Dogs received either 0, 10, or 20 g biscuits/d (estimated to be 0, 2.5, or 5 g RS/d) that were fed within their daily caloric allowance. In this aim, we determined that fat digestibility decreased with increased treat consumption (i.e., increased RS consumption), but dry matter, organic matter, and crude protein digestibilities were not affected. Fecal fermentative end-products, including short- and branched-chain fatty acids, ammonia, phenols, and indoles, and microbial populations were not affected. Perhaps RS was present in insufficient concentrations to elicit a biological response.

Our fourth aim was to determine the effects of graded concentrations of a corn-based RS source on apparent total tract macronutrient digestibility, fecal fermentative end-products, and fecal microbial populations in healthy adult dogs. Dogs were fed 0, 1, 2, 3, and 4% of diet DM of Hi-maize 260®, a corn-based ingredient that consists of 60% RS and 40% digestible starch. In this aim, dry matter, organic matter, crude protein, fat, and gross energy digestibilities and fecal pH were linearly decreased ($P < 0.05$) with increased RS consumption. Fecal output was linearly increased ($P < 0.05$) with increased RS consumption. Fecal scores and fecal fermentative end-product concentrations, including ammonia, short-chain fatty acids, branched-chain fatty acids, phenols, and indoles were not affected by RS consumption. Predominant bacterial phyla present in all dogs included Firmicutes, Bacteroidetes, Fusobacteria, Proteobacteria, Actinobacteria, and Tenericutes. Although most bacterial taxa were not affected, fecal *Faecalibacterium* spp. were increased ($P < 0.05$) with increased RS consumption.

Whole grains, when minimally processed, can provide generous amounts (>20%) of dietary fiber or RS. The effects of cooking have been previously studied and have been shown to drastically alter the composition of a cereal grain. Thus, a whole-grain containing baked biscuit treat was hypothesized to be the best method of delivering the health benefits of whole grains to the colon of dogs (Chapter 5). However, the consumption of the treats led to minimal changes in indices of gut health. It is possible that because treat intake was limited to 10% of daily metabolizable energy needs, the dose was too low to observe beneficial effects. Additionally, the treats may not have contained as much RS/dietary fiber as intended/estimated.

The most effective dose of a highly pure, single-source RS was not as easily determined as hypothesized (Chapter 6). Many dietary fibers and prebiotics are rapidly fermented by gut microbiota of dogs, demonstrated by a reduced fecal pH, increased fecal short-chain fatty acids,

and altered microbiota populations (Vickers et al., 2001; Swanson et al., 2002a; 2002b; Flickinger et al., 2003; Propst et al., 2003; Zentek et al., 2003; Middelbos 2007a; 2007b; Beloshapka et al., 2012; 2013). Given data from humans, pigs, and rodents, a similar response was anticipated for RS. Whether it was due to the relatively short transit time or the simplistic large bowel anatomy (i.e., unsacculated colon; colonic fermenter vs. cecal as is in rodent models) of the dog, the microbiota present in the canine gut, or some other factor, the fermentation capacity of RS was not as extensive as expected in the dog. Source of RS may also be in question. The source of RS used in Chapter 6 (Hi-maize 260®), which is projected to be 60% RS and 40% digestible starch, is resistant to cooking and may be less fermentable than other sources. Because data from Chapter 5 seem to agree, however, it may just be that RS is too poorly and/or slowly fermented to elicit any measurable effects of GI health indices in dogs. Although it may be useful in reducing caloric content and promoting laxation, our research suggests that RS does not appear to possess prebiotic-like properties in dogs. However, polydextrose demonstrated beneficial fermentable properties and based on our research (Chapter 4) would be best fed at 1.5% or lower. At this inclusion level, fecal fermentative end-product concentrations and microbial populations were altered as expected, while fecal quality and laxation were maintained at optimal levels.

Although the results obtained through this research provide an in depth compositional analyses of carbohydrate sources used in pet diets, more research is needed to further evaluate the effects of feeding whole grains to pets. Additionally, this research increased our knowledge about the use of non-digestible, glucose-based carbohydrate sources, including polydextrose, whole grain-containing RS and dietary fiber, and corn-based RS, for use in canine diets. The results obtained herein may potentially impact future pet food formulations through the use of

alternative carbohydrate sources, or functional ingredient fractions derived from glucose-based carbohydrate sources.

CHAPTER 8: LITERATURE CITED

Abrams, S. A., I. J. Griffin, K. M. Hawthorne, L. Liang, S. K. Gunn, G. Darlington, and K. J. Ellis. 2005. A combination of prebiotic short- and long-chain inulin-type fructans enhances calcium absorption and bone mineralization in young adolescents. Am. J. Clin. Nutr. 82:471-476.

Aldrich, G. 2006. Oats: cheerios for pets? Petfood Ind. 49:40-41.

Aldrich, G. 2012. Pea proteins: Alternative protein source for petfoods? Petfood Ind. 54:54-55.

Allingham, R. P. 1982. Polydextrose—A new food ingredient: Technical aspects. In Chemistry of Foods and Beverages: Recent Developments, pp. 293-303. Academic Press, New York.

American Association of Cereal Chemists (AACC). 1983. Approved methods. 8th ed. Amer. Assoc. Cereal Chem., St Paul, MN

American Association of Cereal Chemists International (AACC). 2009. Whole grain task force and definition. Available from: http://www.aaccnet.org/definitions/wholegrain.asp.

American Pet Products Manufacturers Association Inc (APPMA). The 2013-2014 APPMA national pet owners survey. Greenwich, Conn: American Pet Products Manufacturers Association Inc, 2013.

Anderson, J. W. 2003. Whole grains protect against atherosclerotic cardiovascular disease. Proc. Nutr. Soc. 62:135-142.

Association of American Feed Control Officials (AAFCO). 2012. Official Publications. Association of American Feed Control Officials, Inc., Oxford, IN.

Association of Official Analytical Chemists (AOAC). 2006. Official methods of analysis. 17th ed. Assoc. Off. Anal. Chem., Gaithersburg, MD.

Bamforth, C. W., and A. H. P. Barclay. 1993. Malting technology and the uses of malt. In: A. W MacGregor and R. S. Bhatty, editors, Barley chemistry and technology. Amer. Assoc. Cereal Chem., St. Paul, MN. p. 297-354.

Barry, K. A.., I. S. Middelbos, B. M. Vester Boler, S. E. Dowd, J. S. Suchodolski, B. Henrissat, P. M. Coutinho, B. A. White, G. C. Fahey, Jr., and K. S. Swanson. 2012. Effects of dietary fiber on the feline gastrointestinal metagenome. J. Proteome Res. 11:5924-5933.

Beaton, L. 2014. Grain-free petfood: A top trend in the U.S. pet market. Petfood Ind. 56:68-70.

Beloshapka, A. N., L. Duclos, B. M. Vester Boler, and K. S. Swanson. 2012. Effects of inulin or yeast cell wall extract on nutrient digestibility, fecal fermentative end-product concentrations, and blood metabolite concentrations of adult dogs fed raw diets. Am. J. Vet. Res. 73: 1016-1023.

Beloshapka, A. N., S. E. Dowd, J. S. Suchodolski, J. M. Steiner, L. Duclos, and K. S. Swanson. 2013. Fecal microbial communities of healthy adult dogs fed raw meat-based diets with or without inulin or yeast cell wall extracts as assessed by 454 pyrosequencing. FEMS Microbiol. Ecol. 84:532-541.

Bird, A. R., C. Flory, D. A. Davies, S. Usher, and D. L. Topping. 2004. A novel barley cultivar (*Himalaya 292*) with a specific gene mutation in starch synthase Ila raises large bowel starch and short-chain fatty acids in rats. J. Nutr. 134:831-835.

Bird, A. R., M. Vuaran, I. Brown, and D. L. Topping. 2007. Two high-amylose maize starches with different amounts of resistant starch vary in their effects on fermentation, tissue and digesta mass accretion, and bacterial populations in the large bowel of pigs. Br J Nutr 97: 134-144.

Bradshaw, J. W. S. 2006. The evolutionary basis for the feeding behavior of domestic dogs (*Canis familiaris*) and cats (*Felis catus*). J. Nutr. 136:1927S-1931S.

Budde, E. F. 1952. The determination of fat in baked biscuit type of dog foods. J. Assoc. Off. Agric. Chem. 35:799-805.

Buléon, A., P. Colonna, V. Planchot, and S. Ball. 1998. Starch granules: structure and biosynthesis. Int. J. Biol. Macromol. 23:85-112.

Caporaso, J. G., J. Kuczynski, J. Stombaugh, K. Bittinger, F. D. Bushman, E. K. Costello, N Fierer, A. G. Peña, J. K. Goodrich, J. I. Gordon, G. A. Huttley, S. T. Kelley, D. Knights, J. E. Koenig, R. E. Ley, C. A. Lozupone, D. McDonald, B. D. Muegge, M. Pirrung, J. Reeder, J. R. Sevinsky, P. J. Turnbaugh, W. A. Walters, J. Widmann, T. Yatsunenko, J. Zaneveld, and R. Knight. 2010. QIIME allows analysis of high-throughput community sequencing data. Nat. Methods 7:335-336.

Carter, P. R., D. R. Hicks, E. S. Oplinger, J. D. Doll, L. G. Bundy, R. T. Schuler, and B. J. Holmes. 1989. Grain sorghum (milo). Alternative Field Crops Manual. University of Wisconsin-Extension, University of Minnesota: Center for Alternative Plant and Animal Products, and the Minnesota Extension Service. Available from: http://www.hort.purdue.edu/newcrop/afcm/sorghum.html

Case, L. P., D. P. Carey, and D. A. Hirakawa. 1995. Canine and feline nutrition: A resource for companion animal professionals. Mosby, Inc., St. Louis, MO.

Cephas, K. D., J. Kim, R. A. Mathai, K. A. Barry, S. E. Dowd, B. S. Meline, and K. S. Swanson. 2011. Comparative analysis of salivary bacterial microbiome diversity in edentulous infants and their mothers or primary care givers using pyrosequencing. PLoS ONE 6:e23503.

Chaban, B., M. G. Links, and J. E. Hill. 2012. A molecular enrichment strategy based on cpn60 for detection of epsilon-proteobacteria in the dog fecal microbiome. Microbiol. Ecol. 63:348-357.

Chaney, A. L., and E. P. Marbach. 1962. Modified reagents for determination of urea and ammonia. Clin. Chem. 8:130-132.

Chen, J., X. He, and J. Huang. 2014. Diet effects in gut microbiome and obesity. J. Food Sci. in press.

Cicero, A. F. G., and A. Gaddi. 2001. Rice bran oil and γ-oryzanol in the treatment of hyperlipoproteinaemias and other conditions. Phytother. Res. 15:277-289.

Collier, C. T., M. R. Smiricky-Tjardes, D. M. Albin. 2003. Molecular ecological analysis of porcine ileal microbiota responses to antimicrobial growth promoters. J. Anim. Sci. 81: 3035-3045.

Connolly, M. L., J. A. Lovegrove, and K. M. Tuohy. 2010. In vitro evaluation of the microbiota modulation abilities of different sized whole oat grain flakes. Anaerobe 16:483-488.

Connolly, M. L., J. A. Lovegrove, and K. M. Tuohy. 2012. In vitro fermentation characteristics of whole grain wheat flakes and the effect of toasting on prebiotic potential. J. Med. Food 15:33-43.

Costabile, A., A. Klinder, F. Fava, A. Napolitano, V. Fogliano, C. Leonard, G. R. Gibson, and K. M. Tuohy. 2008. Whole-grain wheat breakfast cereal has a prebiotic effect on the human gut microbiota: a double-blind, placebo-controlled, crossover study. Br. J. Nutr. 99:110-120.

Craig, S. A. 2008. Polydextrose: Analysis and physiological benefits. In: B. V. McCleary and L. Prosky, editors, Advanced Dietary Fibre Technology. Blackwell Science Ltd, Oxford, UK.

Craig, S. A. S., J. F. Holden, J. P. Troup, M. H. Auerbach, and H. I. Frier. 1998. Polydextrose as soluble fiber: Physiological and analytical aspects. Cereal Food World 43:370-376.

Cummings, J. H., and G. T. Macfarlane. 1991. The control and consequences of bacterial fermentation in the human colon. J. Appl. Bacteriol. 70:443-459.

de Godoy, M. R. C., K. R. Kerr, and G. C. Fahey, Jr. 2013. Alternative dietary fiber sources in companion animal nutrition. Nutrients 5:3099-3117.

Dehghan-Banadaky, M., R. Corbett, and M. Oba. 2007. Effects of barley grain processing on productivity of cattle. Anim. Feed Sci. Tech. 137:1-24.

De Moura, F. F., K. D. Lewis, and M. C. Falk. 2009. Applying the FDA definition of whole grains to the evidence for cardiovascular disease health claims. J. Nutr. 139:2220S-2226S.

DePlancke, B., O. Vidal, D. Ganessunker, S. M. Donovan, R. I. Mackie, and H. R. Gaskins. 2002. Selective growth of mucolytic bacteria including *Clostridium perfringens* in a neonatal piglet model of total parenteral nutrition. Am. J. Clin. Nutr. 76:1117-1125.

Desai, A. R., K. M Musil, A. P. Carr, and J. E. Hill. 2009. Characterization and quantification of feline fecal microbiota using cpn60 sequence-based methods and investigation of animal-to-animal variation in microbial population structure. Vet. Microbiol. 137:120-128.

Dust, J. M., A. M. Gajda, E. A. Flickinger, T. M. Burkhalter, N. R. Merchen, and G. C. Fahey, Jr. 2004. Extrusion conditions affect chemical composition and in vitro digestion of select food ingredients. J. Agric. Food Chem. 52:2989-2996.

Englyst, H. N., S. M. Kingman, and J. H. Cummings. 1992. Classification and measurement of nutritionally important starch fractions. Eur. J. Clin. Nutr. 46:S33-S50.

Engstrom, D. F., G. W. Mathison, and L. A. Goonewardene. 1992. Effect of β-glucan, starch, and fibre content and steam vs. dry rolling of barley grain on its degradability and utilization by steers. Anim. Feed Sci. Tech. 37:33-46.

Erwin, E. S., G. J. March, and E. M. Emery. 1961. Volatile fatty acid analyses of blood and rumen fluid by gas chromatography. J. Dairy Sci. 44:1768-1771.

Food and Agriculture Organization of the United Nations (FAO). 2014. World food situation: FAO cereal supply and demand brief. Available from: www.fao.org/worldfoodsituation/csdb/en

Fava, F., H. Mäkivuokko, H. Siljander-Rasi, H. Putaala, K. Tiihonen, J. Stowell, K. Tuohy, G. Gibson, and N. Rautonen. 2007. Effect of polydextrose on intestinal microbes and immune functions in pigs. Brit. J. Nutr. 98:123-133.

Figdor, S. K., and J. R. Bianchine. 1983. Caloric utilization and disposition of [^{14}C]polydextrose in man. J. Agric. Food Chem. 31:389-393.

Flickinger, E. A., E. M. W. C. Schreijen, A. R. Patil, H. S. Hussein, C. M. Grieshop, N. R. Merchen, and G. C. Fahey, Jr. 2003. Nutrient digestibilities, microbial populations, and

protein catabolites as affected by fructan supplementation of dog diets. J. Anim. Sci. 81:2008-2018.

Fuller, M. F.1991. *In vitro* Digestion for Pigs and Poultry. C.A.B. International, Oxfordshire.

Gajda, M., E. A. Flickinger, C. M. Grieshop, L. L. Bauer, N. R. Merchen, and G. C. Fahey, Jr. 2005. Corn hybrid affects in vitro and in vivo measures of nutrient digestibility in dogs. J. Anim. Sci. 83:160-171.

German, A. J., S. L. Holden, G. L. Moxham, K. L. Holmes, R. M. Hackett, and J. M. Rawlings. 2006. A simple, reliable tool for owners to assess the body condition of their dog or cat. J. Nutr. 136:2031S-2033S.

Gibson, G. R., and M. B. Roberfroid. 1995. Dietary modulation of the colonic microbiota: Introducing the concept of prebiotics. J. Nutr. 125:1401-1412.

Gibson, G. R., H. M. Probert, J. A. E. Van Loo, R. A. Rastall, and M. B. Roberfroid. 2004. Dietary modulation of the human colonic microbiota: Updating the concept of prebiotics. Nutr. Res. Rev. 17:257-259.

Gibson, G. R., K. P. Scott, R. A. Rastall, K. M. Tuohy, A. Hotchkiss, A. Dubert-Ferrandon, M. Gareau, E. F. Murphy, D. Saulnier, G. Loh, S. Macfarlane, N. Delzenne, Y. Ringel, G. Kozianowsk, R. Dickman, I. Lenoir-Wijnkoop, C. Walker, and R. Buddington. 2010. Dietary prebiotics: current status and new definition. IFIS Functional Foods Bulletin 7:1-19.

Ginsberg, J. 2007. Development of dehydration processes: Potato flakes. American Chemical Society.

Goudez, R., M. Weber, V. Biourge, and P. Nguyen. 2011. Influence of different levels and sources of resistant starch on faecal quality of dogs of various body sizes. Br. J. Nutr. 106:S211-S215.

Grieshop, C. M., E. A. Flickinger, K. J. Bruce, A. R. Patil, G. L. Czarnecki-Maulden, and G. C. Fahey, Jr. 2004. Gastrointestinal and immunological responses of senior dogs to chicory and mannan-oligosaccharides. Arch. Anim. Nutr. 58:483-493.

Güçlü-Üstündag, Ö, and G. Mazza. 2007. Saponins: properties, applications and processing. Crit. Rev. Food Sci. Nutr. 47:231-258.

Haenen, D., J. Zhang, C. S. da Silva, G. Bosch, I. M. van der Meer, J. van Arkel, J. J. van den Borne, O. Pérez Gutiérrez, H. Smidt, B. Kemp, M. Müller, and G. J. Hooiveld. 2013. A diet high in resistant starch modulates microbiota composition, SCFA concentrations, and gene expression in pig intestine. J. Nutr. 143:274-283.

Handl, S., S. E. Dowd, J. Garcia-Mazcorro, J. M. Steiner, and J. S. Suchodolski. 2011. Massive parallel 16S rRNA gene pyrosequencing reveals highly diverse fecal bacterial and fungal communities in healthy dogs and cats. FEMS Microbiol. Ecol. 76:301-310.

Haraldsson, A. K., L. Rimsten, M. L. Alminger, R. Andersson, T. Andlid, P. Åman, and A. S. Sandberg. 2004. Phytate content is reduced and β-glucanase activity suppressed in malted barley steeped with lactic acid at high temperature. J. Sci. Food Agric. 84:653-662.

Hasjim, J., E. Li, and S. Dhital. 2013. Milling of rice grains: Effects of starch/flour structures on gelatinization and pasting properties. Carbohydr. Polym. 92:862-690.

Hernot, D. C., T. W. Boileau, L. L. Bauer, K. S. Swanson, and G. C. Fahey, Jr. 2008. In vitro digestion characteristics of unprocessed and processed whole grains and their components. J. Agric. Food Chem. 56:10721-10726.

Hironaka, R., N. Kimura, and G. C. Kozub. 1979. Influence of feed particle size on rate and efficiency of gain, characteristics of rumen fluid and rumen epithelium, and numbers of rumen protozoa. Can. J. Anim. Sci. 589:395-402.

Hooda, S., B. M. Vester Boler, M. C. Rossoni Serao, J. M. Brulc, M. A. Staeger, T. W. Boileau, S. E. Dowd, G. C. Fahey, Jr., and K. S. Swanson. 2012. 454 Pyrosequencing reveals a shift in fecal microbiota of healthy adult men consuming polydextrose or soluble corn fiber. J. Nutr. 142:1259-1265.

Hooda, S., B. M. Vester Boler, K. R. Kerr, S. E. Dowd, and K. S. Swanson. 2013. The gut microbiome of kittens is affected by dietary protein:carbohydrate ratio and associated with blood metabolite and hormone concentrations. Br. J. Nutr. 109:1637-1646.

Institute of Medicine. 2001. Dietary Reference Intakes: Proposed definitions of dietary fiber. Washington, D.C.: National Academy Press.

Institute of Medicine of the National Academies (IMNA). 2005. Dietary, functional, and total fiber. In: Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids, pp. 339-421 Washington, D.C.: The National Academies Press.

Janeczko, S., D. Atwater, E. Bogel, A. Greiter-Wilke, A. Gerold, M. Baumgart, H. Bender, P. L. McDonough, S. P. McDonough, R. E. Goldstein, and S. W. Simpson. 2008. The relationship of mucosal bacteria to duodenal histopathology, cytokine mRNA, and clinical disease activity in cats with inflammatory bowel disease. Vet. Microbiol. 128:178-193.

Jia, J., N. Frantz, C. Khoo, G. R. Gibson, R. A. Rastall, and A. L. McCartney. 2010. Investigation of the faecal microbiota associated with canine chronic diarrhea. FEMS Microbiol. Ecol. 71:304-312.

Jie, Z., L. Bang-yao, X. Ming-jie, L. Hai-Wei, Z. Zu-Kang, W. Ting-Song, and S. A. Craig. 2000. Studies on the effects of polydextrose intake on physiologic functions in Chinese people. Am. J. Clin. Nutr. 72:1503-1509.

Jonnalagadda, S., D. P. Lee, and R. Menon. 2011. Metabolomics analysis of grain samples. FASEB J 25:581.2.

Kalra, S., and S. Joad. 2000. Effect of dietary barley β-glucan on cholesterol and lipoprotein fractions in rats. J. Cereal Sci. 31:141-145.

Kaczmarczyk, M. M., M. J. Miller, and G. G. Freund. 2012. The health benefits of dietary fiber: beyond the usual suspects of type 2 diabetes mellitus, cardiovascular disease and colon cancer. Metabolism 61:1058-1066.

Kanakupt, K., B. M. Vester Boler, B. R. Dunsford, and G. C. Fahey, Jr. 2011. Effects of short-chain fructooligosaccharides and galactooligosaccharides, individually and in combination, on nutrient digestibility, fecal fermentative metabolite concentrations, and large bowel microbial ecology of healthy adult cats. J. Anim. Sci. 89:1376-1384.

Kim, Y. S., O. D. Tsa, A. Morita, and A. Bella. 1982. Effect of sodium butyrate and three human colorectal adenocarcinoma cell lines in culture. Falk. Symp. 31:317-323.

Kerr, K. R., A. N. Beloshapka, and K. S. Swanson 2013. 2011 and 2013 early careers achievement awards: Use of genomic biology to study companion animal intestinal microbiota. J. Anim. Sci. 91:2504-2511.

Kim, H., K. S. Stote, K. M. Behall, K. Spears, B. Vinyard, and J. M. Conway. 2009. Glucose and insulin responses to whole grain breakfasts varying in soluble fiber, β-glucan: a dose response study in obese women with increased risk for insulin resistance. Eur. J. Nutr. 48:170-175.

Knapp, B. K., C. M. Parsons, K. S. Swanson, and G. C. Fahey, Jr. 2008. Physiological responses to novel carbohydrates as assessed using canine and avian models. J. Agric. Food Chem. 56:7999-8006.

Konikoff, M. R., and L. A. Denson. 2006. Role of fecal calprotectin as a biomarker of intestinal inflammation in inflammatory bowel disease. Inflamm. Bowel Dis. 12:524-534.

Kozich, J. J., S. L. Westcott, N. T. Baxter, S. K. Highlander, and P. D. Schloss. 2013. Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the MiSeq Illumina sequencing platform. Appl. Environ. Microbiol. 79:5112-5120.

Laflamme, D. P., S. K. Abood, A. J. Fascetti, L. M. Fleeman, L. M. Freeman, K. E. Michel, C. Bauer, B. L. E. Kemp, J. R. Van Doren, and K. N. Willoughby. 2008. Pet feeding practices of dog and cat owners in the United States and Austrailia. J. Am. Vet. Med. Assoc. 232:687-694.

Lahtinen, S. J., K. Knoblock, A. Drakoularakou, M. Jacob, J. Stowell, G. R. Gibson, and A. C. Ouwehand. 2010. Effect of molecule branching and glycosidic linkage on the degradation of polydextrose by gut microbiota. Biosci. Biotechnol. Biochem. 74:2016-2021.

Liu, L., L. Zubik, F. W. Collins, M. Marko, and M. Meydani. 2004. The antiatherogenic potential of oat phenolic compounds. Atherosclerosis 175:39-49.

Liu, W. T., T. L. Marsh, H. Cheng, and L. J. Forney. 1997. Characterization of microbial diversity by determining terminal restriction fragment length polymorphisms of genes encoding 16S rRNA. Appl. Environ. Microbiol. 63:4516-4522.

Lohse, M., A. M. Bolger, A. Nagel, A. R. Fernie, J. E. Lunn, M. Stitt, and B. Usadel. 2012. RobiNA: a user-friendly, integrated software solution for RNA-Seq-based transcriptomics. Nucleic Acids Res. 40:W622-W627.

Macfarlane, G. T., and S. Macfarlane. 2012. Bacteria, colonic fermentation, and gastrointestinal health. J. AOAC Int. 95:50-60.

Mäkivuokko, H., H. Kettunen, M. Saarinen, T. Kamiwaki, Y. Yokoyama, J. Stowell, and N. Rautonen. 2007. The effect of cocoa and polydextrose on bacterial fermentation in gastrointestinal tract simualations. Biosci. Biotechnol. Biochem. 71:1834-1843.

Mäkivuokko, H., J. Nurmi, P. Nurminen, J. Stowell, and N. Rautonen. 2005. *In vitro* effects on polydextrose by colonic bacteria and Caco-2 cell cyclooxygenase gene expression. Nutr. Cancer 52:94-104.

Malinen, E., A. Kassinen, T. Rinttilä T, and A. Palva. 2003. Comparison of real-time PCR with SYBR Green I or 5'-nuclease assays and dot-blot hybridization with rDNA-targeted oligonucleotide probes in quantification of selected faecal bacteria. Microbiol. 149:269-277.

Mathison, G. W. 1996. Effects of processing on the utilization of grain by cattle. Anim. Feed Sci. Tech. 58:113-125.

Matsuki, T., K. Watanabe, J. Fujimoto, Y. Miyamoto, T. Takada, K. Matsumoto, H. Oyaizu, and R. Tanaka. 2002. Development of 16S rDNA gene-targeted group specific primers for the detection and identification of predominant bacteria in human feces. Appl. Environ. Microbiol. 68:5445-5451.

Matsuura, H. 2001. Saponins in garlic as modifiers of the risk of cardiovascular disease. J. Nutr 131:1000S-1005S.

McOrist, A.L., R. B. Miller, A. R. Bird, J. B. Keogh, M. Noakes, D. L. Topping, and M. A. Conlon. 2011. Fecal butyrate levels vary widely among individuals but are usually increased by a diet high in resistant starch. J. Nutr. 141:883-889.

Middlebos, I. S., N. D. Fastinger, and G. C. Fahey, Jr. 2007a. Evaluation of fermentable oligosaccharides in diets fed to dogs in comparison to fiber standards. J. Anim. Sci. 85:3033-3044.

Middelbos, I. S., M. R. Godoy, N. D. Fastinger, and G. C. Fahey, Jr. 2007b. A dose-response evaluation of spray-dried yeast cell wall supplementation of diets fed to adult dogs: Effects on nutrient digestibility, immune indices, and fecal microbial populations. J. Anim. Sci. 85:3022-3032.

Middelbos, I. S., B. M. Vester Boler, A. Qu, B. A. White, K. S. Swanson, and G. C. Fahey, Jr. 2010. Phylogenetic characterization of fecal microbial communities of dogs fed diets with or without supplemental dietary fiber using 454 pyrosequencing. PLoS ONE 5:e9768.

Moloney, R. D., L. Desbonnet, G. Clarke, T. G. Dinan, and J. F. Cryan. 2014. The microbiome: stress, health and disease. Mamm. Genome 25:49-74.

Morrison, W. R., and J. Karkalas. 1990. In: P. M. Dey and J. B. Harborne, editors, Methods in Plant Biochemistry. Academic Press, London, vol. 2. pp. 323-352.

Mua, J. P., and D. S. Jackson. 1997. Fine structure of corn amylose and amylopectin fractions with various molecular weights. J. Ag. Food Chem. 45:3840-3847.

Muir, J. G., and K. O’Dea. 1992. Measurement of resistant starch: Factors affecting the amount of starch escaping digestion in vitro. Am. J. Clin. Nutr. 56:123-127.

Muir, J.G., and K. O’Dea. 1993. Validation of an in vitro assay for predicting the amount of starch that escapes digestion in the small intestine of humans. Am. J. Clin. Nutr. 57:540-546.

Murray, S. M., E. A. Flickinger, A. R. Patil, N. R. Merchen, J. L. Brent, Jr., and G. C. Fahey, Jr. 2001. In vitro fermentation characteristics of native and processed cereal grains and potato starch using ileal chyme from dogs. J. Anim. Sci. 79:435-444.

Mussato, S. I., and I. M. Mancilha. 2006. Non-digestible oligosaccharides: A review. Carbohyd. Polym. 68:587-597.

Muyzer, G., E. C. de Waal, and A. G. Uitterlinden. 1993. Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes encoding for 16S rRNA. Appl. Environ. Microbiol. 59:695-700.

National Research Council (NRC). 2006. Nutrient requirements for dogs and cats. Washington, D.C.: National Academy Press.

Neeson, R. 2011. Organic spelt production. Industry and Investment NSW. Available from: www.industry.nsw.gov.au/publications

Nepelska, M., A. Cultrone, F. Béguet-Crespel. K. Le Roux, J. Doré, V. Arulampalam, and H. M. Blottière. 2012. Butyrate produced by commensal bacteria potentiates phorbol esters induced AP-1 response in human intestinal epithelial cells. PLoS ONE 7:e52869.

Oelke, E. A., E. S. Oplinger, D. H. Putnam, B. R. Durgan, J. D. Doll, and D. J. Undersander. 1990. Millets. Alternative Field Crops Manual. University of Wisconsin-Extension, University of Minnesota: Center for Alternative Plant and Animal Products, and the Minnesota Extension Service. Available from: http://www.hort.purdue.edu/newcrop/afcm/millet.html

Oelke, E. A., D. H. Putnam, T. M. Teynor, and E. S. Oplinger. 1992. Quinoa. Alternative Field Crops Manual. University of Wisconsin-Extension, University of Minnesota: Center for Alternative Plant and Animal Products, and the Minnesota Extension Service. Available from: http://www.hort.purdue.edu/newcrop/afcm/quinoa.html

Okarter, N., and R. H. Liu. 2010. Health benefits of whole grain phytochemicals. Crit. Rev. Food Sci. Nutr. 50:193-208.

Oplinger, E. S., E. A. Oelke, A. R. Kaminski, K. A. Kelling, J. D. Doll, B. R. Durgan, and R. T. Schuler. 1990. Spelt. Alternative Field Crops Manual. University of Wisconsin-Extension, University of Minnesota: Center for Alternative Plant and Animal Products, and the Minnesota Extension Service. Available from: http://www.hort.purdue.edu/newcrop/afcm/spelt.html

Otten, J. J., J. P. Hellwig, and L. D. Meyers. 2006. Pages 111-121 in Dietary Reference Intakes: The Essential Guide to Nutrient Requirements: Fiber. Washington DC: Natl. Acad. Press.

Ouwehand, A. C., M. Derrien, W. de Vos, K. Tiihonen, and N. Rautonen. 2005. Prebiotics and other microbial substrates for gut functionality. Curr. Opin. Biotech. 16:212-217.

Owens, F. N., D. S. Secrist, W. J. Hill, and D. R. Gill. 1997. The effect of grain source and grain processing on performance of feedlot cattle: a review. J. Anim. Sci. 75:868-879.

Petrosino, J. F., S. Highlander, R. A. Luna, R. A. Gibbs, and J. Versalovic. 2009. Metagenomic pyrosequencing and microbial identification. Clin. Chem. 55:856-866.

Phillips-Donaldson, D. 2012. Grow pet ownership to grow the market. Petfood Ind. 54:8.

Phillips-Donaldson, D. 2013. Why dry petfood rules the world. Petfood Ind. 55:6.

Pinkel, D., T. Straume, and J. W. Gray. 1986. Cytogenetic analysis using quantitative, high-sensitivity, fluorescence hybridization. Proc. Natl. Acad. Sci. USA 83:2934-2938.

Probert, H. M., J. H. A. Apajalahti, N. Rautonen, J. Stowell, and G. R. Gibson. 2004. Polydextrose, lactitol, and fructo-oligosaccharide fermentation by colonic bacteria in a three-stage continuous culture system. Appl. Environ. Microbiol. 20:4505-4511.

Propst, E. L., E. A. Flickinger, L. L. Bauer, N. R. Merchen, and G. C. Fahey, Jr. 2003. A dose-response experiment evaluating the effects of oligofructose and inulin on nutrient digestibility, stool quality, and fecal protein catabolites in healthy adult dogs. J. Anim. Sci. 81:3057-3066.

Prosky, L., N. G. Asp, I. Furda, J. W. DeVries, T. F. Schweizer, and B. F. Harland. 1985. Determination of total dietary fiber in foods and products: Collaborative study. J. Assoc. Off. Anal. Chem. 67:1044-1052.

Prosky, L., N. G. Asp, T. F. Schweizer, J. W. De Vries, and I. Fruda. 1992. Determination of insoluble and soluble dietary fiber in foods and food products: Collaborative study. J. AOAC 75:360-367.

Putnam, D. H., E. A. Oelke, E. S. Oplinger, J. D. Doll, and J. B. Peters. 1990. Annual Canarygrass. Alternative field crops manual. University of Wisconsin-Extension, University of Minnesota: Center for Alternative Plant and Animal Products, and the Minnesota Extension Service. Available from: http://www.hort.purdue.edu/newcrop/afcm/cangrass.html

Ragaee, S., E. S. Abdel-Aal, and M. Noaman. 2006. Antioxidant activity and nutrient composition of selected cereals for food use. Food Chem. 98:32-38.

Richards, J. D., J. Gong, and C. F. M. de Lange. 2005. The gastrointestinal microbiota and its role in monogastric nutrition and health with an emphasis on pigs: Current understanding, possible modulations and new technologies for ecological studies. Can. J. Anim. Sci. 85:421-435.

Rideout, T. C., L. Qiang, P. Wood P, and M. Z. Fan. 2008. Nutrient utilisation and intestinal fermentation are differentially affected by the consumption of resistant starch varieties and conventional fibres in pigs. Br. J. Nutr. 99:984-992.

Rigottier-Gois, L., A. G. Le Bourhis, G. Gramet, V. Rochet, and J. Doré. 2003. Fluorescent hybridization combined with flow cytometry and hybridization of total RNA to analyse

the composition of microbial communities in human fecal samples using 16S rRNA probes. FEMS Microbiol. Ecol. 43:237-245.

Risely-Curtiss, C. 2010. Social work practitioners and the human companion animal bond. A National Study. Soc. Work 55:38-46.

Ritchie, L. E., J. M. Steiner, and J. S. Suchodolski. 2008. Assessment of microbial diversity along the feline intestinal tract using 16S rRNA gene analysis. FEMS Microbiol. Ecol. 66:590-598.

Roberfroid, M. 2007. Prebiotics: The concept revisited. J. Nutr. 137:830S-837S.

Roberfroid, M. R., G. R. Gibson, L. Hoyles, A. L. McCartney, R. Rastall, I. Rowland, D. Wolvers, B. Watzl, H. Szajewska, B. Stahl, F. Guarner, F. Respondek, K. Whelan, V. Coxam, M. J. Davicco, L. Léotoing, Y. Wittrant, N. M. Delzenne, P. D. Cani, A. M. Neyrinck, and A. Meheust. 2010. Prebiotic effects: metabolic and health benefits. Brit. J. Nutr. 104:S1-S63.

Rolfe, V. E., C. A. Adams, R. F. Butterwick, and R. M. Batt. 2002. Relationship between faecal character and intestinal transit time in normal dogs and diet-sensitive dogs. J. Small Anim. Pract. 43:290-294.

Rong, N., L. Ausman, and R. Nicolosi. 1997. Oryzanol decreases cholesterol absorption and aortic fatty streaks in hamsters. Lipids 32:303-309.

Sadiq Butt, M., M. Tahir-Nadeem, M. K. Khan, R. Shabir, and M. S. Butt. 2008. Oat: unique among the cereals. Eur. J. Nutr. 47:68-79.

Sajilata, M. G., R. S. Singhal, and P. R. Kulkarni. 2006. Resistant starch: a review. Compr. Rev. Food Sci. Food Saf. 5:1-17.

Samal, L., A. Pattanaik, C. Mishra. 2011. Application of molecular tools for gut health of pet animals: A review. J. Adv. Vet. Res. 1:38-46.

Saunders, R. M. 1990. The properties of rice bran as a foodstuff. Cereal Foods World. 35:632-636.

Scheppach, W., and F. Weiler. 2004. The butyrate story: old wine in new bottles? Curr. Opin. Clin. Nutr. Metab. Care 7:563-567.

Schütte, U. M. E., Z. Abdo, S. J. Bent, C. Shyu, C. J. Williams, J. D. Pierson, and L. J. Forney. 2008. Advances in the use of terminal restriction fragment length polymorphism (T-RFLP) analysis of 16S rRNA genes to characterize microbial communities. Appl. Microbiol. Biotechnol. 80:365-380.

Seetharamaiah, G. S., and N. Chandrasekhara. 1993. Comparative hypocholesterolemic activities of oryzanol, curcumin and ferulic acid in rats. J. Food Sci. Tech. Mys. 30:249-252.

Shams-Ud-Din, M. D., and K. R. Bhattacharaya. 1978. On the meaning of the degree of milling of rice. J. Food Technol. 13:99-105.

Shewry, P. R., M. J. Hawkesford, V. Piironen, A. Lampi, K. Gebruers, D. Boros, A. A. M. Andersson, P. Åman, M. Rakszegi, Z. Bedo, and J. L. Ward. 2013. Natural variation in grain composition of wheat and related cereals. J. Agric. Food Chem. 61:8295-8303.

Simpson, K.W., B. Dogan, M. Rishniw, R. E. Goldstein, S. Klaessig, P. L. McDonough, A. J. German, R. M. Yates, D. G. Russell, S. E. Johnson, D. E. Berg, J. Harel, G. Bruant, S. P. McDonough, and Y. H. Schukken. 2006. Adherent and invasive *Escherichia coli* is associated with granulomatous colitis in boxer dogs. Infect. Immun. 74:4778-4792.

Singh, R., S. De, and A. Belkheir. 2013. Avena sativa (oat), a potential neutraceutical and therapeutic agent: an overview. Critical Reviews in Food Science and Nutrition. 53:126-144.

Slavin, J. L. 1987. Dietary fiber: Classification, chemical analyses, and food sources. J. Am. Diet. Assoc. 87:1164-1171.

Slavin, J. 2004. Whole grains and human health. Nutr. Res. Rev. 17:99-110.

Smith, E. A., and G. T. Macfarlane. 1997. Formation of phenolic and indolic compounds by anaerobic bacteria in the human large intestine. Microb. Ecol. 33:180-188.

Spears, J. K., and G. C. Fahey, Jr. 2004. Resistant starch as related to companion animal nutrition. J. AOAC Int. 87:787-791.

Stevens, E. J., K. W. Armstrong, H. J. Bezar, W. B. Griffin, and J. G Hampton. 2004. Fodder oats: An overview. In: J. M. Suttie and S. G. Reynolds, editors, Fodder Oats: a world overview. Rome, Italy. Food and Agriculture Organization of the United Nations. Plant Production and Protection Series No. 33. Available from: www.fao.org/docrep

Suchodolski, J. S. 2011a. Companion animal symposium: Microbes and gastrointestinal health of dogs and cats. J. Anim. Sci. 89:1520-1530.

Suchodolski, J. S. 2011b. Intestinal microbiota of dogs and cats: A bigger world than we thought. Vet. Clin. North Am. Small Anim. Pract. 41:261-272.

Suchodolski, J., J. Camacho, and J. Steiner. 2008. Analysis of bacterial diversity in the canine duodenum, jejunum, ileum, and colon by comparative 16S rRNA gene analysis. FEMS Microbiol. Ecol. 66:567-578.

Suchodolski, J., S. E. Dowd, E. Westermarck, J. M. Steiner, R. D. Wolcott, T. Spillmann, and J. A. Harmoinen. 2009. The effect of the macrolide antibiotic tylosin on microbial diversity in the canine small intestine as demonstrated by massive parallel 16S rRNA gene sequencing. BMC Microbiol. 9:210.

Suchodolski,J. S., S. E. Dowd, V. Wilke, J. M. Steiner, and A. E. Jergens. 2012a. 16S rRNA gene pyrosequencing reveals bacterial dysbiosis in the duodenum of dogs with idiopathic inflammatory bowel disease. PLoS ONE 7:e39333.

Suchodolski, J. S., M. E. Markel, J. F. Garcia-Mazcorro, S. Unterer, R. M. Heilmann, S. E. Dowd, P. Kachroo, I. Ivanov, Y. Minamoto, E. M. Dillman, J. M. Steiner, A. K. Cook, and L. Toresson. 2012b. The fecal microbiome in dogs with acute diarrhea and idiopathic inflammatory bowel disease. PLoS ONE 7:e51907.

Suchodolski, J. S., P. G. Xenoulis, C. G. Paddock, J. M. Steiner, and A. E. Jergens. 2010. Molecular analysis of the bacterial microbiota in duodenal biopsies from dogs with idiopathic inflammatory bowel disease. Vet. Microbiol. 142:394-400.

Suh, M. H., S. H. Yoo, P. S. Chang, and H. G. Lee. 2005. Antioxidative activity of microencapsulated gamma-oryzanol on high cholesterol-fed rats. J. Ag. Food Chem. 53:9747-9750.

Swanson, K. S., S. E. Dowd, J. S. Suchodolski, I. S. Middelbos, B. M. Vester, K. A. Barry, K. E. Nelson, M. Torralba, B. Henrissat, P. M. Coutinho, I. K. Cann, B. A. White, and G. C. Fahey, Jr. 2011. Phylogenetic and gene-centric metagenomics of the canine intestinal microbiome reveals similarities with humans and mice. ISME J. 5:639-649.

Swanson, K. S., C. M. Grieshop, E. A. Flickinger, L. L. Bauer, J. Chow, B. W. Wolf, K. A. Garleb, and G. C. Fahey, Jr. 2002a. Fructooligosaccharides and *Lactobacillus acidophilus* modify gut microbial populations, total tract nutrient digestibilities and fecal protein catabolite concentrations in healthy adult dogs. J. Nutr. 132:3721-3731.

Swanson, K. S., C. M. Grieshop, E. A. Flickinger, L. L. Bauer, H. P. Healy, K. A. Dawson, N. R. Merchen, and G. C. Fahey, Jr. 2002b. Supplemental fructooligosaccharides and mannanoligosaccharides influence immune function, ileal and total tract nutrient digestibilities, microbial populations and concentrations of protein catabolites in the large bowel of dogs. J. Nutr. 132:980-989.

Tachon, S., J. Zhou, M. Keenan, R. Martin, and M. L. Marco. 2013. The intestinal microbiota in aged mice is modulated by dietary resistant starch and correlated with improvements in host responses. FEMS Microbiol. Ecol. 83:299-309.

Taylor, J. 2011. Update: the global petfood market for 2011. Petfood Ind. 53:22-24.

Taylor, J. 2014. Petfood nutrition trends in 2013. Petfood Ind. 56:34-35.

Tester, R. F., J. Karkalas, and X. Qi. 2004. Starch—composition, fine structure and architecture. J. Cereal Sci. 39:151-165.

Thivend, P., M. Christiane, and A. Guilbot. 1972. Determination of starch with glucoamylase. Methods Carbohydr. Chem. 6:100-105.

Topping, D. L., and P. M. Clifton. 2001. Short-chain fatty acids and human colonic function: roles of resistant starch and nonstarch polysaccharides. Physiol. Rev. 81:1031-1064.

Twomey, L. N., D. W. Pethick, J. B. Rowe, M. Choct, J. R. Pluske, W. Brown, and M. C. Laviste. 2002. The use of sorghum and corn as alternatives to rice in dog foods. J. Nutr. 132:1704S-1705S.

USDA, Agricultural Research Service. 2013. USDA National Nutrient Database for Standard Reference. Nutrient Data Laboratory Home Page. Available from: http://www.ars.usda.gov/ba/bhnrc/ndl.

Vester Boler, B. M., M. C. Rossoni Serao, L. L. Bauer, M. A. Staeger, T. W. Boileau, K. S. Swanson, and G. C. Fahey, Jr. 2011. Digestive physiological outcomes related to polydextrose and soluble maize fibre consumption by healthy adult men. Br. J. Nutr. 106:1864-1871.

Vickers, R. J., G. D. Sunvold, R. L. Kelley, and G. A. Reinhart. 2001. Comparison of fermentation of selected fructooligosaccharides and other fiber substrates by canine colonic microflora. Amer. J. Vet. Res. 62:609-615.

Vieira, A. T., M. M. Teixeira, and F. S. Martins. 2013. The role of probiotics and prebiotics in inducing gut immunity. Front. Immunol. 4:1-12.

Wang, R. F., W. W. Cao, W. Franklin, W. Campbell, and C. E. Cerniglia. 1994. A 16S rDNA-based PCR method for rapid and specific detection of *Clostridium perfringens* in food. Mol. Cell. Probes 8:131-137.

Weaver, C. M., B. R. Martin, C. H. Nakatsu, A. P. Armstrong, A. Clavijo, L. D. McCabe, G. P. McCabe, S. Duignan, M. N. C. Schoterman, and E. G. H. M. van den Heuvel. 2011. Galactooligosaccharides improve mineral absorption and bone properties in growing rats through gut fermentation. J. Agric. Food Chem. 59:6501-6510.

Weinstock, G. M. 2012. Genomic approaches to studying the human microbiota. Nature 489:250-256.

Welch, R., J. Brown, and J. Leggett. 2000. Interspecific and intraspecific variation in grain and groat characteristics of wild oat (Avena) species: Very high groat (1-3), (1-4)-b-D-glucan in an Avena atlantica genotype. J Cereal Sci 31:273-279.

Willard, M. D., R. B. Simpson, N. D. Cohen, and J. S. Clancy. 2000. Effects of dietary fructooligosaccharide on selected bacterial populations in feces of dogs. Amer. J. Vet. Res. 61:820-825.

Wilson, T. A., R. J. Nicolosi, B. Wollfrey, and D. Kritchevsky. 2007. Rice bran oil and oryzanol reduce plasma lipid and lipoprotein cholesterol concentrations and aortic cholesterol ester accumulation to a greater extent than ferulic acid in hypercholesterolemic hamsters. J. Nutr. Biochem. 18:105-112.

Winfield, K., M. Hall, and B. Paynter. 2007. Milling oat and feed oat quality: What are the differences. Bulletin 4703. Department of Agriculture and Food, Government of Western Austrailia, pp. 10-12.

Xenoulis, P.G., B. Palculict, K. Allenspach, J. M. Steiner, A. M. Van House, and J. S. Suchodolski. 2008. Molecular-phylogenetic characterization of microbial communities imbalances in the small intestine of dogs with inflammatory bowel disease. FEMS Microbiol. Ecol. 66:579-589.

Yu, Z., and M. Morrison. 2004. Improved extraction of PCR-quality community DNA from digesta and fecal samples. Biotechniques 36:808-812.

Zeng, H., D. L. Lazarova, and M. Bordonaro. 2014. Mechanisms linking dietary fiber, gut microbiota and colon cancer prevention. World J. Gastrointest. Oncol. 6:41-51.

Zentek, J., B. Marquart, T. Pietrzak, O. Ballevre, and F. Rochat. 2003. Dietary effects on bifidobacteria and *Clostridium perfringens* in the canine intestinal tract. J. Anim. Physiol. Anim. Nutr. 87:397-407.

Zhang, G., and B. R. Hamaker. 2009. Slowly digestible starch: concept, mechanism, and proposed extended glycemic index. Crit. Rev. Food Sci. Nutr. 49:852-867.

Zhang, J., K. Kobert, T. X. Flouri, and A. Stamatakis. 2014. PEAR: a fast and accurate Illumina Paired-End read merger. Bioinformatics 30:614-620.

Zinn, R. A. 1993. Influence of processing on the comparative feeding value of barley for feedlot cattle. J. Anim. Sci. 71:3-10.

Zoetendal, E. G., A. D. L. Akkermans, and W. M. de Vos. 1998. Temperature gradient gel electrophoresis analysis from human fecal samples reveals stable and host-specific communities of active bacteria. Appl. Environ. Microbiol. 64:3854-3859.