Production, absorption, and metabolic fate of fatty acids in pigs fed high-fiber diets

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A Thesis Submitted to the Faculty of Graduate Studies of The University of Manitoba

In partial fulfilment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

in the

Department of Animal Science

Faculty of Agricultural and Food Sciences

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ABSTRACT

In recent years, the use of agro-industrial co-products has significantly increased in pig diets resulting in increased dietary fiber content. It is, however, intriguing that nutrient utilization and performance are depressed if pigs consume nutritionally balanced high-fiber diets that are composed of co-products plus supplementary fat. The first study investigated the effects of adding soluble and insoluble fiber in growing pig diets on growth performance, fecal fat digestibility, serum lipids, and concentrations of volatile fatty acids (VFA) and bile acids (BA), histomorphological characteristics, and microbiota composition in the intestines. Soluble fiber depressed growth performance. Soluble and insoluble fiber increased intestinal VFA and BA contents, but reduced fecal fat digestibility. Soluble and insoluble fiber induced variable effects on histological characteristics of small intestines, and altered the composition and function of digesta- and mucosa-associated microbiota in the ileum and cecum of growing pigs. The second study investigated the effects of soluble and insoluble fiber on production and absorption of hindgut VFA, digestibility of DF and fatty acids (FA), and flows of FA and BA in the terminal ileum and feces. Soluble and insoluble fiber reduced, ileal digested energy, FA digestibility, and absorption, gastrointestinal flows of FA and excretion of BA and induced variable effects on digestibility of DF fractions, hindgut VFA production and fecal flows of unsaturated FA. The third study investigated the interactive effects of DF solubility and lipid type on digestibility of DF and FA, and flows of FA and BA in the ileum, cecum and colon, and VFA production and absorption in the cecum and colon of growing pigs. The production and absorption of VFA and DF fermentability in the cecum and colon was greater in soluble fiber-fed pigs compared with pigs fed insoluble fiber, and animal fat supplementation depressed fermentation compared to vegetable oil. The interaction between DF solubility and lipid type modulates digestibility of lipids, flows of FA and BA and fermentability of DF fractions but differs for soluble and insoluble fiber, and saturated and unsaturated fatty acids. Lastly, the fourth study investigated the effects of soluble and insoluble

fiber on uptake and metabolism of colonic VFA, and colonic and hepatic expression of genes that are involved in lipid metabolism in growing pigs. Soluble and insoluble fiber increased the VFA uptake, and transportation and induced comparable effects on expressions of mRNA of colonic and hepatic genes involved in lipid metabolism. Taken together, soluble and insoluble fiber reduce fatty acids digestibility and induce variable effects on growth performance, intestinal fermentation, histomorphology, expression of genes involved in lipid metabolism and gastrointestinal microbiota composition.

DEDICATION

In loving memory of my adorable parents Noleen and Posset, gone too soon before giving me a

signal on how to 'treat' them because they are here with us today.

FOREWORD

Materials used in this thesis have been presented as poster and oral presentations at the 2015 ADSA-ASAS Joint Annual Meeting, Orlando, FL. Manuscripts in this thesis were formatted according to the Journal of Animal Science and comprise of 7 papers. The details of the contributing authors and information about the research articles and their stage of publications are as follows:

Paper 1: Ndou, S. P., E. Kiarie, S. J. Thandapilly, M. C. Walsh, N. Ames, and C. M. Nyachoti. 2017. Flaxseed meal and oat hulls supplementation modulates growth performance, blood lipids, intestinal fermentation, bile acids, and neutral sterols in growing pigs fed corn-soybean meal-based diets. J. Anim. Sci. 95:3068-3078. doi:10.2527/jas.2016.1328.

Paper 2: Ndou, S. P., H. M. Tun, E. Kiarie, M. C. Walsh, E. Khafipour, and C. M. Nyachoti. 2018. Dietary supplementation with flaxseed meal and oat hulls modulates intestinal histomorphometric characteristics, digesta- and mucosa-associated microbiota in pigs. Sci. Rep. 8:5880. doi:10.1038/s41598-018-24043-5.

Paper 3: Ndou, S. P., E. Kiarie, and C. M. Nyachoti. 2018. Flaxseed meal and oat hulls supplementation: Impact on predicted production and absorption of volatile fatty acids and energy from hindgut fermentation in pigs. J. Anim. Sci. doi:10.1093/jas/sky399.

Paper 4: Ndou, S. P., E. Kiarie, N. Ames, and C. M. Nyachoti. 2018. Flaxseed meal and oat hulls supplementation: Impact on dietary fiber digestibility and flows of fatty acids and bile acids in growing pigs. Targeted Journal: J. Anim. Sci. doi:10.1093/jas/sky398.

Paper 5: Ndou, S. P., E. Kiarie, M. C. Walsh, C. F. M. de Lange, and C. M. Nyachoti. 2018. Predicted production and absorption of short chain fatty acids in the cecum and colon of growing pigs: Interactive effects of dietary fiber and lipid type. Targeted Journal: J. Nutr. (Under preparation)

Paper 6: Ndou, S. P., E. Kiarie, M. C. Walsh, N. Ames, C. F. M. de Lange, and C. M. Nyachoti. 2018. Dietary fiber and fatty acids utilization in growing pigs: Interactive effects of dietary fiber solubility and lipid types. Targeted Journal: Br. J. Nutr. doi:10.1017/S0007114518003434.

Paper 7: Ndou, S. P., S. Liu, E. Kiarie, C. Yang, and C. M. Nyachoti. 2018. Diet supplementation with flaxseed meal and oat hulls modulates transepithelial uptake of volatile fatty acids in the colon assessed using the everted sac technique, and colonic and hepatic gene expression in growing pigs. Targeted Journal: Livest. Sci. (Under review).

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LIST OF ABBREVIATIONS

AA	Amino acids
ACSL5	Acyl-CoA synthetase long-chain Family Member 5
ADF	Acid detergent fiber
ADFI	Average daily feed intake
ADG	Average daily gain
AOAC	Association of Official Analytical Chemists
AOCS	American Oil Chemists' Society
APOA4	Apolipoprotein A IV
ASAE	American Society of Agricultural Engineers
ATTD	Apparent total tract digestibility
BA	Bile acids
BCVFA	Branched chain volatile fatty acids
BD	Bulk density
BSH	Bile salt hydrolase activity
BW	Body weight
CB	Cellulose and beef tallow-containing diet
CC	Cellulose and corn oil-containing diet
CCAC	Canadian Council on Animal Care
CD	Crypt depth
CD147	Basigin
CON	Control diet
CP	Crude protein
CYP2C49	Cytochrome P450 2C49
CYP7A1	Cholesterol 7α-hydroxylase

DCA	Deoxycholic acid
DDGS	Distiller's dried grains with solubles
DM	Dry matter
DMI	Dry matter intake
DNA	Deoxyribonucleic acid
EHC	Enterohepatic circulation
ELF	Endogenous losses of fat
EST	Everted sac technique
FA	Fatty acids
FABP1	Liver-type fatty acid binding protein
FFAR2	Free fatty acid receptor 2
FFAR3	Free fatty acid receptor 3
FM	Flaxseed meal-containing diet
FXR	Farnesoid X receptor
G:F	Weight gain : feed intake ratio
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase
GE	Gross energy
GIT	Gastrointestinal tract
GLP-2R	Glucagon-like peptide-2 receptor
IDCA	Isodeoxycholic acid
IDF	Insoluble dietary fiber
IV	Iodine value
IVP	Iodine value of the product
KEGG	Kyoto Encyclopaedia of Genes and Genomes
LCA	Lithocholic acid

LDA	Linear discriminant analysis
LEfSe	Linear discriminant analysis of effect size
MCT1	Monocarboxylate transporter 1
mRNA	Messenger RNA
MUC2	Mucin
MUFA	Monounsaturated fatty acids
N	Nitrogen
NDF	Neutral detergent fiber
nMDS	Non-metric multidimensional scaling
NRC	National research council
NS	Neutral sterols
NSP	Non-starch polysaccharides
OH	Oat hulls-containing diets
OM	Organic matter
OTU	Operational taxonomic units
PB	Pectin and beef tallow-containing diet
PC	Pectin and corn oil-containing diet
PCR	Polymerase chain reaction
PEPCK1	Phosphoenolpyruvate carboxykinase 1
PERMANOVA	Permutational multivariate analysis of variance
PICRUSt	Phylogenetic investigation of communities by reconstruction of unobserved states
PYY	Peptide YY
qRT-PCR	Quantitative real time-PCR ()
RNA	Ribonucleic acid

SCFA	Short-chain fatty acids
SDF	Soluble dietary fiber
SEM	Standard error of means
SFA	Saturated fatty acids
SID	Standardized ileal digestible
SMCT1	Sodium-coupled monocarboxylate transporter 1
SWC	Swelling capacity
TDF	Total dietary fiber
TFA	Total fatty acids
TNS	Total neutral sterols
UDCA	Ursodeoxycholic acid
VCR	Villi height : crypt depth ratio
VFA	Volatile fatty acids
VH	Villi height
WHC	Water holding capacity

CHAPTER ONE

1. GENERAL INTRODUCTION

1.1. Background and Problem Statement

Continuous volatility and increase in prices for the common feedstuffs calls for need to come up with alternative protein and energy sources to feed pigs. In recent years, the use of low-cost agro-industrial co-products has significantly increased in pig diets resulting in increased dietary fiber (**DF**) content and reduced dietary energy density. High-fiber diets are supplemented with animal fat or plant oil to meet the energy content of a nutritionally-balanced diet (Bakker et al., 1996). In theory, performance of growing pigs fed high-fiber diets will not decline if diet formulations are such that pigs consume adequate amounts of energy, ileal digestible amino acids and other essential nutrients. However, it has been demonstrated that nutrient utilization and performance is poor in growing pigs fed nutritionally-balanced diets with a similar energy supply but composed of fibrous co-products plus supplementary fat, compared to pigs given diets based on cereals or high-fiber co-products without supplemental fat (Bakker et al., 1996; Ball et al., 2010; Gutierrez et al., 2013). This discrepancy in performance has been attributed to overestimation of fat energy value or interaction between DF and supplementary fat. However, a repeated observation is the variation between calculated energy values during feed formulation and measured energy values when fibrous ingredients are used in pig diets (O'Doherty et al., 2002; Beaulieu et al., 2009; Gutierrez et al., 2013). Therefore, it is difficult to predict growth performance when pigs are fed fiber-rich co-products with supplementary fat to increase the dietary energy density based on individual ingredients energy values.

The idiosyncrasy of the interaction between DF and lipid type on growth performance and gastrointestinal fermentation in pigs has been associated with microbial activity. This implies that

microbial activity are greatly influenced by inclusion level of DF and/or its solubility as well as the degree of saturation of dietary fatty acids (**FA**). Depending on their solubility in water or weak alkali, DF sources can be classified as soluble or insoluble (Bach Knudsen, 2001). Soluble DF is mostly fermented before reaching the colon, in pigs (Jiménez-Moreno et al., 2009; Gutierrez et al., 2013; Jaworski and Stein, 2017). In contrast, insoluble DF have greater proportion of cell wall components that are difficult and/or may delay microbial degradation but are partially fermentable after passing the cecum (Bach Knudsen, 2001; Agyekum and Nyachoti, 2017). The diverse physicochemical properties of supplemental FA as well as their potential interactions with DF, and minerals may affect microbiological activities and animal performance by compromising absorption of FA and energy metabolism. Non-starch polysaccharides (**NSP**) and lignin are the main constituent entities of DF that determine its solubility and fermentability in the gastrointestinal tract (**GIT**) of pigs (Bach Knudsen, 1997; Agyekum and Nyachoti, 2017).

Non-starch polysaccharides can be hydrolyzed by gastrointestinal microbiota to produce short chain fatty acids (**SCFA**) that are used as fuel by the host (Bergman, 1990). Apart from their role as the main sources of energy to the host (Bergman, 1990), together with bile acids (**BA**), SCFA not only modulate gastrointestinal microbiota composition but also acts as signalling molecules that regulate energy and lipid metabolism in pigs (Den Besten et al., 2013; Ferrebe and Dawson, 2015). In turn, gastrointestinal microbiota also regulate synthesis and composition of SCFA. However, data on the comparative effects of adding either a soluble or insoluble DF on bioavailability of SCFA produced during microbial fermentation hindgut is scantily documented. From the literature in ruminants it can be deduced that a high dietary fat content can inhibit fermentation by gastrointestinal microbiota (Brook et al., 1954) or reduce microbiota population and diversity (Mallet and Rowland, 1988). This is one aspect of pig nutrition that has not been

systematically studied and will require further clarification.

Short chain fatty acids are major links between microbiota and the host. Energy contribution from metabolism of SCFA produced during hindgut fermentation ranges from 7 to 17 % of total available energy, of which it contributes up to 30% of the host's maintenance energy supply (Varel et al., 1997; Anguita et al., 2006). With the introduction of co-products with different DF compositions and solubility, these estimates are likely to be altered but detailed information to facilitate clear understanding of the metabolic fate and bioenergetics of SCFA in growing pigs is scant. Bioenergetics of SCFA consists of a system of transporters, precursors, cofactors, hormones, enzymes, genes and metabolic pathways by which SCFA are converted into metabolizable energy or modulate energy or lipid metabolism. Therefore, DF effects on gastrointestinal flows, absorption and metabolic fates of SCFA and dietary FA, in relation with expression of genes involved in energy or lipid metabolism warrants further investigations.

There is a general speculation that between 85 to 95% of SCFA are absorbed and metabolized in the pig GIT. Nevertheless, a repeated observation indicated that dietary inclusion of fermentable fiber increased SCFA fluxes at the same time increasing the amount of fecal fat (Bach Knudsen and Hensen, 1991; Galassi et al., 2004). It is also peculiar that total tract digestibility of fat was more negative (-54%) than ileal fat digestibility (-19%) in pigs (Graham et al., 1986) and fermentation was observed to increase fecal fat excretion by 47% and fecal BA by 35% compared with the control group in humans (Judd and Truswell, 1981). Increased fat in feces because of increased fermentability is obviously counter-productive to dietary inclusion of fiber-containing co-products, as alternative feed ingredients in pig diets. Therefore, it is still a matter of debate on whether fecal fat could be attributable to sub-optimal absorption of added lipids predisposed by encapsulation of FA within the DF matrices or by deconjugation of BA and

microbial FA production following increased bacterial activities in the cecum and colon (Ahn et al., 2003). Moreover, fecal fat could also be ascribed to endogenous losses from the gastrointestinal secretions (Kil et al., 2010) caused by DF, however, data to support this phenomenon is scarce. These mechanisms are likely to influence homeostasis of lipid metabolism-related blood constituents, such as cholesterol and triglycerides. There is also accumulating evidence that cholesterol derivatives such as BA and neutral sterols (**NS**) facilitate a cross-talk between intestinal microbiota and energy or lipid metabolism (Tan et al., 2014; Ferrebee and Dawson, 2015). Gastrointestinal microbiota play a central role during biotransformation of primary to secondary BA and alter BA composition (Nie et al., 2015; Wahlström et al., 2016). Interestingly, a growing body of research has also pinpointed that BA, in turn, alter the community structure of gastrointestinal microbiota that produce SCFA (Nie et al., 2015; Wahlström et al., 2016). The mechanism involved during fat digestibility, BA flows, and gastrointestinal microbiota are complex and the effects of DF solubility remain elusive and difficult to decipher. There is no detailed information and clear understanding of the compositional and functional alterations of intestinal digesta- or mucosa-associated microbiota in response to DF solubility.

If the usefulness of DF as energy sources is to be met, there is need to also understand the bioavailability of SCFA. A plethora of studies have demonstrated distinct differences in trans-epithelial transport of intestinal SCFA in various non-ruminant species (Engelhardt et al., 1994; Busche et al., 2002). SCFA are transported through epithelial cells either in the ionized (transporter-mediated) or non-ionized (without transporter) forms (Den Besten et al., 2013). Further interests in understanding SCFA absorption kinetics and metabolic adaptations to SCFA fluxes and dietary FA in pigs fed high-fiber diet has emerged, but limited research in this area has

occurred in relation to DF solubility. There is, therefore, need to investigate how epithelial cells develop in response to DF or to maximize SCFA absorption.

Physiological effects of SCFA during the post absorptive phase are mediated by receptors that regulate lipid metabolism (Den Besten et al., 2013). To what extent DF solubility or SCFA fluxes stimulate gene expression in relation to lipid and energy metabolism in pigs fed fibrous diets under growing conditions remains a mysterious question which needs an answer. Microbial biotransformation of BA modulates the signaling properties of BA via the nuclear farnesoid X receptor and the G protein-coupled membrane receptor 5, which regulate numerous metabolic pathways including BA synthesis, lipid and energy metabolism by the host (Ferrebee and Dawson, 2015, Nie et al., 2015). It is also still not clear whether these genes are expressed in hepatocytes the same way as in colonocytes. There is, therefore, a paucity of information on metabolic adaptations and mechanisms regulating adaptations that occur during bioenergetics of SCFA in pigs fed high-fiber diets. Worse still, there is no quantitative data on hepatic precursors that act as fuels during the post absorptive phase of SCFA. This makes it, even more difficult to appreciate the role of SCFA in energy metabolism. Therefore, the influence SCFA or BA on gene expression is difficult to decipher, since they are probably indirect and involve interplay among multiple biochemical changes and metabolic pathways that warrant further investigations. The need to characterize the flows of SCFA and FA, their absorption kinetics, and gene expressions indicative of lipid and energy metabolism and adaptation mechanisms that occur when growing pigs are fed different sources of DF should not be undermined. It was hypothesized that dietary supplementation with soluble and insoluble fiber induce comparable effects on growth performance, lipid nutrition, and gastrointestinal development, metabolites and gut microbiota in growing pigs. Therefore, the broad objective of the studies described in this thesis was to

investigate the effects of DF sources or types (solubility) on growth performance, production, absorption, and metabolism of FA in growing pigs fed high-fiber diets.

1.2. Justification of The Study

High-fiber diets need to be described in such a way that it is possible to predict the effects of fiber solubility on the contribution of energy from SCFA produced during hindgut microbial activity to total tract digestible energy and growth performance. To achieve a more comprehensive understanding of the contribution of DF, there is need to characterize not only SCFA flows but also fluxes of other gastrointestinal metabolites (BA and NS) that are influenced by DF and gastrointestinal microbiota. The elucidation of knowledge on the comparative effects of adding either a soluble or insoluble DF on gastrointestinal microbiota and absorption of FA, DF fermentability and metabolic consequences of SCFA is crucial to better understand and develop strategies to improve utilization of fibrous feedstuffs in swine. The quantification of SCFA production is pivotal for predicting the extent or amount of metabolizable energy contributed by SCFA to the host. Knowledge of descriptors of trans-epithelial absorption and intra-epithelial metabolism of SCFA, FA and other metabolic substrates enables feed compounders to ensure that ingestion of sufficient nutrients by pigs given high-fiber diets occurs without excretion of dietary FA or SCFA needed for energy supply to the host. Understanding transepithelial absorption and metabolism of SCFA and its regulation also gives new insights on the adaptive capacity of colonocytes to react to luminal SCFA availability and dietary status. The assessment of colonic and hepatic gene expressions involved in lipid nutrition and energy metabolism will be an important step towards understanding the role of DF solubility on energy metabolism, growth performance and adaptation of pigs to fiber-enriched diets.

CHAPTER TWO

2. LITERATURE REVIEW

2.1. INTRODUCTION

Feed resources are scarce and, if available, prices are increasingly becoming prohibitive, energy and protein being the greatest part of the costs. In recent years, because of the ever-increasing feed costs, there is continued pressure for pig feed compounders to seek alternative cost-effective ingredients options such co-products from agro-industrial processing plants. A characteristic feature of co-products is that they increase dietary fiber (**DF**) content and subsequently dilute energy density of feed. Supplementation with animal fats or vegetable oils fortifies the dietary caloric density to meet requirements for pig. Dietary fiber and fat interact and alter fatty acids (**FA**) absorption, gastrointestinal microbial composition, activities and metabolites, and consequently growth performance. Together with short chain fatty acids (**SCFA**) produced from fermentation, medium- and long-chain fatty acids (**MCFA** and **LCFA**) of dietary origin are not only important substrates for energy and lipid metabolism but have a plethora of other beneficial effects.

To optimise usefulness of fibrous ingredients, identify potential targets for optimising absorption and metabolism of FA and elucidate knowledge on metabolic attributes of SCFA, this chapter reviewed different aspects of DF solubility and dietary lipid types in gastrointestinal fermentation and FA nutrition in pigs. Specifically, the existing knowledge on sources, sites for production and absorption, regulation of metabolism and contribution of energy produced from SCFA, and dietary FA to host, will be discussed in relation with DF fermentability, FA digestibility, gastrointestinal microbiota and bile acids (**BA**) metabolism. This review will also open a discussion on questions that, when answered, will open way for identifying potential targets

for optimizing the usefulness of fibrous feedstuffs and dietary lipids in pigs. The contribution to total digestible energy and bioenergetics of SCFA are also assessed in relation to DF solubility and FA nutrition.

2.2. AN OVERVIEW OF FERMENTATION SUBSTRATES AND FATTY ACIDS

The major constituents of gastrointestinal fermentation substrates are dietary fibers which mainly consist of either indigestible structural carbohydrates that escape endogenous digestion or those dietary components that are physically masked from digestive enzymes because they are embedded within fiber matrices. These include NSP namely hemicelluloses, celluloses, oligosaccharides, pectins, inulin and gums (Bach-Knudsen, 2001). Cellulose, hemicellulose and pectin are the main constituent entities of DF that can be fermented in the gastrointestinal tract (**GIT**) of pigs (Bach-Knudsen, 2001; Agyekum and Nyachoti, 2017). The DF fraction also consists of lignins, lignans, waxes, phenolic acids and glyco-protein complexes (Bach-Knudsen, 1997). The NSP portions of DF play a central role in governing their physiological actions. Apart from NSP and other undigested non-fibrous feed components including proteins and lipids, a fraction of starch known as resistant starch and endogenous secretions are also fermented by intestinal microbiota to produce SCFA (Jha and Berrocoso, 2016). Thus, DF should not be considered as a singular entity, as it is a term that in fact embraces a several contributors with divergent monomeric compositions.

High-fiber diets need to be characterized to open ways for predicting the interactive effects of DF and lipids on nutrient absorption, gastrointestinal fermentation, physiological and metabolic processes, and subsequently growth performance in pigs, and lipid profile of pork products. Based on the NSP and lignin composition, and their solubility in water or weak alkali, DF can be

categorized as soluble or insoluble fiber (Bach-Knudsen, 2001). Soluble DF are highly fermentable and believed to influence the process of digestion in the stomach and small intestines. Conversely, insoluble DF are less fermentable and primarily modulate processes in the cecum and colon where, due to their physical presence, they effectively dilute gastrointestinal contents, increase mouth to anus passage rate and increase fecal bulk (Bach Knudsen, 1997; 2001).

Pigs will eat at a level to meet its nutrient requirement and allow potential performance, subject to its capacity to deal with some constrains which are linked to the physicochemical measures of bulkiness of feed and digesta (Whittemore et al., 2003; Nyachoti et al., 2004). The feed bulking capacity is entirely influenced by DF source and inclusion level (Emmans and Kyriazakis, 1995; Ndou et al., 2013a,b). During transit of digesta in the GIT, the rate and overall degree of degradation of DF is mainly influenced by the chemical nature of the fiber, the solubility, and the degree of lignification (Bach Knudsen et al., 2000; 2012). β-glucans, soluble arabinoxylans (**AX**), and pectin fibers are all fermented in the cecum and proximal colon (Bach Knudsen et al., 1993; Canibe and Bach Knudsen, 1997; Glitsø et al., 1998) whilst the more insoluble plant fiber, e.g. cellulose and insoluble AX, are fermented more slowly at more distal locations of the colon (Bach Knudsen et al., 1993; Glitsø et al., 1998). Apart from the ratio of soluble to insoluble DF, other physicochemical properties such as feed bulkiness (fiber inclusion level), water holding capacity (**WHC**), bulk density, viscosity, and swelling capacity (**SWC**) can also be used to classify DF and predict its fermentability. The WHC of feed describes the ability of fiber to trap water, swell and form gels with high water contents. The WHC of the feed emerges as the most accurate predictor of voluntary feed intake in growing pigs fed fibrous diets with a wide range of physicochemical properties (Whittemore et al., 2003; Ndou et al., 2013a,b). A fiber with a high WHC is likely to be highly fermentable as it will create films of moist within the fiber matrices

thereby creating a conducive atmosphere for microbial activity. The capacity of DF to hold water also have direct influence on SWC (Serena et al., 2008). Thus, a fibrous ingredient with a high SWC may increase the surface area over which microbial activity can occur. Viscosity of fibrious feedstuffs refers to their ability to thicken or form gels in solution (Dikeman and Fahey, 2006). Although viscosity is a well-characterized physicochemical property known to influence endogenous enzymatic digestion and absorption of nutrients, previous results have reported conflicting results on its influence on microbial populations (Kiarie et al., 2007). Fibrous co-products such as flaxseed meal and citrus pulp that contain high proportions of soluble DF and increase digesta viscosity are likely to induce variable effects when compared to insoluble DF-rich co-products such as oat hulls and sunflower husks. The influence of physicochemical measures of bulkiness on DF fermentability warrants further investigations.

Fatty acids (FA) present in both animal and plant tissues can be classified into short-, medium-, and long-chain fatty acids (**SCFA**, **MCFA** and **LCFA**). The SCFA is a broad term which describes a group of saturated aliphatic organic acids that consist of 1-6 carbon atoms in a straight- or branched-chain arrangement (Bergman, 1990; Cook and Sellin, 1998). Therefore, the term SCFA embroils short carbon chain organic acids and volatile fatty acids (**VFA**). Examples of SCFA are acetic (C2), propionic (C3), butyric (C4), valeric (C5), formic (C1), isobutyric, isovaleric, 2 methyl-butyric, hexanoic, heptanoic and lactic acids (Bergman, 1990). Together with BA, VFA are the main products from gastrointestinal fermentation that principally serve as the major links between the host and gastrointestinal microbiota. The first three are the pre-dominant fermentation by-products and metabolic substrates of substantive value in non-ruminants (Bergman, 1990). Fatty acids of total carbon atom from 7 to 12 are defined as MCFA, where those with greater than 12 carbons are classified as LCFA (Schönfeld and Wojtczak, 2016).

Within the digesta milieu or during absorption, VFA exists either in their dissociated or undissociated forms. In pigs, the colonic molar ratio of acetate, propionate, and butyrate production is 60:25:15, respectively (Tazoe et al., 2008), and the concentration of these VFA in the gastrointestinal tract ranges between 70-140 mmol/L. The proportions VFA can vary depending on factors such as diet, microbiota composition, site of fermentation, and host (Hamer et al., 2008) but information on the effects of DF solubility in growing pigs is scarce.

Some VFA volatilize under room temperature, whereas, MCFA and LCFA exists as liquids or solids (Rauen, 1964; Schönfeld and Wojtczak, 2016). Fatty acids with shorter chains, up to 9 total carbon atoms, are liquid at room temperature (Rauen, 1964). Depending on the presence of double bonds within the carbon chains, monomeric composition and arrangements, MCFA and LCFA can also be categorized into saturated (**SFA**) and unsaturated (**UFA**) fatty acids. Moreover, depending on the number of double bonds within the carbon chain, the MCFA and LCFA can be further categorized into either monounsaturated (**MUFA**) or polyunsaturated (**PUFA**) (Schönfeld and Wojtczak, 2016). Omega 3 and 6 forms of LCFA have also been classified and comprehensively reviewed with regards to human nutrition and health-eating quality of pork but little in relation to the interactive effects of DF solubility and FA types in pig nutrition. High-fat diets can inhibit ruminal fermentation by gastrointestinal microbiota (Brooks et al., 1954) or reduce microbial population (Mallet and Rowland, 1988) and diversity. To the best of our knowledge, the interaction between DF solubility and lipid type on performance and gastrointestinal fermentation in pigs has not been reported but is likely to be associated with microbial activity. Therefore, it can be postulated that the response of pigs to supplemented lipids in high-fiber diets is greatly influenced by inclusion level of DF and/or its solubility. The interactive effects of DF and lipid

types needs to be elucidated so that high-fiber diets can be formulated to optimize growth performance with minimum nutrient losses.

2.3. SOURCES AND PRODUCTION OF FATTY ACIDS

2.3.1. Sites of fermentation and VFA production

During transit of digesta, VFA are produced at different concentrations following microbial activity within each GIT segment (Bergman *et al.*, 1990; Smiricky-Tjardes *et al.*, 2003; Bindelle *et al.*, 2008). Among these GIT segments, microbial degradation produces the largest quantities of VFA mainly in the distal regions of the lower parts of the cecum and colon (Wang et al., 2003). Conversely, minimum amounts of VFA have been detected in the stomach and small intestines (Wang et al., 2003). Supporting this phenomenon are observations that approximately 24 and 17% of ingested neutral detergent (**NDF**) and total dietary (**TDF**) fiber, respectively, are digested before the end of ileum, depending on DF solubility (Graham et al., 1986; Urriola et al., 2010). Because both the amount of soluble or insoluble fiber has been reported by Gutierrez et al. (2013) to modulate the degree of fermentation before the end of the ileum, and have no effects on fermentation measurements in other studies reported by Graham et al. (1986), the effects of DF solubility is still a matter of debate. Furthermore, distinctive differences in VFA concentration within each segment had been reported, for example, proximal region of colon and cecum are rich in VFA compared to the distal regions. In some cases, some researchers reported that the VFA concentration between different segments are the same (Montoya et al., 2016). More work is needed to explain these differences as these VFA fluxes only indicates net numerical results but not quantities absorbed or produced.

The VFA are produced by gastrointestinal bacterial communities through various mechanism that had been elegantly reviewed by Macfarlane and Macfarlane (2003), Bindelle et al. (2008) and Den Besten et al. (2014). In brief, acetate is produced though reductive methylation of CO_2 via the Wood-Ljungdahl pathway (Pyrde et al., 2002; Ragsdale and Pierce, 2008), whereas propionate is formed by two major routes namely; the methylmalonic-CoA pathway and the acrylate pathway (Miller et al., 1996). Den Besten et al. (2014) indicated that in addition to propionate produced through the methylmalonic-CoA pathway, one acetate molecule will be formed per two molecules of propionate produced. Exogenously derived acetate can also be used by butyrate-producing bacteria to generate butyrate and acetyl-CoA (Den Besten et al., 2014). Therefore, abovementioned mechanisms are likely to shift depending on DF solubility which modulates the structure and function of gastrointestinal microbiota, but studies to support these assumptions are scarce.

Apart from microbial fermentation, multiple reports indicate that cellular metabolic activities, in particular FA oxidation, amino acids and glucose metabolism are indirect progenitors of VFA, in humans (Sakakibara et al., 2009; Layden et al., 2013). For example, fasting increases acetate production in the liver (Sakakibara et al., 2009). For newborn mammals, including piglets, the sow's milk constitutes an important source of not only VFA but also of MCFA. The effects of DF on VFA in milk is scantily documented but could be a culmination towards efforts to improve gut health. With the introduction of fibrous co-products such as canola meal, distiller's dried grains with solubles (**DDGS**) and flaxseed meal in lactating sow diets, DF solubility is likely to influence the VFA fluxes in milk and gut health in piglets, but data to confirm this hypothesis have not been reported.

Fermentation of diets and liquid feeding are other crucial feed technologies that increase the concentration of VFA in feed. It remains unclear how modern feed technologies such as fermentation of feed ingredients or liquid feeding influence VFA production in the GIT but are also emerging sources of FA. However, research on the impact of circulating VFA has been ignored in farmed pigs especially in the gestating sows were feeding can be once or twice a day followed by prolonged fasting periods or restriction periods during precision feeding. Therefore, VFA produced through hindgut fermentation and those through cellular metabolism can be referred to as exogenous and endogenous VFA, respectively. Henceforth, for the purpose of this review, exogenous VFA can be further categorized into dietary VFA produced during feed preparations and microbial VFA originating from gut fermentation. Quantification of the balance between exogenous and endogenous VFA and their response to DF solubility in pigs elucidate knowledge on biological attributes of VFA. This also facilitates effective manipulation of high-fiber diets fed to pigs to increase VFA production and promote growth performance. The infusion of isotopes in catheterized or cannulated pig models may assist the characterization of other sources of VFA and how solubility of DF influence production and absorption kinetics.

2.3.2. Origins of MCFA and LCFA

Fatty acids detected in digesta and feces originate from the feed, endogenous secretions, as well as microbial-produced MCFA/LCFA and VFA produced by gastrointestinal fermentation. It is also important to note that infeed FA originate from lipids embedded within the ingredient matrices and/or supplemental fat. Furthermore, supplemental fat can be of animal (beef tallow, fish oil and lard) or plant (corn oil, flax, camelina, canola, coconut oil) origin. These dietary fat sources have diverse chemical structures and FA profiles that interact with other dietary

components including DF and gut microbiota thereby modulating nutrient utilization and pig performance (Jorgensen et al., 2000). Dietary fat supplementation may not only increase the dietary energy density and palatability of feed but may also offer an opportunity for manipulating the health-eating quality of pork (Wood et al., 2003). Little attention has been given to the influence of different dietary fat sources or their degree of saturation on intestinal flows and digestibility of FA and DF fractions in pigs fed fibrous co-products.

Microbiota in the intestinal lumen can utilize SCFA to produce LCFA, or elongate and desaturate other FA, henceforth contributing to the FA pools in pig digesta and fecal contents (Martínez-Ramírez et al., 2013). For example, biosynthesis of 9c11t-CLA from 18:2n-6 facilitated by bacteria such as *Butyrivibrio fibrisolvens* and conversion of 11t-18:1 to 9c11t-CLA by Δ9 desaturases has been established in ruminants (Bauman and Griinari, 2003; Martínez-Ramírez et al., 2013). Chin et al. (1994) and Adlof et al. (2000) postulated that rats and humans can also synthesize CLA isomers in the upper gut, however, it remains unclear how these processes influences gastrointestinal FA flows in intestines of pigs fed different DF types. There is accumulating evidence also that hindgut microbiota convert unsaturated FA into stearic acid probably as a result biohydrogenation of PUFA (Reis De Souza et al., 1995; Jorgensen et al., 2000). Accordingly, the synthesis of branched-chain fatty acids is an important indicator of fermentation facilitated by gastrointestinal microbiota (Htoo et al., 2007; Serena et al., 2008). These processes are likely to be modulated by DF solubility and species-specific mechanisms that modulate the FA profiles of microbial-produced fat may exist depending of the microbiota population and composition, so further studies are required to test different DF sources.

Endogenous FA that predominantly originate from various digestive secretions (pancreatic secretions, gastric secretions and intestinal secretions), desquamated gastro intestinal epithelial

cells also contribute lipid pool in digesta contents (Kil et al., 2010). As is the case in amino acids, if quantified, the endogenous flow of FA at the terminal ileum is the net result of the overall dynamics of endogenous FA sources along the digestive tract. It also represents the net algebraic difference between the secreted and reabsorbed endogenous FA pool. It is also worth noting that because losses of endogenous fat, including microbial fat, are increased in the hindgut of pigs compared to small intestines, values for the apparent ileal digestibility of fat are sometimes greater than values for total tract digestibility of fat (Kil et al., 2010; Gao et al., 2015). Although values for total tract digestibility of fat may not, therefore, appear to give a better estimate of fat bioavailability than those of ileal digestibility (Gao et al., 2015), the quantification of true or standardized ileal digestibility of individual FA should be given careful consideration. Furthermore, soluble DF may reduce absorption of nutrients including dietary FA and increase gastric secretions by increasing digesta viscosity. On the other hand, insoluble DF may induce the same responses by reducing digesta transit time. These mechanisms are likely to compromise FA utilization and endogenous loss of FA but studies to compare different DF sources are ignored. As a consequence of these processes, the source of endogenous FA and the entry point into the digestive tract appear to be critical in determining these dynamics. However, considering the complicated nature of the processes and metabolic pathways involved, it is difficult to pinpoint the most accurate contributors to fecal fat. Thus, as mentioned above, the use of cannulation techniques and infusing isotopes in different intestinal segments will open a way for identifying closer to actual descriptors of fecal fat.

2.3.3. Factors affecting production of VFA and microbial FA

Various factors that influence production of VFA and microbial fat can be categorised into dietary, digesta quality and flow (bulking properties, transit time), host or animal-related (breed,

gut development and capacity) and microbiological factors. Production and absorption of VFA are not mutually exclusive events and may occur at the same time. Therefore, the use of concentrations of VFA in the gastrointestinal tract may not be used to define VFA production but represent net fluxes resulting from both production and absorption (Montoya et al., 2016). The physicochemical properties of DF and supplementary FA influence the bioavailability VFA. Thus, when quantifying the amount of VFA produced, the complexity in the influence of these factors on fermentation warrants a holistic approach which concurrently evaluates all key factors including physicochemical properties of digesta that influence absorption of both dietary FA and VFA from gastrointestinal fermentation. Furthermore, understanding the quantification of microbial fat in conjunction with VFA production is of paramount importance as the latter is a substrate for production of the former. Alternatively, the crosstalk between BA with either microbial communities or fat acids profiles also influence VFA production but the role of DF solubility in this regard is unclear.

2.3.3.1. Diet-related factors

Diet-related factors are mainly those components of the feed that influence digesta quality and quantity of substrate that can be available for supporting microbial activity during hindgut fermentation. Examples of these diet constituents include indigestible carbohydrates and proteins, DF and lipid types or sources and inclusion levels in feed, minerals and bulking properties. Processing of feed ingredients, such as grinding, extruding, pelleting, micronizing, enzyme supplementation, fermenting, liquid feeding and ensiling are likely to alter bulking properties, DF, lipid, VFA and microbiota compositions of high-fiber diets. Thus, influencing the availability of fermentation substrates, and consequently VFA production in the gut. The inclusion level, types

of fiber (soluble or insoluble DF) and/or source of substrate (carbohydrate or protein) have been indicated to affect VFA profiles (Bergman, 1990; Awati et al., 2006; Montoya et al., 2016). Further works are required to illustrate the influence of including fibrous ingredients with different physicochemical properties in terms of solubility, water holding capacity and viscosity. For example, some fibers are soluble and easily fermentable (pectin), whereas insoluble fibers (cellulose) are partially fermented or may escape both foregut degradation and hindgut degradation. The inclusion level of the fiber also determines the amount of feed that can be ingested (Nyachoti et al., 2004; Ndou et al., 2013a,b) and consequently quantities of DF that are available for both endogenous and exogenous VFA production, depending of fiber source.

A plethora of studies have also indicated that different fiber sources are fermented at different rates to give different profiles of VFA (Anguita et al., 2006; Awati et al., 2006; Jha and Berosocco, 2016). In rats, pectin stimulated production of acetate while guar gum and resistant starch induced formation of cecal propionate and butyrate, respectively (Henningson et al., 2002). VFA profiles can also be shaped by the interaction between different diet properties or changes in physicochemical nature of digesta during transit from one gut segment to the other. For example, a reduction in carbohydrates is associated with fermentation of approximately 30% of proteins into branched-chain VFA (mainly iso-butyrate, valerate and iso-valerate), ammonia, biogenic amines, phenolic and phenolic components, and volatile S-compounds (Jha and Berosocco, 2016; Millet et al., 2010). The two major sources of protein that are available for hindgut fermentation are usually resistant protein that escape endogenous enzymatic hydrolysis and non-dietary indigestible protein (pancreatic enzyme, mucus, and exfoliated epithelial cells) from endogenous secretions and degenerated gastrointestinal cells (Hughes et al., 2000). An increase in soluble DF is likely to increase digesta viscosity and subsequently modulate the quantity of substrate available for

gastrointestinal fermentation. For example, high protein co-products (flaxseed, canola and camelina meals) that may contain anti-nutritional factors (trypsin inhibitors, DF and glycosinolates) that increase deposition of undigested dietary components into the hindgut are likely to promote protein fermentation, so further studies are required. The digestibility of most amino acids in corn DDGS may be reduced due to heat damage during fermentation or drying (Pahm et al., 2008; Kim et al., 2012), and consequently increasing the protein deposition in the hindgut. Ileal endogenous proteins from intestinal and pancreatic secretions, and mucoproteins that are also thought to resist enzymatic hydrolysis could contribute to the pool of proteins that serve as substrate for hindgut fermentation (Montagne et al., 2003). Branched VFA (**BCVFA**) such as isovaleric and isobutyric acid are produced exclusively from deamination and decarboxylation of amino acids. The major progenitors of BCVFA are valine, leucine and isoleucine (Jha and Berossocco, 2016). The most abundant amino acids in the ileal endogenous proteins are glutamic acids, aspartic acid, threonine, proline, serine and glycine. Evidence from studies by Nyachoti et al. (1997) suggest that the components of endogenous secretions are influenced by fiber type and inclusion level, with effect of insoluble fiber being pronounced more on mucin whereas that of soluble and viscous fiber is mainly on the bacterial component. Because it is well-established that mucin can represent up to 11% of total endogenous N losses at the ileum of pigs, depending on dietary fiber and protein (Lien et al., 1997; Montagne et al., 2004). Therefore, BCVFA constitute between 16 and 23% of the total VFA pool, depending on the substrate type or ration of soluble:insoluble DF (Cone et al 2005), but role of practical sources of DF that are rich in protein is not well-characterized.

Furthermore, charged regions with the fiber matrices and divalent minerals (such as calcium) also interact with dietary FA thereby altering the overall absorption kinetics in the small

intestines and quantity of FA deposited in the hindgut (Jorgensen et al., 1992; Dove 1993; Li and Sauer, 1994). An increase of dietary crude fiber content by 1% depresses the ATTD of FA by 1.3-1.5 (Stahly, 1984). During digesta transit, interactions of DF and lipid types are also likely to play a central role in altering fermentation patterns. There is a paucity of information on influence of oil holding capacity of fibrous co-products and its influence in fermentability. The role played by either animal fat or plant fat with either soluble or insoluble DF on VFA has not been documented in modern genotypes. An increase in PUFA content in a high-fiber diet increase microbial fat. Previous research on chemostat using pure cultures of saccharolytic gut micro-organisms demonstrated that acetate and formate are the major bifidobacterial fermentation products during growth under C-limitations, whereas acetate and lactate are produced when carbohydrates is in excess (Macfarlane and Macfarlane, 2003). With the introduction of enzymes that solubilize NSP and promote their degradation, the levels of production of VFA following microbial degradation in different compartments are likely to be altered and warrant further investigations *in vivo*. Tannins in diets depress digestibility in a number of ways by forming stable tannin–protein and tannin–cellulose complexes, inhibiting microbial fermentation and through reactions with endogenous proteins on the intestinal mucosa (McLeod, 1974). Therefore, fibrous feedstuffs that are also rich in tannins, such as sorghum DDGS and leguminous leaf meals are likely to modulate protein fermentation.

2.3.3.2. Digesta quality, transit time and retention time

Bulking properties of the diet has great impact on changes in physicochemical properties of digesta and processes occurring along the GIT. Changes in these properties alter gastrointestinal microbiota that participate in fermentation to produce VFA. Apart from digesta quality, the extent

of VFA production from fiber fermentation is also influenced by the passage rate of digesta. Tendencies were observed whereby WHC reduced the transit time in the hindgut but prolonged transit time in the upper gut segments (Leeuwen and Jansman, 2007). It can be postulated that a fibrous ingredient with a high WHC causes digesta to swell and allow a film of moisture to build up within the fiber matrices, thus creating a conducive atmosphere for microbial activity.

Prolonged transit time or a gradual passage rate is likely to promote microbial growth, increase the efficacy of the digestion process, and subsequently increasing VFA production. In this regard, the high mucilaginous NSP in flaxseed meal may impair nutrient absorption by increasing digesta viscosity (Bhatty, 1993). Interestingly, the consumption of flaxseed reduced ileal SCFA concentrations, anaerobic spore formation and NSP digestibility suggesting pre-cecal suppression of microbial activity (Kiarie et al., 2007). A reduction in ileal microbial activity in pigs fed flaxseed contradicts the results in poultry (Langout et al., 2000) and piglets (McDonald et al., 2001), which demonstrated that high intestinal viscosity is associated with high bacterial activity. These discrepancies are attributable to the difference in the physicochemical nature between flaxseed meal and the viscous polysaccharides, such as guar gum, carboxymethylcellulose, pearl barley and citrus pectin used in the aforementioned studies (Langout et al., 2000; McDonald et al., 2001). Wheat bran is one of the most effective fiber sources for increasing the rate of passage in the digestive tract (Jha and Berrocoso, 2015), however, the underlying mechanisms behind are yet to be characterized. If a sound feeding strategy that maximise VFA production without compromising digestibility of other dietary components is to be pursued, there is need to consider passage rate as a culmination of two entities namely digesta transit time and digesta retention time (Millet et al., 2010). The former is considered as the time it takes for gastrointestinal contents to pass through a

particular section of the GIT, while the later quantifies the time period it takes for individual portions of digesta to be retained in the gut (Millet et al., 2010).

One paradoxical way by which soluble fiber reduce passage rate thereby constraining the gut capacity and depressing the intake of more DF. Moreover, an increase in transit time increases the duration of microbial activity and consequently fermentation and production of VFA. Another controversial relationship is by which insoluble fiber depresses fermentation by reducing transit time and time of expose of substrate to microbial degradation but at the same time reducing the constraining effect on gut capacity by promoting gut emptying (Millet et al., 2010; Ndou et al., 2013a). There is also a possibility that exceptionally high passage rate of digesta in the upper GIT (stomach and small intestine) may automatically lead to poor nutrient absorption and high nutrients bypass. Moreover, soluble DF including fractions of β-glucan and arabinoxylans may also suppress absorption of lipids by increasing digesta viscosity, a phenomenon that had been repeatedly reported in poultry. An increase in digesta viscosity interrupts enzymatic hydrolysis of lipids, and disturbs micelle formation with BA and mucosal uptake of FA in upper gut (Smith and Annison, 1996). It is, therefore, highly likely that reduction in nutrient absorption in the upper gut may increase the amounts of substrate that can be available for fermentation in the hindgut. Therefore, increment level of fermentable DF increase digesta passage rate in the upper gut and reduce hindgut passage rate of digesta thereby increasing the quantity of fermentation substrate that can be deposited for hindgut fermentation (Leeuwen and Jansman, 2007). To date, little efforts had been made to determine the influence of physicochemical properties of diets on digesta quality in relation to passage rate and VFA production.

In pigs, an increase in viscosity of gut chyme stimulate epithelial cell proliferation, and may also contribute to increased cell lesions and exfoliation especially in the apical part of the intestinal

villus (Gee et al., 1996; Schiavon et al., 2004). The inclusion of DF also increases pancreatic secretions (Schneeman et al., 1982). In this respect, there is a reasonable chance that through these postulated mechanisms, both soluble and insoluble fiber could, simultaneously, increase the quantity of indigestible nutrients that are potential substrates for microbial activity in the lower intestines. Thus, creating a conducive environment that favours growth of beneficial microbiota, as well as, production of VFA and microbial fat. Colonic transit time is influenced by VFA in the lumen of the large intestine but the underlying mechanisms are scantily documented (Fukumoto et al., 2003). With the introduction of alternative co-products with varying dietary fiber properties, further interests in investigating the effects of fiber solubility on hindgut VFA, FA flows and lipid metabolism-related blood metabolites in growing pigs has emerged, but limited research in this area has occurred. Such research is of paramount importance as a culmination to clarify suggestions raised by above-mentioned paradoxical observations and assess response in modern pig genotypes.

2.3.3.3. Host or Animal-related factors

There is an inter-relationship among various animal-related factors that are linked to the digestive and absorptive capacity for lipids, GIT fermenting pattern and capacity to shape VFA profiles, and subsequently microbial fat production in pigs. These include the age, breed and species, pig weight, gender and reproductive physiological status, GIT segment, GIT capacity, adaptation period or duration of exposure to diets, stress and neuroendocrine system activity, and pancreatic and other secretions in the GIT and have been elegantly reviewed by Macfarlane and Macfarlane (2003) and Millet et al. (2010). Although these factors are mainly linked with anatomical differences that probably detect the composition and diversity of gastrointestinal

microbiota, they also play the central role in determining the amount of substrate available for microbial degradation. Besides, these factors also detect enzyme activity and absorption capacity of not only VFA but also FA in the upper gastrointestinal segments. Microbiota communities in the GIT establishes at or immediately after birth and increase and diversify with aging. For example, an increase in enterobacteriaceae and a decrease in anaerobes and bifidobacteria in elderly humans (Hebuterne, 2003). Matured pigs such as sows and finisher pigs, as well as indigenous breeds have a greater fermentation capacity compared to weaned and grower pigs and exotics breeds. Lindemann et al. (1986) postulated that pigs have a complete digestive capacity for all dietary lipids at approximately 40 kg, and weanling pigs have a poor lipid digestive and absorptive capacity as well as fermentation capability. The ability to digest fat and absorb dietary FA is poorly developed in piglets probably due to disturbances in the GIT as diets shift from liquid to solid. At the same time, the hindgut of young pigs is not fully developed to accommodate microbial populations that facilitate DF fermentation. Pigs fed fibrous diets adapts by developing heavier gastrointestinal tracts than those fed low-fiber diets (Rijnen et al., 2001; Yen, 2001), but the influence of DF solubility is often ignored in research.

2.3.3.4. Gastrointestinal microbiota-related factors

The anaerobic bacterial population in the pig GIT ranges from log 7-8 in the stomach and small intestine, to log 11-12 in the large intestine (Peng et al., 2013). These gastrointestinal microbial communities are diverse, for example, up to 10^{11} bacteria per gram of feces classified into more than 50 genera and over 400 species (Savage, 1977; Dunne et al., 2001). Of these, approximately 90% of bacterial constituents in the pig colon are Gram-positive, strictly of the *Streptococcus*, *Lactobacillus*, *Eubacterium*, *Clostridium* and *Peptostreptococcus* genus (Ridlon et

al., 2006). Gram negative bacteria represent about 10% of the microbial population and comprise of the *Bacteroides* and *Prevotella* groups (Ridlon et al., 2006). The major amylolytic bacteria belong to the genera *Bifidobacterium*, *Bacteroides*, *Fusobacterium*, and *Butyrivibrio* (Macfarlane and Englyst, 1986). The predominant proteolytic species in the hindgut belong to the genera *Bacteroides*, *Propionibacterium*, *Clostridium*, *Fusobacterium*, *Streptococcus*, and *Lactobacillus* (MarFarlane and Cummings, 1991). Relationships among GIT microbiota involve commensalism, mutualism, and competition, with individual species involved in more than one type of interaction during fermentation of DF, and biosynthesis and biotransformation of FA and BA.

The crucial role played DF solubility in modulating the gastrointestinal microbiota activities that facilitate the interplay among various abovementioned effectors of lipid digestion, GIT fermenting pattern and capacity to shape VFA profiles, and subsequently microbial FA production in pigs should not be understated. The GIT of the pig is characterized of dark, warm, moist and anaerobic conditions that harbour abovementioned magnanimous microbial communities as an ecosystem in digesta (Bergman, 1990) and mucosal walls. The proportion of VFA produced from hindgut fermentation solely depends on how DF type and inclusion level shapes the microbiota population, activities and metabolic by-products and their interrelationships. The most abundant phyla in the intestines are *Bacteroidetes* (Gram-negative), *Firmicutes* (Gram-postive), and *Actinobacteria* (Gram-positive) (Den Besten et al., 2014). The *Bacteroidetes* phylum has acetate and propionate as their primary metabolic end product, whereas the *Firmicutes* phylum mainly produce butyrate (Macfarlane and Macfarlane, 2003). There is accumulating evidence that BA pool size and composition also regulate structural and functional characteristics of gastrointestinal microbiota in intestinal digesta and mucosa that influence VFA and BA flows (Tan et al., 2014; Ferrebee and Dawson, 2015; Nie et al., 2015). Therefore, because DF solubility influence the

concentration of intestinal BA that play a central role in FA absorption, this has stimulated a need for future studies to investigate VFA production in conjunction with gastrointestinal flows of FA and BA and digesta and mucosa-associated microbiota composition and metagenomic function.

The GIT microbiota also exhibit symbiotic associations with the host that are likely to be modulated by DF solubility, for example, molecular hydrogen produced during acetate formation is used by other bacterial populations to prevent inhibition NADH oxidation (Den Besten et al., 2013). Additionally, the CO_2 used during the primitive electron transfer is partly produced by the host, but some of it is excreted into the GIT lumen as HCO^{-3} in exchange for VFA anions (Den Besten 2014). Lactobacilli and streptococci are major species in the pig intestine and can convert carbohydrates into lactic acid (Pluske et al., 2002). The cross-feeding theory also suggests that lactic acid can be utilized by *Veillonella* spp as a carbon source and converted into propionate and acetate (Hugenholtz et al., 2013). Moreover, *Bacteroidetes* are part of a community, stabilized by mutual cross feeding, where other members of the community consume these gasses. For instance, Archaea produce CH_4 from CO_2 and H_2, while acetogens convert CO_2 into acetate. Depending on the source of DF, increase in abundance of *Lactobacillus* was associated with a lower abundance of *Faecalibacterium* (Berggren et al., 1993). Therefore, the mechanism by which the former suppresses the growth of the latter species is by competitive exclusion as well changes in enzymatic activities (Dierick and Decuypere, 1996).

The production of VFA and other organic acids including lactic acid by hindgut fermentation decrease intestinal pH and consequently inhibits the growth of entero-pathogens (*Escherichia coli*, *Salmonella* spp. and *Clostridium* spp) and alleviate post-weaning diarrhoea (Montagne et al., 2003). Other bacterial species such as *Methanobrevibacter smithii* and Firmicutes have been reported to promote energy bioenergetics of fermentation products in hindgut of rodents and

human. However, the influence of these relationships on VFA profiles warrants further elucidations in growing pigs. A plethora of studies reviewed by Hugenholtz et al. (2013) in human and rodents revealed that microbial composition and probiotics influence the VFA profile, such studies are ignored in pigs fed different fiber sources. These results suggest that diets can be manipulated in such a way that the VFA production is improved as well as their absorption especially when the growth of butyrate producing bacteria populations is promoted. Butyrate is a major and preferred metabolic fuel for intestinal epithelium providing at least 60-70% of their energy requirements for proliferation and differentiation and ensuring that dietary nutrients and VFA are absorbed by the host at the best possible efficiency (Bergman, 1990; Suzuki et al., 2008). Eventually, butyrate producing species within the bacterial order *Clostridiales* are expected to be beneficial for animal performance (Rinttilä and Apajalahti, 2013). At the same time, production of butyrate in the GIT will reduce nutrient emissions.

Unlike in ruminants, it has become clear that in monogastric animals, gastrointestinal microbial communities, FA and DF are in continuous exposure to BA. The role played by BA in modulating the microbiota composition has been reviewed in humans, particularly in relation to metabolic diseases. Microbes shape BA profiles directly through BA hydrolysis, dehydrogenation and dihydroxylation, and indirectly by producing VFA that reduce GIT pH, and results in deconjugation of bile salts (Ridlon et al., 2006). There is also accumulating evidence that apart from BA, cholesterol derivatives including neutral sterols (NS) facilitates interrelationships between intestinal microbiota and energy or lipid metabolism (Tan et al., 2014; Ferrebee and Dawson, 2015; Nie et al., 2015). It remains unknown, however, how the interaction between BA and microbes influence growth performance, energy metabolism and FA utilization in growing pigs fed soluble or insoluble DF-enriched diets.

2.4. GASTROINTESTINAL ABSORPTION OF VFA AND DIETARY MCFA AND LCFA

Digestion of dietary fat starts in the stomach, continues into the duodenum and increases in the jejunum, whereas passive absorption of MCFA and LCFA takes place in all regions of the small intestines up to the distal ileum. The absorption of MCFA and LCFA is considered to be negligible in the cecum and colon (Den Besten et al., 2013; Schönfeld and Wojtczak; 2016), however, these FA undergoes through biotransformation into microbial FA forms. On the other hand, production and absorption of VFA from microbial fermentation occurs in the colonocytes across the epithelial walls of different intestinal segments (Clemens et al., 1975; Schönfeld and Wojtczak; 2016; Montoya et al., 2016). As far as we know, most studies on absorption kinetics of VFA have been extensively performed in cecal and colonic epithelial, but very few, if any studies have been reported for epithelium in stomach or small intestines in pigs. Incremental levels of DF from wheat bran and kiwi fruit have been reported to increase hindgut VFA production (Montoya et al., 2016; Iyayi and Adeola, 2015) but influence of DF solubility is not well researched. If the usefulness of DF as energy sources and that of fat supplemented to fortify energy contents of high-fiber diets is to be met, there is need to understand the influence of DF solubility on bioavailability and absorption kinetics of VFA from fermentation, as well as dietary MCFA and LCFA.

In humans, 95% of the produced VFA are rapidly absorbed by the colonocytes while the remaining 5% are excreted in feces (Den Besten *et al.*, 2013). In pigs, up to 85% of the total VFA produced in the gut are absorbed. Other studies have demonstrated that about 90% of VFA produced are rapidly absorbed, stimulating water and sodium absorption (Topping and Clifton, 2001). Jorgensen et al. (1997) reported that less than 1% of infused VFA were excreted in feces. The disagreements in these studies stimulates a need for a holistic approach that quantify VFA

produced concurrently with other factors including physicochemical properties of digesta such as mineral, pH, water content, WHC and viscosity. A plethora of studies have used VFA concentrations that are in gut segments and have drawn conclusions regarding the production of VFA. The VFA concentrations in the GIT represent net results of VFA productions and absorption and are not accurate descriptors for assessing fermentability (Montoya et al., 2016). To counter for this limitation, the use of *in vitro* fermentation technique in conjunction with *in vivo* assay models (catheterized and intestine-cannulated pigs) to quantify VFA production and absorption in the GIT is increasingly getting attention in pig nutrition (Iyayi and Adeola, 2015; Montoya et al., 2016; Ingerslev et al., 2017).

Volatile fatty acids are actively and passively transported through epithelial cells either in the ionized or non-ionized (without transporter) forms (Den Besten et al., 2013). Recent studies have demonstrated that trans-epithelial absorption of ionized VFA through active transport across the colonocytes is facilitated by 3 channels, namely unidentified transporter (employs exchange of bicarbonate ion), monocarboxylate transporter 1 (MCT 1, use H+-dependent electroneutrality) and electrogenic sodium-dependent monocarboxylate transporter 1 (SMCT1) (Den Besten *et al.*, 2013). Gene expressions of both MCT-1 and SMCT-1 occurs on colonocytes and also along the entire GIT including the small intestine and the cecum (Iwanaga et al., 2006). In pigs, Agyekum et al. (2015), demonstrated dietary addition of DF from DDGS and exogenous enzymes initiated expression of ileal MCT1. However, more work is needed to establish effects of different DF sources and transporter mRNA expressions in different segments of the GIT. The anionic form of VFA can also be absorbed in exchange of Cl- instead of HCO3- only (Rajendran and Binder, 1991; Mascolo et al., 1991). Passive diffusion of undissociated VFA directly across both the apical and basolateral membrane have also been proposed (Den Besten, 2013). Considering that DF

fermentation results in production of lactic acid that may lower the pH of the GIT, dietary inclusion of highly fermentable fiber that supports growth of lactic acid-producing bacteria is likely to increase the ratio of anionic:undissociated VFA.

The absorption of VFA is greatly influenced by pH of the gut milieu. The pH in the GIT revolves between 5.4 and 6.5, but Marlett et al. (2002) reported that with a pK_a of approximately 4.8 and a luminal pH around 6.0 (pH 5.5-6.5) only a very small proportion of VFA exists in its undissociated forms. Thus, it is highly unlikely that active transport plays the central role during trans-epithelial absorption of VFA. However, it can be postulated that if deposition of digesta in lower gut is reduced, pH is likely to increase thereby opening an opportunity for passive diffusion of the undissociated forms of VFA to play a major role. This assumption can be supported by a pig study in which the concentration of butyrate in the portal vein started to increase 4 h postdosing (i.e., the time when the bulk of digesta reaches the hindgut (Bach-Knudsen, 2003, 2005). Conversely, the slight acidification of luminal pH, possibly by bacterial metabolic activities will increase the prevalence of protonated forms of VFA (Schönfeld and Wojtczak, 2016).

Dietary fiber composition, amount and monomeric arrangements within its building blocks are the major determinants of net portal absorption of VFA (Bach-Knudsen, 2000, 2015; Ingerslev et al., 2014). Moreover, a strong relation between net butyrate absorption and butyrate concentration in portal blood has been observed by Bach-Knudsen (2000) and Ingerslev et al. (2014), but no studies have linked these fluxes with SCFA in GIT lumen, including butyrate. The absorption of VFA in the colon, and the bacterial fermentation product formation varied quantitatively and qualitatively in different regions of the colon, principally in relation to butyrate production (Macfarlane et al. 1994). In bovine, Stevens and Stettler (1967) found that among other SCFA, propionate is the only acid that is markedly increased in blood by the presence of other

gastrointestinal VFA especially butyrate. Butyrate is the main source of energy for trans-epithelial absorption of VFA in the gut (Bergman, 1990). However, there is still a paucity of data on the influence of DF types on absorption kinetics of individual VFA in different gut segments, uptake of VFA by hepatocytes and other visceral organs, as well as that of the transporters involved. Moreover, information on metabolic adaptations to VFA absorption in modern pig breeds fed fiber-rich diets is still a meagre. Henceforth, the lack of actual quantitative data on fluxes of VFA has hampered swine nutritionists to elucidate the contribution of VFA to the host's metabolizable energy.

In contrast to VFA and MCFA, which directly enter the portal vein from the intestinal tract as free acids, LCFA are esterified to triglycerides in enterocytes, incorporated into chylomicrons, and then enter the lymphatic system (Schonfeld and Wojtczak, 2016). However, supplementation with fermentable fiber increased VFA flows but also increased the amount of fat in the feces (Bach Knudsen and Hensen, 1991; Galassi et al., 2004; Kritchevsky and Tepper, 2005). It is difficult to ascertain whether fecal fat is due to sub-optimal absorption of dietary FA or inefficient digestion and absorption of dietary fat due to deconjugation of bile acids by increased bacterial activity in the upper gut. Poor absorption of FA is also exacerbated by the presence of insoluble DF which acts as a sequestrating agent by masking BA thus reducing their fat emulsifying capacity. In addition, excretion of dietary fat suggests that dietary FA or endogenous BA could have been masked within the fiber matrices thereby compromising emulsification and lipase activity in the upper GIT. These mechanisms are likely to influence homeostasis of lipid metabolism-related blood constituencies, such as cholesterol and triglycerides, and consequently energy metabolism and growth performance. However, an increase in fecal fat due to fermentation and/or poor absorption of FA in the GIT is undesirable and defeats the whole purpose of dietary inclusion of

fermentable fibrous ingredients or fortifying high-fiber diets with supplemental fat needed for energy supply. Furthermore, not only did DF intake influence the MCFA and LCFA absorption, but the absorption of FA from gut lumen vary, depending on other dietary-related (FA profile, FA inclusion levels, FFA content, degree of saturation, extracted and intact fat, mineral content) and animal-related factors (age, breed, body weight, gut morphology) as well as physiological processes (BA and FA transporters, digesta quality). The interaction effects of DF sources with these factors have not been extensively studied in pigs and few experiments conducted in bovine, humans and rodents have produced conflicting results.

Fat digestibility increased probably due to a high proportion of UFA that can be absorbed with high efficiency in the small intestines (Dégen et al., 2009; Kil et al 2010; Hooda et al., 2011). Similarly, Davis et al., demonstrated that ileal digestible C18:2 and PUFA increased with inclusion of tallow independent of DDGS supplemental level and indicated that the increment was a function of dietary concentration of the FA. In the same study, they also reported that although ileal digestible C16:0, C18:0, and SFA increased with addition of tallow to 0% DDGS-containing diets, the increment was less prominent in pigs fed diets with 30% DDGS. Supplementing grower-finisher pig diets with high DDGS and supplemental oil increases PUFA, linoleic acid (C18:2) content, and iodine value (**IV**) of pork fat (Stein and Shurson, 2009) thus reducing belly firmness (Xu et al., 2010; Widmer et al., 2008). Davis et al. (2015) suggested that the use of tallow to reduce deposition of MUFA and PUFA may not work because the digestibility of SFA was depressed with increase in dietary addition of a fibrous co-product. These observations trigger the need to investigate the impact of the SFA:PUFA/MUFA ratios in pigs fed other types or sources of DF such as pectin, cellulose, flaxseed meal and oat hulls because the DDGS are commonly rich in arabinoxylans (Agyekum et al., 2015). Although the digestibility of individual FA was not

determined in the study by Kil et al. (2010) who found that the apparent digestibility of fat increased with incremental levels of dietary extracted or intact fat, the true digestibility of fat was not influenced by the dietary concentration of fat. One reason for this observation is that endogenous losses of fat (**ELF**) affects apparent digestibility, but not the true digestibility. Therefore, determination of individual FA profiles and ELF or endogenous losses of FA that has been ignored in most studies attempt to characterize fat absorption may provide closer to accurate values.

2.5. METABOLISM OF SHORT-CHAIN FATTY ACIDS

2.5.1. Sites and pathways of VFA metabolism

There is general assumption that VFA are rapidly absorbed and/or metabolized in the hindgut. However, previous reports have demonstrated that butyrate does not pass in portal blood, but is completely used up during active transport in the ceco-colonic epithelial cells, whereas other VFA reduce as they pass through the liver, muscles and adipose tissues (Bergman, 1990; Layden et al., 2013). The colonocytes are the first host cells that take up VFA and depend largely on butyrate for their energy supply (Binder, 2010). Other studies demonstrated that the majority of VFA are utilized by colonic epithelial cells especially butyrate, but small proportions reaches the liver (Roediger, 1982; Reilly and Rombeau, 1993) and are completely metabolized together with propionate and up to 70% of acetate for gluconeogenesis (Bergman, 1990; Bloemen et al., 2009; Roberfroid, 2007). Conversely, Bach Knudsen et al. (2003) and Bach Knudsen, (2015) reported that a small proportion of the butyrate that is produced in the GIT is also recovered in the portal vein, where it flows to the liver, heart and lungs. Augmenting these results are findings by Ingerslev et al. (2014) who reported that the clearance rate of butyrate is approximately 82%.

Furthermore, the adipose tissue and milk fat do not contain butyrate suggesting that most of it is used up either in the colon epithelial tissue or by liver cells (Kien et al., 2000).

A previous study demonstrated that the ratio of acetate: propionate: butyrate had been found to be 69:23:8 in the hepatic portal vein and 57:22:21 in the large intestine (Binder et al., 2010). Such observations may not only further confirm metabolism of VFA during epithelial transport but also indicates that colonocytes have high affinity for butyrate. However, it is still not clear how much of the butyrate is used by the colonocytes and hepatocytes in growing pigs that had been fed fermentable fiber-rich diets. Bach Knudsen (2005) also reported that an increase in DF intake is associated with traces of micromolar concentrations of all 3 VFA reaching the peripheral tissues. These studies demonstrate contradictory revelations that seriously questioned the general view that butyrate is completely metabolized within the vicinity of the colonic epithelium and warrants further investigations in growing pigs fed practical sources of DF (Cummings et al., 1987a; Roediger, 1992; Scheppach and Weiler, 2004).

In the liver, all VFA, especially propionate and acetate are used as substrates of energy producing tri-carboxylic acid cycle and efficiently metabolized to produce glucose at clearance rates of approximately 94 and 41%, respectively (Den Besten et al., 2013; Ingerslev et al., 2014). Both the exogenous acetate (product of hindgut microbial fermentation) and endogenous acetate (released by host tissues and organs) enters the blood compartment and are used as substrates for various metabolic changes (Knowles et al., 1974; Bloemen et al., 2009). The actual contributions of the exogenous and endogenous acetate sources to the pool of acetate in blood and energy supply had not been established. However, up to 70% of the acetate is used up in the liver and the remaining 30% is metabolized by other tissues including the heart, adipose tissue, kidney and muscle (Knowles et al., 1974; Bloemen et al., 2009; Peng et al., 2013). Previous studies have

established that not all of the acetate that goes to the liver is used for energy supply to the hepatocytes, some is used for de novo synthesis of cholesterol and long chain fatty acids, and as a substrate for glutamine and glutamate synthesis (Layden et al., 2013; Zhang et al., 2014). There is need to quantify the amount of acetate that is used up during each of these events and how DF solubility modulates these fluxes. It has been suggested that under certain physiological condition (relatively high colonic concentration of acetate compared with butyrate), acetate is at least as important as butyrate for energy supply to the colonocytes in human and rats (Den Besten et al., 2013). Therefore, further research is needed to investigate whether this postulation is valid in growing pigs fed different DF sources and lipid types.

Previous studies with the application of the isotope dilution technique suggest that between 45 and 65% of propionate is used in glucose synthesis, but differences between hepatic portal blood and venous blood suggest that around 30% of propionate is taken by the liver (Cummings et al., 1987; Bloemen et al., 2009). Another study in humans estimated that hepatocytes use up to 50% of propionate for gluconeogenesis (Den Bestegen et al., 2013). Therefore, it is clear that a large proportion of propionate from the portal circulation is used by the liver, however, it is difficult to ascertain the extent to which propionate contributes to energy metabolism in pigs fed different DF sources. One reason for this is lack of quantified data on true production rates not only of propionate but also other major VFA. It has been reported that the peripheral venous blood content of propionate is 23% lower than hepatic venous blood (Reilly and Rombeau, 1993), but there is still lack of actual energy contributions of propionate to each of the peripheral tissue in growing pigs. The use of the catheterized and cannulated pig models will open way for researchers to quantify the absorption kinetics and uptake of VFA from colonocytes, hepatocytes and the rest of body cells. Furthermore, the catheterized pig model would provide a better picture of the

disappearance of blood constituents in the body if it is combined with infusion of isotope-labelled VFA. The catheterized model has been used extensively to determine metabolism of other nutrients, very few studies have determined VFA absorption and metabolism. The integration of the catheterized and cannulated pigs model may raise animal welfare concerns. These data collectively suggest that VFA are metabolized in three major regions, that is: 1) by colonocytes that use them as an energy source, 2) by hepatocytes that use propionate for glycogenesis, and 3) by muscles and adipose tissue for muscular activities and fat deposition.

2.5.2. Role of VFA as energy sources to the host

The contribution of energy from VFA produced by gastrointestinal fermentation to the host's maintenance energy should not be doubted. It is conditioned by the absorption and metabolic utilization of hindgut microbial activity by-products by the host. To be specific among DF fermentation products, VFA are the main if not the only substrates for energy generation to the pig (Bindelle et al., 2008). It is also worth noting that secondary BA are also another group of microbial metabolites that indirectly influence energy metabolism by altering the microbial communities that might participate in hindgut fermentation. The BA also modulates the farnesoid X receptor (**FXR**), a receptor that participate in energy metabolism. The equivalent energy produced by acetate, propionate, butyrate and valerate is estimated to be 208, 364, 520, and 676 kcal/mol, respectively (Weast, 1977). The energy values of these selected VFA might be lower than the 8.4 and 9.2 kcal/g reviewed by Bach and Babayan (1982), Papamandjaris et al., (1998) and Marten et al. (2006) for MCFA and LCFA, respectively, but the distinct differences in the roles that each of the VFA play to energy contribution should not be undermined. Although, other VFA like valerate have a higher energy value, great emphasis had been given to the first three, most likely because they constitute

major proportion of the total VFA produced. Several studies have revealed that energy produced from hindgut VFA contributes up to approximately 30% of total digestible energy in finishing pigs and sows (Dierick et al., 1989; Varel and Yen, 1997; Anguita et al., 2006). Volatile fatty acids can also contribute between 24 and 30% of the energy needs of growing pigs (Yen et al., 1991), and 25% have been postulated in sows (Iyayi and Adeola, 2015). These estimates were influenced by level of inclusion of DF, however, the impact of DF solubility and other physicochemical properties is not well-characterized. The integration of *in vitro-in vivo* models could offer opportunities to predict energy contribution from gastrointestinal VFA. According to an *in vitro* study done in rats by Roediger (1982) and Suzuki et al. (2008), carbon dioxide production measurements in isolated colonocytes indicated that colonocytes derive 60-70% of their energy supply from VFA oxidation.

Conclusions on energy supply from hindgut fermentation have been made based on *in vivo or in vitro* methods or an integration of the two (Coles et al., 2005; Awati et al., 2006; Iyayi and Adeola, 2015; Pecka-Kielb et al., 2016). Although these methods take care of the immediate absorption of VFA produced *in vivo*, they ignore the difference in luminal contents and microbiota communities in different section of the hindgut (colon and cecum). However, it is well-established that DF fractions and fermentability differ from cecum to colon (Jarwoski and Stein, 2016) and the microbiota composition between the cecum and colon are also different. Furthermore, the distribution of VFA transporter along the GIT also indicates that absorption kinetics differ in different GIT segments. Thus, results obtained using the *in vivo-in vitro* fermentation assay using ileal cannulated pigs are likely to be inaccurate and they ignore the influence of DF source. Interestingly, the use of substrate from double (ileal and cecal) cannulation may open way for estimating the production and absorption of VFA in the cecum and colon. Although these authors

reported that these calculations are influenced by host-related factors (age, breed and body weight) and fermentable carbohydrate content of the diets, there is a paucity of information on the influence of physicochemical properties of the fibrous ingredients and metabolic fates of various VFA in growing pigs. There are distinct differences in the roles that each of the VFA namely acetate, propionate and butyrate play to energy contribution (**Table 2.1**). Some proportion of VFA are used for boosting fat production, however, their efficiency is lower compared to glucose (Bindelle et al., 2008). Future research should focus on quantifying the actual changes in VFA fluxes from the gut, visceral organs and the rest of the body, in conjunction with other energy-related blood metabolites, such as glucose, insulin, bile acids and fatty acids.

2.5.3. Gene expression indicative of transport and metabolism of fatty acids and bile acids

Next to their role as metabolic substrates for energy supply, physiological effects of VFA during the post absorptive phase are mediated by receptors namely, peroxisome proliferator-activated receptor (PPAR)γ, G protein-coupled receptors namely, free fatty acid receptor (FFA) 2 (also known as GPR43) and FFA3 (also called GPR41) (Gao et al., 2009; Den Besten et al., 2013; Zhang et al., 2014). It is well established that both FFA2 and FFA3 are well expressed in the pig, human and mouse colon, are localized on the same chromosome, and share 43% amino acid sequence identity, but they differ in affinity for individual VFA, tissue distribution and physiological roles (Den Besten et al., 2013; Zhang et al., 2014). Furthermore, it is still a matter of debate whether FFA2 and FFA3 reside on apical or basolateral membranes (Den Besten et al. 2013) and/or are both expressed in hepatocytes the same way as in colonocytes. Recent research in pigs has reported the existence of a duplicated FFA2 gene known as FFA2L (Zhang et al., 2014). This implies that more orphaned G protein-coupled receptors exist and warrant further investigations in growing pigs fed high-fiber diets. The role of VFA in regulating metabolism of

Table 2.1. Role of short chain fatty acids as energy sources for the host

C2	C3	C4	Energy contribution	References
		↑	-Energy source for colonocytes -Induce cellular differentiation in the colon cell lines	Roedieger, 1982; Reilly and Rombeau, 1993; Binder, 2010; Peng *et al.*, 2013
↑	↑		-Substrates for gluconeogenesis in the liver (hepatocytes)	Bloemen et al., 2010; Peng *et al.*, 2013; Besten *et al.*, 2013
↑			-Energy source to the adipose tissue	Lin *et al.*, 2012; Hugenholtz *et al.*, 2013
↑	↑		-Energy source to the muscle	Lin *et al.*, 2012; Peng *et al.*, 2013
↑	↑		-Energy source to the heart	Lin *et al.*, 2012
↑	↑		-Energy source to the brain	Lin *et al.*, 2012; Hugenholtz *et al.*, 2013
↑			-Energy source to the kidney	Hugenholtz *et al.*, 2013

C2 = acetate; C3 = propionate; C4 = butyrate; ↑ = is a preferred energy source.

FA, glucose and cholesterol had been extensively reported in mice, rats and humans. Each of the three major VFA has unique cellular functions. Therefore, the influence of individual VFA metabolism on cecal and colonic gene expression is difficult to decipher, since they are probably indirect and involve interplay among multiple biochemical processes. For example, butyrate is involved in absorption of other VFA at the same time influence gene expression via selective effects on G-proteins within cells or by more directly interaction with regulatory DNA sequences. To what extent VFA fluxes stimulate gene expression of bioenergetics in pigs fed different fiber sources under growing conditions remains a mystery. Continuous exposure of pigs to fiber-rich diets may lead to direct and/or indirect modification of mRNA expression of genes involved in lipid and energy metabolism. Gene expression by certain dietary components, such as DF or products from their degradation (VFA and BA) can be by indirect or direct interaction with regulatory elements in the genome, and consequently changing the transcription rate of a given gene (Cousins, 1999).

Apart from that, the inclusion of xylanase in corn bran dietary fiber-rich diets fed to rats was reported to improve mRNA expression of genes involved in lipids metabolism (Hu et al., 2008). Exogenous enzymes are increasingly incorporated in pig diets to solubilize the DF components and promote fermentability of insoluble fiber. Moreover, previous reports have demonstrated that fermentable fiber increase fecal fat content (Bach Knudsen, 1991; Galassi et al., 2004; Bakker et al., 1996). It is not clear whether the source of fecal fat is due to suboptimal absorption of VFA or dietary fat, whichever way, excretion of fat is undesirable and counter-productive to use of fibrous co-products in pig diets. One cannot meaningfully address these paradoxes unless studies are carried out to determine whether inclusion of fermentable DF diets given to pigs improves lipid, BA and VFA homeostasis through influencing of genes involved in both lipid and VFA

metabolism. The liver, ileum, cecum and colon are the primary sites for VFA and BA metabolism. On the other hand, the effects of VFA on metabolism of FA and lipid distribution are not fully understood, but it is suggested that VFA, particularly propionate may change adipose tissue lipolysis, adipocyte size and differentiation, and consequently body fat distribution. Therefore, assessment of cecal, colonic and hepatic genes expression indicative of VFA and BA regulation warrant further investigation in pigs fed different DF sources. Interestingly, BA can activate FXR and modulate gastrointestinal microbiota responsible for producing VFA (Nie et al., 2015). Bile acids-activated FXR can induce the expression of genes, such as *Ang1*, *iNos*, *Il18*, CYP7A1 and CYP2C49 which are involved in enteroprotection and energy metabolism (Inagaki *et al.,* 2006). Therefore, these genes alter processes that contribute to the inhibition of microbial overgrowth and mucosal damage as well as cholesterol metabolism. Moreover, increase in intestinal concentrations of chenodeoxycholic acid and ursodeoxycholic acid activate the expression of cathelicidin through FXR and vitamin D receptor. Cathelicidin is an antimicrobial peptide which prevents microbial invasion in the biliary epithelium (D'Aldebert et al., 2009). Taken together, BA indirectly modulate gastrointestinal microbiota via nuclear receptors.

CHAPTER THREE

HYPOTHESES AND OBJECTIVES

3.1. HYPOTHESES

The specific hypotheses tested in this thesis are that:

1. Dietary supplementation with soluble and insoluble fiber induce comparable effects on growth performance, blood lipids, gastrointestinal metabolites, histomorphology, microbiota composition in pigs;

2. There is no difference in digestibility of fatty acids (**FA**) and dietary fiber (**DF**) fractions, flows of FA and secondary bile acids (**BA**) in the gastrointestinal tract, and production and absorption of hindgut volatile fatty acids (**VFA**) in pigs fed soluble and insoluble fibers in diets;

3. The interaction between DF solubility and lipid type will reduce fermentability of DF more in soluble fiber-fed pigs compared to insoluble fiber-fed pigs, thereby reducing fat digestibility and hindgut VFA production and absorption and increasing gastrointestinal flow of FA and BA more in animal fat-containing diets than vegetable oil-containing diet; and

4. Dietary inclusion of soluble fiber increases intestinal VFA fluxes, and consequently stimulates colonic and hepatic expression of genes involved in VFA, FA and BA transportation and metabolism of VFA and FA in colonic mucosa tissue and liver of growing pigs.

3.2. OBJECTIVES

1. To determine the effect of DF solubility on growth performance, fat digestibility, serum lipids, and concentrations of VFA, BA and NS, histomorphological characteristics of intestines, and digesta- and mucosa-associated microbiota in gastrointestinal tract of growing pigs.

2. To investigate the effects of DF solubility on digestibility of DF and FA, and flows of FA and BA in the ileum and feces, and use an integration of the *in vivo* and *in vitro* fermentation technique to predict hindgut VFA production and absorption in ileal-cannulated pigs.

3. To determine the interactive effects of DF solubility and lipid type on digestibility of DF and FA, and flows of FA and BA in the ileum, cecum and colon and use a combination of *in vivo* and *in vitro* fermentation methodology to predict VFA production and absorption in the cecum and colon of ileal- and cecal-cannulated pigs.

4. To investigate the effects of DF solubility on transepithelial transport, intra-epithelial metabolism and uptake of VFA in the colon and the colonic and hepatic expression of genes involved in lipid metabolism in growing pigs.

CHAPTER FOUR

MANUSCRIPT 1

Dietary supplementation with flaxseed meal and oat hulls modulates performance, blood lipids, fat digestibility, gastrointestinal metabolites, histomorphometric characteristics, and microbiota in growing pigs[1-3]

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[1]Materials used in this chapter were presented in part at the 2015 ADSA-ASAS Joint Annual Meeting, Orlando, FL.

[2]The other version of the materials presented in this chapter have been published as follows: S. P. Ndou, E. Kiarie[1], S. J. Thandapilly, M. C. Walsh, N. Ames and C. M. Nyachoti. 2017. J. Anim. Sci. 95:3068-3078. doi:10.2527/jas.2016.1328. (**Paper 1**)

[3]Materials used in this chapter have been used in the following publication: S. P. Ndou, H. M. Tun, E. Kiarie[1], M. C. Walsh, E. Khafipour[2] and C. M. Nyachoti. 2018. Sci. Rep. 8:5880. doi:10.1038/s41598-018-24043-5. (**Paper 2**)

Authors' contributions

S.P.N., E.K.[1]. and C.M.N. designed the experiment. S.P.N conducted the animal study, lab and statistical analyzes, analyzed the data and wrote the manuscripts. S.P.N., H.M.T. and E.K.[2] developed the bioinformatics models. E.K.[1], S.J.T, E.K.[2], M.C.W., N.A. and C.M.N. critically reviewed the manuscripts. C.M.N. was the principal investigator who supervised all aspects of the study.

4.1 ABSTRACT

The present study investigated the effects of feeding soluble and insoluble fiber on growth performance, apparent total tract digestibility (ATTD) of fat, serum lipids, and concentrations of volatile fatty acids (VFA), bile acids (BA) and neutral sterols (NS), histomorphological characteristics, digesta- and mucosa-associated microbiota in pig intestines. Forty-Eight Genesus barrows (initial BW: 25.0 ± 0.32 kg) were housed in pairs. Pigs were assigned to one of the three corn-soybean meal-based diets without (CON, control), or with flaxseed meal (FM) or oat hulls (OH), in a completely randomized design for 28 d. Pigs fed the control or OH diet had greater ($P < 0.05$) final BW, ADFI and ADG compared with FM diet-fed pigs. The apparent total tract digestibility of fat in FM diet was lowest at 70.1%, followed by 79.2% in OH diets and greatest at 92.4% in control diet ($P < 0.05$). Total serum cholesterol content was 2.25 and 1.99 mmol/L and lower ($P < 0.001$) in pigs fed FM and OH diets, respectively, than 2.36 mmol/L in pigs fed the control diet. Pigs fed FM and OH diets had greater ($P < 0.01$) ileal and cecal total VFA, and ileal deoxycholic acid, and cecal and fecal cholesterol concentrations than those fed the control diet. The concentrations of coprostanol in cecal digesta and feces were higher ($P < 0.05$) in pigs fed FM and OH diets than in pigs fed the control diet. In comparison with the CON and OH diets, the consumption of the FM diet increased the jejunal villi height (VH) and the ratio of VH to crypt depths and excreted more fecal lithocholic acid and ursodeoxycholic acid ($P < 0.01$). The PERMANOVA analyzes showed distinct ($P < 0.05$) microbial communities in ileal digesta and mucosa, and cecal mucosa in CON and FM-diets fed pigs compared to the OH diet-fed pigs. The predicted functional metagenomes indicated that amino acids and butanoate metabolism, lysine degradation, bile acids biosynthesis, and apoptosis were selectively enhanced at more than 2.2 log-folds in intestinal microbiota of pigs fed the FM diet. In conclusion, soluble fiber from flaxseed

meal depressed growth performance, whereas the insoluble fiber from oat hulls did not have any effect. Addition of soluble and insoluble fiber in growing pigs' diets reduced fat digestibility and serum cholesterol and, stimulated malabsorption of primary bile acids and excretion of secondary bile acids and neutral sterols. Soluble and insoluble fiber induced variable effects on gastrointestinal histological and fermentation characteristics, and altered the composition and function of intestinal microbiota in growing pigs, depending on the intestinal segment and physicochemical property of the dietary fiber source.

Key words: bile acids, dietary fiber solubility, lipid metabolism, pigs, volatile fatty acids.

4.2. INTRODUCTION

Increased global demand for grains and soybean has led to incorporation of low cost agro-industrial co-products in swine diets resulting in increase in dietary fiber (**DF**) content. Dietary fiber can be fermented to produce volatile fatty acids (**VFA**) needed for energy supply by the host, improve gut health and development (Bergman, 1990). Inconsistent results have demonstrated that growth performance declines and nutrient utilization is low when growing pigs are fed nutritionally balanced diets that are supplemented with fibrous co-products and additional fat (Bakker et al., 1996; Gutierrez et al., 2013).

Supplementation with fibrous ingredients may not only lead to malabsorption of dietary fat and, but could also promote bioconversion of cholesterol to non-absorbable bile acids (**BA**) and neutral sterols (**NS**) (Nie et al., 2015). Excretion of fat or cholesterol-derived metabolites (BA and NS) occurs at the expense of metabolizable energy and may compromise growth performance. Gastrointestinal microbiota play a central role during biotransformation of primary to secondary

BA and alter BA composition (Ferrebee and Dawson, 2015; Nie et al., 2015; Wahlström et al., 2016). There is also accumulating evidence that BA facilitates a cross-talk between intestinal microbiota and energy or lipid metabolism (Jha and Berrocoso, 2016; Ferrebee and Dawson, 2015; Nie et al., 2015). Interestingly, a growing body of research has also pinpointed that BA, in turn, alter the community structure of gastrointestinal microbiota (Nie et al., 2015; Wahlström et al., 2016). It is intriguing to note that growth performance is depressed even when pigs are fed nutritionally-balanced diets that are supplemented with fibrous ingredients and added fat, (Bakker, 1996; Gutierrez et al., 2013).

To prevent negative effects that are associated with utilization of fibrous co-products, it may become necessary to understand effects of DF sources and/or types on the cross-talk among gastro-intestinal and lipid metabolism-related blood metabolites, fat digestibility, microbial communities and subsequently their effects on growth performance in pigs fed nutritionally balanced high-DF diets. Therefore, the first objective of this chapter was to investigate the effects of adding soluble and insoluble dietary fiber on growth performance, fat digestibility, blood lipids, and intestinal fermentation products, BA and NS in growing pigs. The second objective was to investigate the effects of supplementation with soluble and insoluble fiber on histomorphological characteristics of small intestines, structural and functional characteristics of mucosa and microbiota and their association with gastrointestinal and blood lipid-related metabolites in growing pigs. Flaxseed meal and oat hulls were selected based on previous studies and preliminary analysis in our lab that showed that flaxseed meal and oat hulls are rich in soluble and insoluble fiber, respectively and can be incorporated in pig diets (Eastwood et al., 2009; Jiménez-Moreno et al., 2009).

4.3. MATERIALS AND METHODS

4.3.1. Diets, Pigs, Experimental Design and Sample Collection

The experimental procedures and use of animals were approved by the Animal Care Committee of the University of Manitoba and pigs were cared for according to the guidelines of the Canadian Council on Animal Care (CCAC, 2009). Expelled flaxseed (*Linum usitatissimum L.*) meal was sourced from Shape Foods Inc. (Brandon, MB, Canada). Oat (*Avena sativa L.*) hulls, a by-product obtained during mechanical extraction of groats (edible huskless grains) from oat kernels were supplied by Grain Millers Inc. (Yorkton, SK, Canada). Both flaxseed meal and oat hulls were ground using a hammer mill fitted with a 1 mm sieve prior to diet mixing (**Table 4.1**). The experimental diets were; a basal corn-soybean meal-containing diet (control diet), a 12% flaxseed meal-containing diet (**FM**), and a diet containing 10% oat hulls (**OH**) (**Table 4.2**). All diets were formulated to be iso-energetic and to contain similar standardized ileal digestible (**SID**) AA contents, and met other nutrient requirements for 25 to 50 kg pigs (NRC, 2012). Titanium dioxide (0.3%) was added in all diets as an indigestible marker.

Forty-eight Genesis crossbred barrows [(Yorkshire-Landrace dam) × Duroc sire] with an initial BW of 25.1 ± 0.32 (mean ± SEM) kg were obtained from University of Manitoba's Glenlea Swine Research Unit (Winnipeg, MB, Canada). Pigs were housed in pairs and assigned to experimental diets in a completely randomized design to give eight replicates per treatment. The experiment lasted 28 d. Room temperature was maintained at 21.2 ± 2.3 (mean ± SD) ℃ throughout the study. Feed and water were provided on an *ad libitum* basis. Feed intake and BW were measured at the beginning and end of study (d 28) to calculate ADG, ADFI and G: F. On d 26 and 27, fecal grab samples were collected through rectal palpation and frozen at -20℃ until analyzed. After overnight fasting, on d 28, blood was sampled through jugular venipuncture into 10 mL serum tubes (BD Franklin Lakes, NJ). Blood serum was extracted by centrifugation at 2,000

Table 4.1. Composition and physical properties of flaxseed meal and oat hulls (as fed)

Item	Ingredients	
	Flaxseed meal	Oat hulls
DM, %	93.6	92.3
Gross energy, kcal/kg	4,661	4,015
CP (N × 6.25), %	33.92	2.46
Starch, %	1.0	7.3
Fat content, %	7.80	1.70
Amino acids, %		
Arg	3.00	0.22
His	0.79	0.35
Ile	0.74	0.08
Leu	1.78	0.21
Lys	1.22	0.15
Met	0.60	0.05
Thr	1.22	0.10
Trp	0.58	0.06
Phe	1.38	0.12
Val	1.33	0.16
NDF, %	41.6	73.5
ADF, %	14.7	39.8
Ca, %	0.41	0.14
P, %	0.77	0.08
NSP constituent sugars, %[1]		
Total NSP	22.4 (7.8)	54.1 (1.7)
Arabinose	3.9 (0.7)	3.9 (0)
Xylose	5.8 (2.3)	24.1 (0.1)
Mannose	0.2 (0.2)	0.2 (0.2)
Galactose	3.5 (1.7)	1.1 (0.1)
Glucose	5.1 (0.9)	24.1 (1.0)
Uronic acids	2.4 (1.4)	0.5 (0.3)
Physical properties		
Bulk density, g/mL	0.74	0.13
Swelling capacity, mL/g	4.05	5.52
Water holding capacity, mL/g	8.31	4.22
Particle size distribution, %[2]		
>1700 μm	3.48	1.96
> 850 μm	16.1	15.6
< 600 μm	80.4	76.6

[1]Values in parentheses are for the soluble portion of non-starch polysaccharides (**NSP**) in the ingredient.

[2]100 g of sample size for each ingredient.

×*g* for 10 min at 4 °C and stored at -20 °C until analyzed.Following blood sampling, pigs were offered their respective treatment diets. On Days 29 and 30 of the study, one pig was randomly selected from each pen and sedated by intramuscular injection of Ketamine: Xylazine (20:0 mg/kg of BW; Bimeda-MTC animal Health Inc., Cambridge, ON, Canada) and subsequently euthanized by intravenous injection of sodium pentobarbital (110 mg/kg of BW; Bimeda-MTC animal Health Inc., Cambridge, ON, Canada). After euthanizing, pigs were immediately eviscerated from sternum to pubis for collection of intestinal digesta contents, feces and tissue sub-samples. The whole digestive tract was carefully removed and segmented by clamping and tying with wire string to partition the: jejunum, duodenum, ileum, cecum and colon. Ileal digesta was collected 20 cm from the ileal-cecal junction. Luminal contents from the descending colon were collected at 20 and 5 cm cranial to the rectum. Digesta from each segment were collected separately, divided into three sub-samples, transferred into sterile tubes and immediately snap-frozen in liquid nitrogen and stored at -80°C until further analyzes. One sub-sample was freeze-dried and used for bile acids (BA) and neutral sterols (NS) assays. The other two sub-samples were analyzed for volatile fatty acids (VFA) concentrations, and DNA extraction and subsequent microbial analyzes. The tissue samples were aseptically collected from the jejunum, duodenum, ileum and cecum, flushed with sterile ice cold phosphate buffered saline solution to remove luminal contents. Tissue samples from the ileum were divided into two sub-samples. The first sub-sampled tissue from the ileum and cecal tissue were immediately transferred into sterile tubes, snap-frozen in liquid nitrogen and transferred to -80°C until used for bacterial genomic DNA extraction and microbial community composition analyzes. Tissues from the jejunum, and duodenum, as well as the second tissue sub-samples from the ileum were placed in individual plastic vials and stored in formalin until assessed for histomorphometric characteristics. Blood and digesta sampling times were adopted based on

Table 4.2. Ingredient composition and calculated provisions of the control, flaxseed meal and oat hulls diets[1]

Ingredients	Diet[1]		
	Control	FM	OH
Corn	64.53	57.40	52.99
Oat Hulls	-	-	10.00
Flaxseed meal	-	12.00	-
Soybean meal, 44% CP	31.00	25.49	31.50
Vegetable oil	1.354	2.048	2.390
Limestone	0.683	0.667	0.640
Monocalcium phosphate	0.752	0.637	0.750
Salt	0.35	0.35	0.35
Vitamin-mineral premix[2]	1.00	1.00	1.00
L-Lysine HCl	-	0.086	0.010
DL-Methionine	0.031	0.010	0.060
Threonine	-	0.012	0.010
Titanium dioxide	0.30	0.30	0.30
Total	100	100	100
Calculated dietary provisions[3]			
ME kcal/kg	3,330	3,334	3,326
CP, %	18.10	18.70	18.01
SID Lys, %	0.99	0.99	0.99
SID Met, %	0.30	0.31	0.30
SID Met + Cys, %	0.58	0.61	0.55
SID Thr, %	0.62	0.60	0.60
SID Trp, %	0.21	0.17	0.18

[1]FM = flaxseed meal-containing diet; OH = oat hulls-containing diet.

[2]Provided the following nutrients per kg of air-dry diet: 8,250 IU retinol (vitamin A); 200 IU cholecalciferol (vitamin D_3); 40 UI α-tocopherol (vitamin E); 4 mg vitamin K; 1.5 mg vitamin B_1; 7 mg vitamin B_2; 2.5 mg vitamin B_6; 25 µg vitamin B_{12}; 14 mg calcium pantothenate; 2 mg folic acid; 21 mg niacin (vitamin B_3); and 200 µg biotin (vitamin B_7). Minerals: 15 mg Cu (as copper sulphate); 0.4 mg iodine (as potassium iodine); 120 mg iron (as ferrous sulphate); 20 mg Mn (as

manganese oxide); 0.3 mg Se (as sodium selenite); 110 mg Zn (as zinc oxide). [3]The nutrient compositions were calculated based on ingredient composition values from NRC (2012) and our laboratory.

the previous reports that VFA and blood lipids are at their highest concentrations during the first 5 h of the post-prandial period (Serena et al., 2009).

4.3.2. Laboratory Analyzes

Diets, freeze-dried ileal, cecal, and colonic contents and feces were ground through a 1-mm screen in a laboratory mill (Thomas Wiley Mill Model 4, Thomas Scientific, Swedesboro, NJ) (**Table 4.3**). Dry matter (method 934.01), GE (method 942.05) and CP (method 968.06) content of flaxseed meal, oat hulls and experimental diets were determined as described by AOAC (1990). The GE value of flaxseed meal, oat hulls and experimental diets were determined using a Parr adiabatic oxygen bomb calorimeter (Parr Instrument, Moline, IL), with benzoic acid as a calibration standard. Flaxseed meal, oat hulls and diets were analyzed for Nitrogen (**N**) content using a Leco NS 2000 Nitrogen Analyzer (Leco Corporation, St. Joseph, MI). Crude protein content was estimated by multiplying the N content by 6.25. The flaxseed meal, oat hulls, experimental diets and feces were analyzed for fat content using an Ankom XT10 extractor (serial number XT10110202; Ankom Technology, Macedon, NY) using hexane according to the manufacturer's instructions. The flaxseed meal, oat hulls and diets were analyzed for neutral detergent fiber (**NDF**) and acid detergent fiber (**ADF**) using an ANKOM Fiber Analyzer (Ankom Technology, NY) as described by Van Soest *et al.* (1991) and Van Soest (1973), respectively. The mono sugars composition of the non-starch polysaccharides (**NSP**) in flaxseed meal, oat hulls and diets was determined by the method of Englyst et al. (1994), as described by Ndou et al. (2015). The water holding capacity (**WHC**) values of flaxseed meal, oat hulls and diets were determined according to Whittemore et al. (2003), whereas swelling capacity (**SWC**) values were determined as described by Canibe and Bach-Knudsen (2002). The bulk density (**BD**) values of flaxseed meal,

Table 4.3. Analyzed composition of the control, flaxseed meal and oat hulls diets[1]

Item	Diet[1]		
	Control	FM	OH
Chemical composition			
CP (N × 6.25), %	19.14	19.33	19.24
Gross energy, kcal/kg	3,879	3,886	3,977
Fat content, %	4.58	5.32	5.94
NDF, %	9.37	18.0	18.6
ADF, %	3.62	4.83	7.20
Ca, %	0.61	0.62	0.60
P, %	0.58	0.63	0.58
NSP constituent sugars, %[2]			
Total NSP	9.48 (0.80)	13.3 (3.50)	15.0 (1.79)
Arabinose	1.80 (0.16)	2.20 (0.36)	1.84 (0.06)
Xylose	1.83 (0.06)	2.76 (0.79)	4.81 (0.38)
Mannose	0.26 (0.00)	0.23 (0.06)	0.22 (0.01)
Galactose	1.50 (0.15)	1.75 (0.50)	1.30 (0.06)
Glucose	2.78 (0.43)	4.26 (1.29)	5.60 (0.94)
Uronic acids	1.32 (0.12)	2.10 (0.50)	1.20 (0.35)
Physical properties			
Bulk density, g/mL	1.56	1.43	1.36
Swelling capacity, mL/g	2.67	4.51	2.85
Water holding capacity, mL/g	3.31	5.98	4.52
Particle size distribution, %[3]			
>1700 µm	19.8	20.6	18.2
> 850 µm	19.3	16.6	22.7
< 600 µm	61.0	62.8	59.1

[1]FM = flaxseed meal-containing diet; OH = oat hulls-containing diet.

[2]Values in parentheses represent the soluble portions of the non-starch polysaccharides (NSP).

[3]100 g of sample size for each diet.

oat hulls and diets were determined following the water displacement method described by Kyriazakis and Emmans (1995). Particle size distribution and geometric mean diameter of flaxseed meal, oat hulls and diets were measured using the methods described by American Society of Agricultural Engineers (ASAE, 1995). Samples for Ca and P analyzes were prepared according to AOAC (1990); method 990.08 and read on a Varian inductively coupled plasma mass spectrometer (Model ICP-OES, Varian Inc., Palo Alto, CA). Titanium dioxide concentration in the diets and feces were determined according to Lomer et al. (2000) using a Varian Inductively Coupled Plasma Mass Spectrometer (Model ICP-OES, Varian Inc., Palo Alto, CA).

Digesta samples were analyzed for VFA concentrations using gas chromatography–mass spectrometry (Varian Chromatograph System, model Star 3400; Varian Medical Systems, Palo Alto, CA, USA) that was equipped with a capillary column (30 m × 0.5 mm; Restek Corp., Belefonte, PA, USA). Prior to gas chromatography analysis, the digesta samples were subjected to an acid-base treatment followed by ether extraction and derivatization according to procedures described by Erwin et al. (1961). The concentrations of BA and NS in freeze-dried digesta contents and feces were extracted according to procedures described by Batta et al. (1999). In brief, between 10 and 15 mg of freeze-dried samples were weighed into culture tubes (12 × 75 mm). Norcholic acid and 5α-cholestane were used as internal standards for BA and NS, respectively. Samples were hydrolyzed with concentrated HCl at 60 °C for 4 h on dry bath and submitted to salinization for 30 min at 55°C. Prior to and after salinization, solvents were evaporated under N flux using a N-EVAP 112 evaporator (Berlin, MA) at 60 and 55°C, respectively. After salinization, 200 µl of hexane was added and the supernatant was recovered after centrifugation at 2,800×g for 20 min at 4°C. Then, aliquots were transferred into vials and analyzed by gas chromatography-mass

spectrometry. Total cholesterol and triglycerides in blood serum were enzymatically determined using a ci8200 analyzer (Abbot Diagnostics, Wiesbaden, Germany).

Frozen ileal and cecal digesta, and tissue samples were thawed at room temperature. Approximately 200 mg of digesta contents and ~ 200 mg of mucosa were separately obtained by scrapping the inner wall of each of the ileal and cecal tissue, and used for genomic DNA extraction using ZR Fecal and Tissue DNA extraction kits, respectively (ZYMO Research Corp., Orange, CA, USA). The kit included a bead-beating step for the mechanical lysis of the microbial cells. The DNA concentration was subsequently quantified using a NanoDrop 2000 spectrophotometer (Thermo Scientific, Waltham, MA, USA). Thereafter, DNA samples were adjusted to 20 ng/µl, and quality checked by PCR amplification of the 16S rRNA gene using universal primers 27F (5'-GAAGAGTTTGATCATGGCTCAG-3') and 342R (5'-CTGCTGCCTCCCGTAG-3') as described by Khafipour et al., 2009. The amplicons were verified by agarose gel electrophoresis.

Library construction and Illumina sequencing were performed as described by Derakhshani et al. 2016. In brief, the V4 region of 16S rRNA was targeted for PCR amplification using modified F515/R806 primers (Caporaso et al., 2012). The reverse PCR primer was indexed with 12-base Golay barcodes allowing for multiplexing of samples. PCR reaction for each sample was performed in duplicate and contained 1.0 µl of pre-normalized DNA, 1.0 µl of each forward and reverse primers (10 µM), 12 µl HPLC grade water (Fisher Scientific, Ottawa, ON, Canada) and 10 µl 5 Prime Hot MasterMix (5 Prime, Inc., Gaithersburg, MD, USA). Reactions consisted of an initial denaturing step at 94°C for 3 min followed by 35 amplification cycles at 94°C for 45 sec, 50°C for 60 sec, and 72°C for 90 sec; finalized by an extension step at 72°C for 10 min in an Eppendorf Mastercycler pro (Eppendorf, Hamburg, Germany). PCR products were then purified using ZR-96 DNA Clean-up Kit (ZYMO Research, Irvine, CA, USA) to remove primers, dNTPs

and reaction components. The V4 library was then generated by pooling 200 ng of each sample, quantified by Picogreen dsDNA (Invitrogen, Burlington, On, Canada). This was followed by multiple dilution steps using pre-chilled hybridization buffer (HT1) (Illumina, San Diego, CA, USA) to bring the pooled amplicons to a final concentration of 5 pM, measured by Qubit 2.0 Fluorometer (Life technologies, Burlington, ON, Canada). Finally, 15% of PhiX control library was spiked into the amplicon pool to improve the unbalanced and biased base composition, a known characteristic of low diversity 16S rRNA libraries. Customized sequencing primers for read1 (5´-TATGGTAATTGTGTGCCAGCMGCCGCGGTAA-3´), read2 (5´-AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT-3´) and index read (5´-ATTAGAWACCCBDGTAGTCCGGCTGACTGACT-3´) were synthesized and purified by polyacrylamide gel electrophoresis (Integrated DNA Technologies, Coralville, IA, USA) and added to the MiSeq Reagent Kit V2 (300-cycle) (Illumina, San Diego, CA, USA). The 150 paired-end sequencing reaction was performed on a MiSeq platform (Illumina, San Diego, CA, USA) at the Gut Microbiome and Large Animal Biosecurity Laboratories, Department of Animal Science, University of Manitoba, Canada. The sequencing data were subjected to the Sequence Read Achive (SRA) of NCBA (http://www.ncbi.nlm.nih.gov/sra) and can be assessed using access number SRR5226958. Hierarchical clustering analysis was performed using R (3.3.1 version) (RDCT, 2014) to show a visual interpretation heat map of the similarity of bacterial taxa based on treatment. Normalized relative abundance of bacterial taxa (row normalize length transformation, PAST, version 2.17) was used to for generating the clustering heat map.

4.3.3. Calculations, Bioinformatic and Statistical analyzes

Bioinformatic analyzes were performed as described by Derakhshani et al. (2016). In brief, the PANDAseq assembler was used to merge overlapping paired-end Illumina fastq files (Masella

et al., 2012). All the sequences with low quality base calling scores as well as those containing uncalled bases (N) in the overlapping region were discarded. The output fastq file was then analyzed by downstream computational pipelines of the open source software package QIIME (Caporaso et al., 2010b). Assembled reads were demultiplexed according to the barcode sequences, chimeric reads were filtered using UCHIME (Edgar et al., 2011) and sequences were assigned to Operational Taxonomic Units (**OTU**) using the QIIME implementation of UCLUST (Edgar et al., 2010) at 97% pairwise identity threshold. Taxonomies were assigned to the representative sequence of each OTU using RDP classifier (Wang et al., 2007) and aligned with the Greengenes Core reference database (DeSantis et al., 2006) using PyNAST algorithms (Caporaso et al., 2010a). Phylogenetic tree was built with FastTree 2.1.3. Further comparisons between microbial communities were performed according to Price et al. (2010).

Within community diversity (α-diversity) was computed using QIIME. Alpha rarefaction curve was generated using Chao 1 estimator of species richness (Chao, 1984) with ten sampling repetitions at each sampling depth. An even depth of approximately 15,700 sequences per sample was used for calculation of richness and diversity indices. For comparison of microbial community composition between samples, the β-diversity was measured by calculating the weighted and unweighted UniFrac and Bray-Curtis distances using QIIME default scripts (Lozupone and Knight, 2005). β-diversity among treatments were visualized using non-metric multidimensional scaling (**nMDS**) ordination plots that were generated using R software (3.1.0) by employing Bray-Curtis similarity matrices with a conventional cut-off of <0.2 for the stress value. Each data point on the graph represents one sample. The spatial distance between points in the plot was interpreted as the relative difference in the bacterial community composition; thus, points that were closer were more similar than points that were more distant. To assess the statistical differences in β-

diversity of bacterial communities among treatment groups, permutational multivariate analysis of variance (**PERMANOVA**) was performed to calculate P-values (Anderson, 2005).

Finally, the open source software PICRUSt (phylogenetic investigation of communities by reconstruction of unobserved states) (Langille et al., 2013) was used to predict functional genes of the classified members of the ileal and cecal digesta and mucosa-associated microbiota (resulting from reference based OTU picking against Greengenes database). Predicted genes were then hierarchically clustered and categorized using the Kyoto Encyclopedia of Genes and Genomes (**KEGG**) (Kanehisa and Goto, 2000) orthologs and pathways (levels 1–3).

The apparent total tract digestibilities (**ATTD**) of fat were calculated relative to TiO_2 concentration, as described by Ndou et al. (2015). All other statistical analyzes were performed using SAS version 9.4 (SAS, Institute, Inc., Cary, NC, 2009). The UNIVARIATE procedure was used to test for outliers and homogeneity of variances among treatments. Statistical analysis was performed using a generalized linear model procedure of SAS. Non-normally distributed data were analyzed using GLIMMIX procedure using the negative binomial or Poison distributions. The goodness of fit for each distribution was determined using Pearson chi-square/DF ratio with values closer to 1 considered better. The models accounted for the effects of diet on growth performance, intake of dietary components, histomorphometric characteristics, ATTD of fat, blood lipids, VFA, BA, NS and α-diversity indices of bacterial communities and communities composition. Pen was considered as the experimental unit. Comparisons of means were performed using the Tukey-Kramer honest significant difference test. Significant differences among means were declared at $P < 0.05$, and trends declared for P values between 0.05 and 0.10. All the phyla were divided into two groups of abundant, above 1% of the community, and low-abundance, below 1% of the community.

Statistical analyzes on the proportion of functional genes and pathways was performed using Linear discriminant analysis (**LDA**) effect size (**LEfSe**) (Segata et al., 2011), a software principally developed to discover metagenomics biomarkers. Analyzes included the non-parametric factorial Kruskal-Wallis sum rank test (Kruskal and Wallis, 1952), followed by LDA to estimate the effect size of each differentially abundant feature. The threshold on the logarithmic LDA score for discriminative features was set at 2.0, so that features with at least 100-fold shift were considered significant.

Associations between bacterial taxa with an abundance above ≥ 0.05% of the community and VFA (acetate, propionate, butyrate), BA (cholic acid (**CA**); chenodeoxycholic acid (**CDCA**); deoxycholic acid (**DCA**); isodeoxycholic acid (**IDCA**); lithocholic acid (**LCA**) and ursodeoxycholic acid (**UDCA**), blood lipids (total cholesterol and triglycerides) and fat digestibility were assessed using non-parametric Spearman's rank correlation (JMP, Version 10; SAS Institute Inc., Cary, NC, USA). For each correlation, correlation coefficient (Spearman's Rho) and *P* value were obtained. The correlation coefficient values ranged from -1 to +1 with the upper limit values indicating the strength of the relationship, while positive and negative symbols indicating the direction of association.

4.4. RESULTS

4.4.1. Performance, fat digestibility, serum lipids and intestinal histomorphology

All pigs were healthy and consumed their daily feed allowance throughout the experiment. Average daily feed intake (*P* = 0.005), ADG (*P* < 0.001) and final BW (*P* < 0.001) were higher for pigs fed the control and OH diets than for pigs fed the FM diets (**Table 4.4**). The G: F ratio in FM diet-fed pigs was lower (*P* = 0.013) than that in control diet-fed pigs. The ATTD of fat in FM

diet-fed pigs was lowest, followed by OH diets and greatest in pigs fed the control diet ($P = 0.020$). Total serum cholesterol content was lower ($P < 0.001$) in pigs fed the FM and OH diets, respectively, than in pigs fed the control diet.

Addition of flaxseed meal and oat hulls in pig diets decreased ($P < 0.001$) the villi height (**VH**) in the duodenum and ileum compared to CON diet (**Table 4.5**). The jejunal VH were higher ($P < 0.001$) in FM diet-fed pigs compared to CON and OH diets-fed pigs. Although there was a tendency in which crypt in duodenum of pigs fed FM diets was deeper ($P = 0.057$) compared to those fed OH diets, no significant differences were observed in jejunal and ileal crypt depth (**CD**) among treatments. Dietary inclusion of flaxseed meal decreased ($P < 0.037$) the ileal villi height: crypt depth (**VCR**) and tended to decrease ($P = 0.073$) the duodenal VCR, but increased ($P < 0.001$) jejunal VCR in comparison to CON and OH diets.

4.4.2. Gastrointestinal Volatile Fatty Acids, Bile Acid and Neutral Sterols

Acetate concentrations were greater ($P = 0.001$) in the ileum of pigs fed the OH diet, followed by the FM diet-fed pigs and lowest in those fed the control diet (**Fig 4.1**). Propionate concentrations were greater ($P < 0.001$) in the ileum of pigs that consumed the FM diet than those fed the control and OH diets. Dietary inclusion of flaxseed meal and oat hulls resulted in higher concentration of acetate in the cecum ($P < 0.001$) and colon ($P = 0.002$) compared to the control diet. Butyrate concentrations in the cecum of pigs fed the FM and control diets were greater ($P = 0.007$) than those fed the OH diets. Pigs fed the FM and OH diets tended to have higher concentrations of valerate in the cecum ($P = 0.056$), and valerate ($P = 0.096$) and total VFA ($P = 0.094$) in the colon, than those that consumed the control diet. Total VFA in the ileal ($P = 0.022$) and cecal ($P = 0.004$) digesta contents were greater in pigs fed the FM and OH diets than in those fed the control diet.

Table 4.4. Least square means of growth performance, apparent total tract digestibility and serum lipids of growing pigs fed the control, flaxseed meal and oat hulls diets[1] (n = 8)

Item	Diet[1] Control	FM	OH	SEM	*P*
Growth performance					
Initial BW, kg	25.1	25.0	25.0	0.32	0.731
Final BW, kg	48.1[a]	42.3[b]	46.0[a]	0.74	<0.001
ADFI, kg/d	1.85[a]	1.65[b]	1.84[a]	0.04	0.005
ADG, g/d	853[a]	639[b]	778[a]	32.1	<0.001
G: F, g/kg	462[a]	390[b]	422[ab]	21.0	0.013
ATTD[2] of fat, %	92.4[a]	70.1[c]	79.2[b]	4.04	0.020
Serum lipids, mmol/L					
Cholesterol	2.36[a]	2.25[b]	1.99[c]	0.04	<0.001
Total triglycerides	0.614	0.601	0.851	0.14	0.232

[1]FM = flaxseed meal-containing diet; OH = oat hulls-containing diet.

[2]ATTD = apparent total tract digestibility.

[a,b,c]Mean values within a row with unlike superscripts differ ($P < 0.05$).

The concentration of ileal CA + CDCA in pigs fed the OH diet was higher ($P = 0.019$) than in pigs fed the control diet (Table 4.6). The total BA, DCA, IDCA, and LCA in the ileum of pigs fed the OH and FM diets were higher ($P < 0.001$) than in pigs fed the control diet. In the cecum, the total BA concentration in pigs fed the FM and OH diets were higher ($P < 0.001$) than in those fed the control diet. The concentration of CA + CDCA in the cecal contents of pigs fed the control diet was lower ($P < 0.001$) than in pigs fed the FM and OH diets. The concentration of total BA excreted in feces of pigs fed the FM and OH diets were higher ($P = 0.003$) than in pigs fed the control diet. Ursodeoxycholic acid was excreted in the largest concentration ($P = 0.001$) in pigs fed FM diet, followed by those fed the OH diet and then those fed the control diet. Pigs fed the FM diet excreted more ($P = 0.002$) fecal LCA compared to those that consumed the OH and control diets.

The coprostanol concentration in the ileum was higher ($P < 0.042$) in pigs fed the FM and control diets than in those fed the OH diet (**Fig 4.2**). Cholestanol concentration ($P = 0.059$) than in the control diet, whereas that of pigs fed the FM diet was intermediate. The concentration of cholesterol and coprostanol in cecal contents of pigs fed the FM and OH diets were higher ($P < 0.001$) than in the control diet-fed pigs. The concentration of sitosterol in the ileal contents were lower ($P < 0.022$) in pigs fed the OH diet compared to those fed the control diet. The concentrations of cholestanol in cecal contents were highest ($P < 0.01$) in pigs fed the FM diet, followed by that of pigs fed the OH diet, and then the control diet. The concentration of sitosterol and stigmasterol in cecal digesta of pigs fed the FM diet were higher ($P < 0.001$) than that of pigs fed the control and OH diets. The concentrations of fecal cholesterol in pigs consuming the FM and OH diets were higher ($P < 0.001$) than in pigs fed the control diet. The concentration of fecal coprostanol in pigs fed the high DF-enriched diets was higher ($P = 0.01$) than in pigs fed the control diet.

Table 4.5. Histomorphometric characteristics of intestinal tissue in growing pigs fed experimental diets[1]

[3]Item	[2]Diet			[4]SEM	*P*-value[5]
	CON	FM	OH		
Duodenum					
VH, µm	507.7[a]	471.6[b]	464.4[b]	10.29	<0.001[G]
CD, µm	344.9[AB]	350.8[A]	324.6[B]	8.27	0.057[G]
VCR	1.47[A]	1.35[B]	1.47[A]	0.038	0.073[G]
Jejunum					
VH, µm	450.1[b]	523.1[a]	458.5[b]	6.77	<0.001[P]
CD, µm	307.4	319.1	301.9	3.50	0.789[G]
VCR	1.46[b]	1.64[a]	1.52[b]	0.027	<0.001[NB]
Ileum					
VH, µm	471.4[a]	420.8[b]	404.3[c]	4.78	<0.001[P]
CD, µm	286.2	295.5	278.1	12.64	0.368[NB]
VCR	1.69[a]	1.43[b]	1.46[ab]	0.106	0.037[G]

[1]Least square mean values for histomorphometric characteristics.

[abc]Within a row, means with unlike superscripts differ (*P* < 0.05)

[ABC]Within a row, means with unlike superscripts differ (*P* ≤ 0.10).

[2]CON, Control diet; FM, Flaxseed meal-containing diet; OH, Oat hulls-containing diet.

[3]VH, villi height; CD, crypt depth; VCR, villi height to crypt depth ratio.

[4]Standard error of treatment mean (n = 8 replicates per treatment).

[5]Methods of analysis denoted by [G] (Gaussian), [NB] (Negative Binomial) and [P] (Poisson).
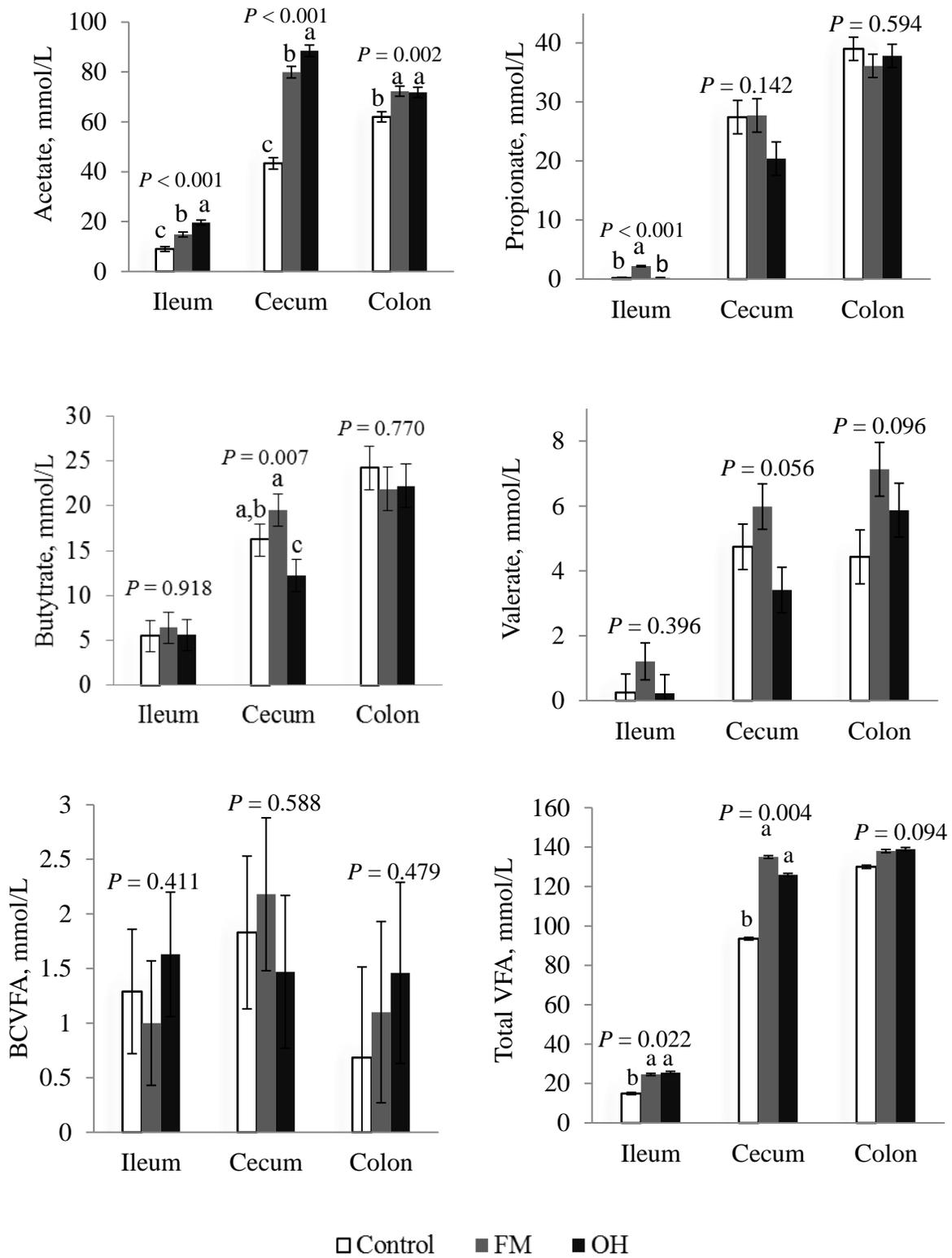
Figure 4. 1. Concentration of volatile fatty acids (VFA) in the ileum, cecum and colon of growing pigs fed experimental diets. Values are least square means; n = 8. [abc]Mean values with unlike letters differ ($P < 0.05$). SEM are represented by vertical bars. BCVFA = branched chain VFA (Sum of isovaleric and isobutyric acid).

Table 4.6. Bile acids (BA) concentration in digesta and feces of growing pigs fed control,

flaxseed meal and oat hulls diets[1] (n = 8)

Item	Diet[1]			SEM	*P*
	Control	FM	OH		
Ileum, mg/g					
Chenodeoxycholic + cholic acid	51.04[b]	61.93[ab]	66.13[a]	3.57	0.019
Deoxycholic acid	8.26[c]	11.81[b]	15.37[a]	0.97	<0.001
Isodeoxycholic acid	0.99[b]	1.49[a]	1.54[a]	0.09	<0.001
Lithocholic acid	0.38[b]	1.19[a]	1.51[a]	0.17	<0.001
Ursodeoxycholic	7.49[b]	8.60[b]	12.93[a]	0.67	<0.001
Secondary BA[2]	17.0[c]	23.1[b]	31.4[a]	1.40	<0.001
Total BA[3]	68.09[b]	85.02[a]	97.48[a]	4.09	<0.001
Cecum, mg/g					
Chenodeoxycholic + cholic acid	0.32[c]	1.04[b]	2.27[a]	0.10	<0.001
Deoxycholic acid	1.43[c]	6.08[a]	5.07[b]	0.28	<0.001
Isodeoxycholic acid	1.37	0.60	0.89	0.31	0.244
Lithocholic acid	2.19[c]	4.34[a]	3.44[b]	0.36	0.002
Ursodeoxycholic	9.00[c]	18.54[a]	13.46[b]	0.99	<0.001
Secondary BA[2]	13.9[c]	29.6[a]	22.4[b]	1.22	<0.001
Total BA[3]	14.32[c]	30.60[a]	25.12[b]	1.27	<0.001
Feces, mg/g					
Chenodeoxycholic + cholic acid	0.26	0.42	0.71	0.01	0.420
Deoxycholic acid	0.81	0.56	2.07	0.51	0.108
Isodeoxycholic acid	0.29	0.37	0.27	0.07	0.605
Lithocholic acid	1.89[b]	3.97[a]	2.26[b]	0.30	0.002
Ursodeoxycholic acid	4.30[c]	13.38[a]	9.99[b]	1.37	0.001
Secondary BA[2]	7.29[b]	18.31[a]	14.59[a]	1.89	0.002
Total BA[3]	7.55[b]	18.72[a]	15.31[a]	2.04	0.003

[1]FM = Flaxseed meal-containing diet; OH = Oat hulls-containing diet.

[2]Calculated as: deoxycholic + isodeoxycholic + lithocholic + ursodeoxycholic acid.

[3]Calculated as: chenodeoxycholic + cholic + deoxycholic + isodeoxycholic + lithocholic +

ursodeoxycholic acid.

[a,b,c]Mean values within a row with unlike superscripts differ ($P < 0.05$).

4.4.3. Gastrointestinal microbiota composition

As illustrated in **Appendix 1 Supplementary Table 4.1 and Fig 4.1**, no significant diet-induced effects were observed on Chao1 richness as well as Shannon and Simpson diversity indices in ileal digesta microbiota. Although there were no significant differences observed on Chao1 richness ($P = 0.828$) and Simpson diversity ($P = 0.365$) indices in ileal mucosa-associated microbiota, there was a tendency ($P = 0.064$) in which the greatest Shannon diversity value was observed in FM diet-fed pigs, followed by OH and CON-diet fed pigs (**Supplementary Table 4.2 and Fig 4.2**). The Shannon diversity index indicated no treatment effects ($P > 0.10$) within cecal digesta microbiota but the Chao1 index revealed a tendency in which species richness in CON diet-fed pigs was lower ($P = 0.050$) compared to pigs consumed OH and FM diets (**Supplementary Table 4.1 and Fig 4.3**). According to Shannon diversity index calculated in cecal mucosa, higher ($P = 0.015$) bacterial diversity was observed in both FM and CON diets-fed pigs compared to OH (**Supplementary Table 4.2 and Figure 4.4**). The comparison of Chao1 index within cecal mucosa revealed that species richness was higher ($P = 0.039$) in FM diet-fed pigs compared to those fed the CON diet. There were no treatment effects on Simpson diversity index in cecal mucosa-associated microbiota ($P = 0.144$). The assessment of β-diversity differences in ileal and cecal
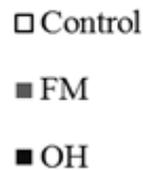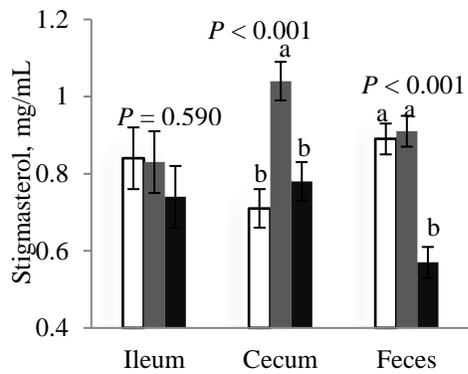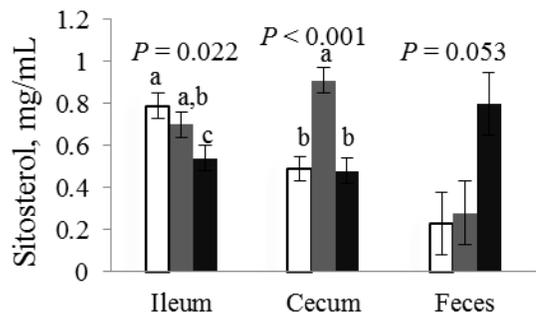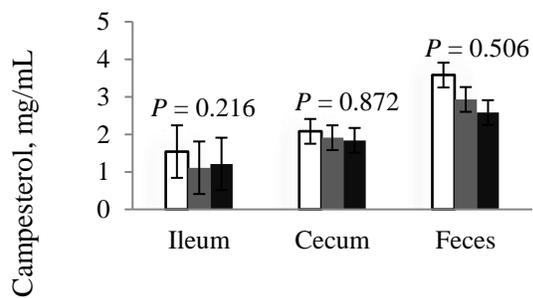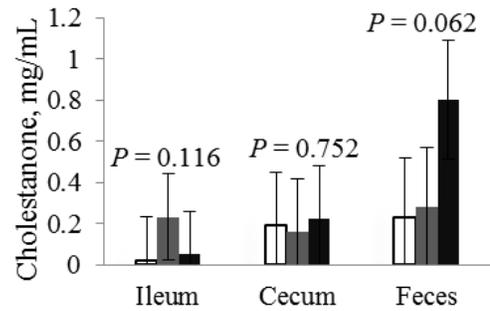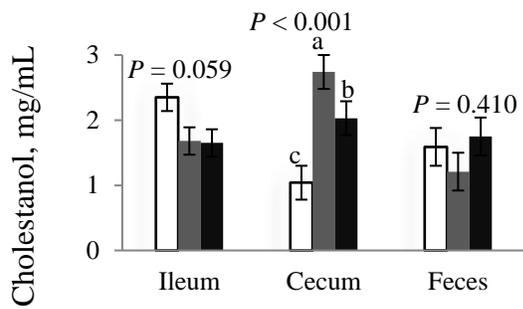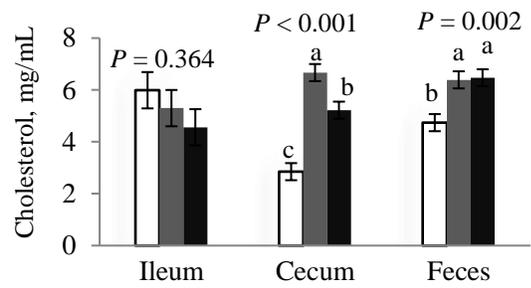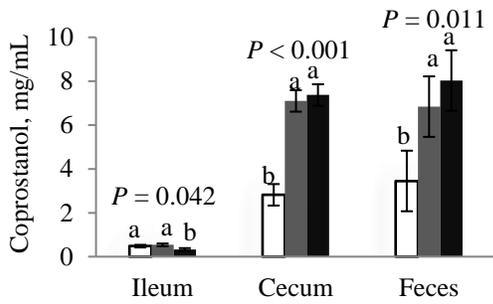
Figure 4. 2. Concentration of neutral sterols (NS) in the ileum, cecum and colon of growing pigs fed the experimental diets. TNS = Total neutral sterols. Values are least square means; n = 8. SEM are represented by vertical bars. [abc]Mean values with unlike letters differ (P < 0.05).

microbiota are illustrated in **Figure 4.3**. The PERMANOVA analyzes of unweighted UniFrac distances revealed distinct clustering patterns between the ileal digesta microbiota of CON and FM ($P = 0.010$), and FM and OH diets-fed pigs ($P = 0.005$). The PERMANOVA analysis of unweighted UniFrac distances also revealed distinctions between cecal mucosa-associated microbiota of the CON and FM-diets fed pigs ($P = 0.030$), and ileal mucosa-associated microbiota of pig fed FM and OH diets ($P = 0.041$).

Taxonomic classification of clustered OTUs in the ileal microbiota revealed the presence of 10 bacterial phyla. While the some of OTUs were identified at the genus (g.) or species levels, others were only classified at the phylum (p.), class (c.), order (o.), or family (f.) level. Firmicutes were the most abundant phylum, followed by Bacteroidetes and Proteobacteria, with pigs fed the FM and OH diets having ($P = 0.081$) the greatest proportion of p. Firmicutes in digesta compared to pigs fed CON diet. **(Fig 4.4a, Supplementary Table 4.3)**. Ileal mucosa-associated microbiota, p. Firmicutes were most ($P = 0.001$) dominant in CON diet-fed pigs compared to those fed the FM and OH diets (**Fig 4.4b, Supplementary Table 4.4**). However, p. Bacteroidetes did not differ ($P = 0.772$) among treatment groups in ileal digesta but was highest ($P < 0.001$) in ileal mucosa of FM diet-fed pigs and lowest in pigs fed the OH diet compared to those fed the CON diet. The compositions of bacterial taxa at the genus level in the ileal digesta and mucosa of pigs fed the experimental diets are presented in **Supplementary Tables 4.5** and **4.6**, respectively. As illustrated by differences in colour codes on the heat map, the cluster analysis of microbial community indicated that the abundance of taxa in ileal digesta (**Fig 4.5**) and mucosa (**Fig 4.6**) differed ($P < 0.05$) between diet treatments. The taxonomic classification of clustered OTUs in the cecal microbiota revealed the presence of 11 bacterial phyla (**Fig 4.4c and 4.4d**, **Supplementary Tables 4.7 and 4.8**). The majority of OTUs were identified at the genus (g.) or species levels, but some

a. Ileal digesta (D) and mucosa (T)
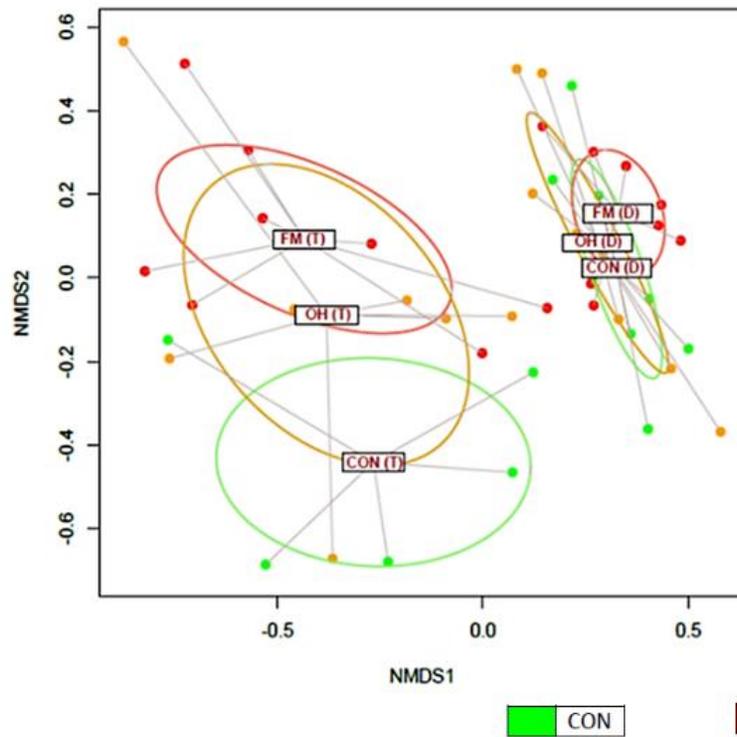
b. Caecal digesta (D) and mucosa (T)

CON   FM   OH
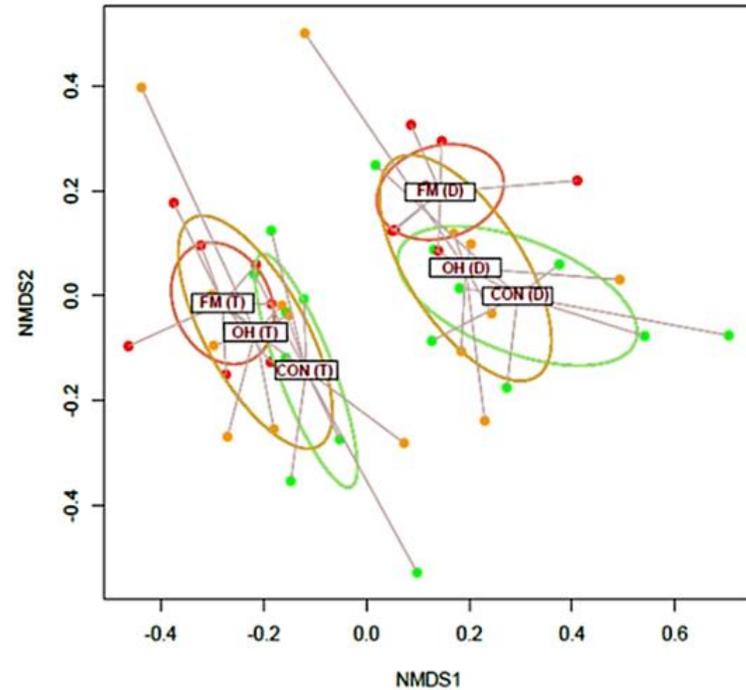
Figure 4.3. Non-metric multidimensional scaling (nMDS) ordination plot, illustrating beta biodiversity differences of bacterial community compositions in the cecal digesta (D) and mucosa-associated microbiota (T) of pigs fed control (CON), flaxseed meal (FM) and oat hulls (OH) diets. The PERMANOVA analyzes of unweighted UniFrac distances revealed distinct clustering patterns between the ileal digesta microbiota of CON and FM (P = 0.010), and FM and OH diets-fed pigs (P = 0.005). The PERMANOVA analysis of

unweighted UniFrac distances also revealed distinctions between cecal mucosa-associated microbiota of the CON and FM-diets fed pigs (P = 0.030), and ileal mucosa-associated microbiota of the FM and OH-diets fed pigs (P = 0.041).

were only classified at the phylum (p.), class (c.), order (o.), or family (f.) level. Among the most dominant phyla in cecal digesta, the proportion of Firmicutes was higher ($P = 0.003$) in pigs fed the FM diet compared to those fed OH and CON diets (**Fig 4.4c**, **Supplementary Table 4.7**). Although no significant differences were observed on the proportion of p. Bacteroidetes across all treatments, the proportion of p. Proteobacteria was higher ($P = 0.049$) in FM diet-fed pigs compared to that of pigs fed the OH and CON diets. As depicted in **Fig 4.4d** and **Supplementary Table 4.8**, p. Bacteroidetes were the most dominant phyla in cecal mucosa and their proportion was lowest ($P = 0.008$) in OH diet-fed pigs compared to the CON. No significant differences were observed in the proportions of p. Firmicutes among treatments. However, the proportion of Proteobacteria in cecal mucosa of OH diet-fed pigs were higher ($P < 0.001$) compared to CON. The compositions of bacterial genera in cecal digesta and mucosa-associated microbiota of pigs fed the experimental diets are presented in **Supplementary Table 4.9** and **4.10**, respectively. As shown by differences in colour codes on the heat map, the cluster analysis of microbial community indicated that the abundance of the taxa in cecal digesta (**Fig 4.7**) and mucosa (**Fig 4.8**) differed ($P < 0.05$) between diet treatments.

4.4.4. Predicted functional capacity of the intestinal microbiota

As illustrated in **Figure 4.9A**, various types of N-glycan biosynthesis mechanisms were overrepresented at more than 2.2 log-folds within ileal digesta microbiota of FM diet-fed pigs, whereas arginine and proline metabolism were enriched in ileal digesta microbiota of CON diet-fed pigs. **Figure 4.9B** depicts that functional pathways including primary BA, secondary BA and carotenoid biosynthesis, phosphatidylinositol signaling system and apoptosis were enriched in ileal mucosa-associated microbiota of FM diet-fed pigs. In addition, the Kyoto Encyclopedia of
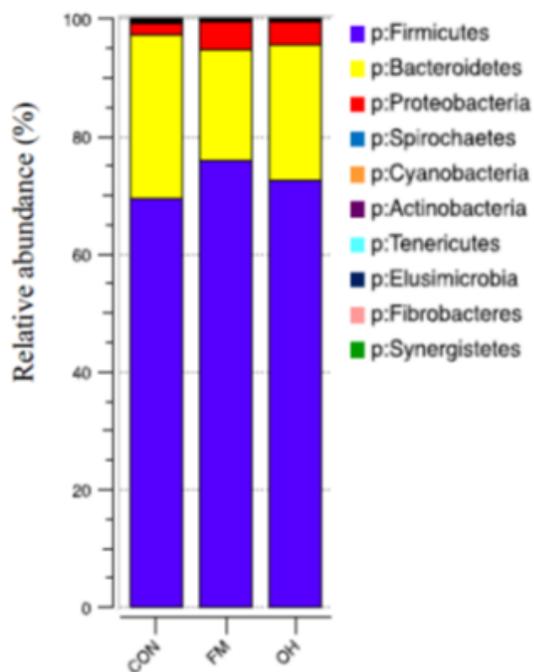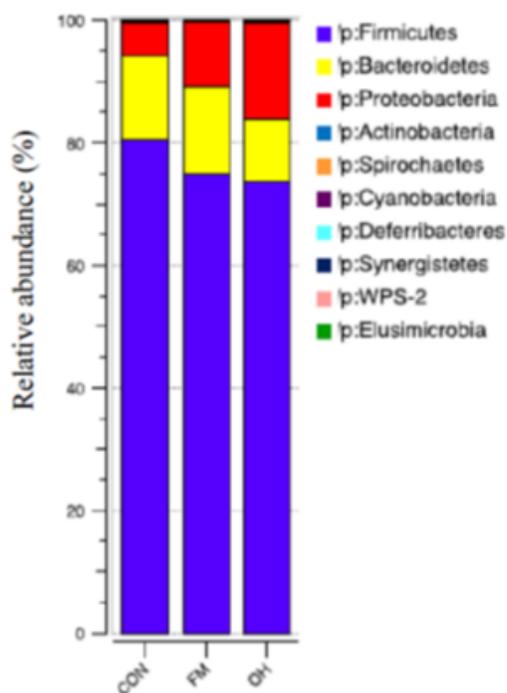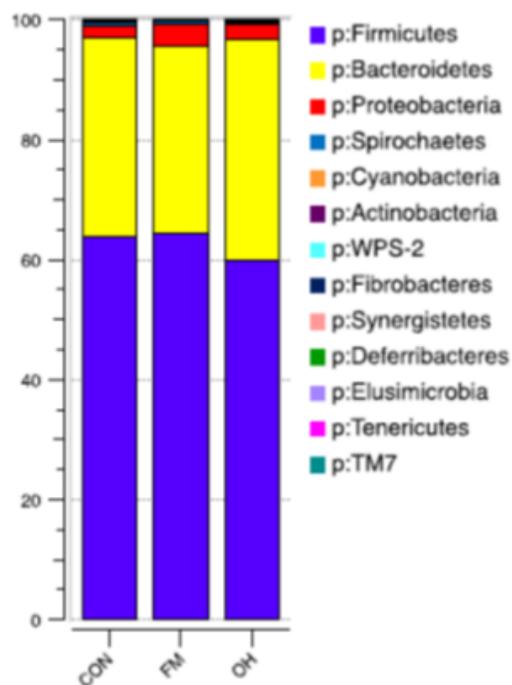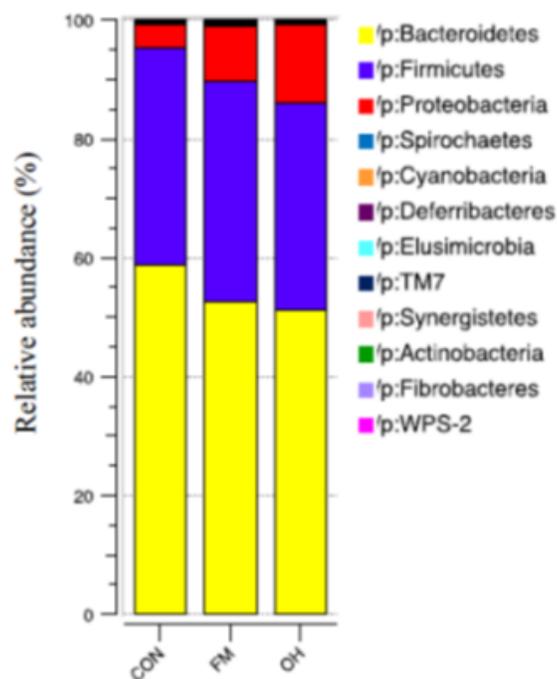
Figure 4.4. Relative abundances of bacterial phyla (p) in ileal digesta (a) and mucosa (b) cecal

digesta (c) and mucosa (D) of pigs. The data was also presented in Supplementry Table 1-4.
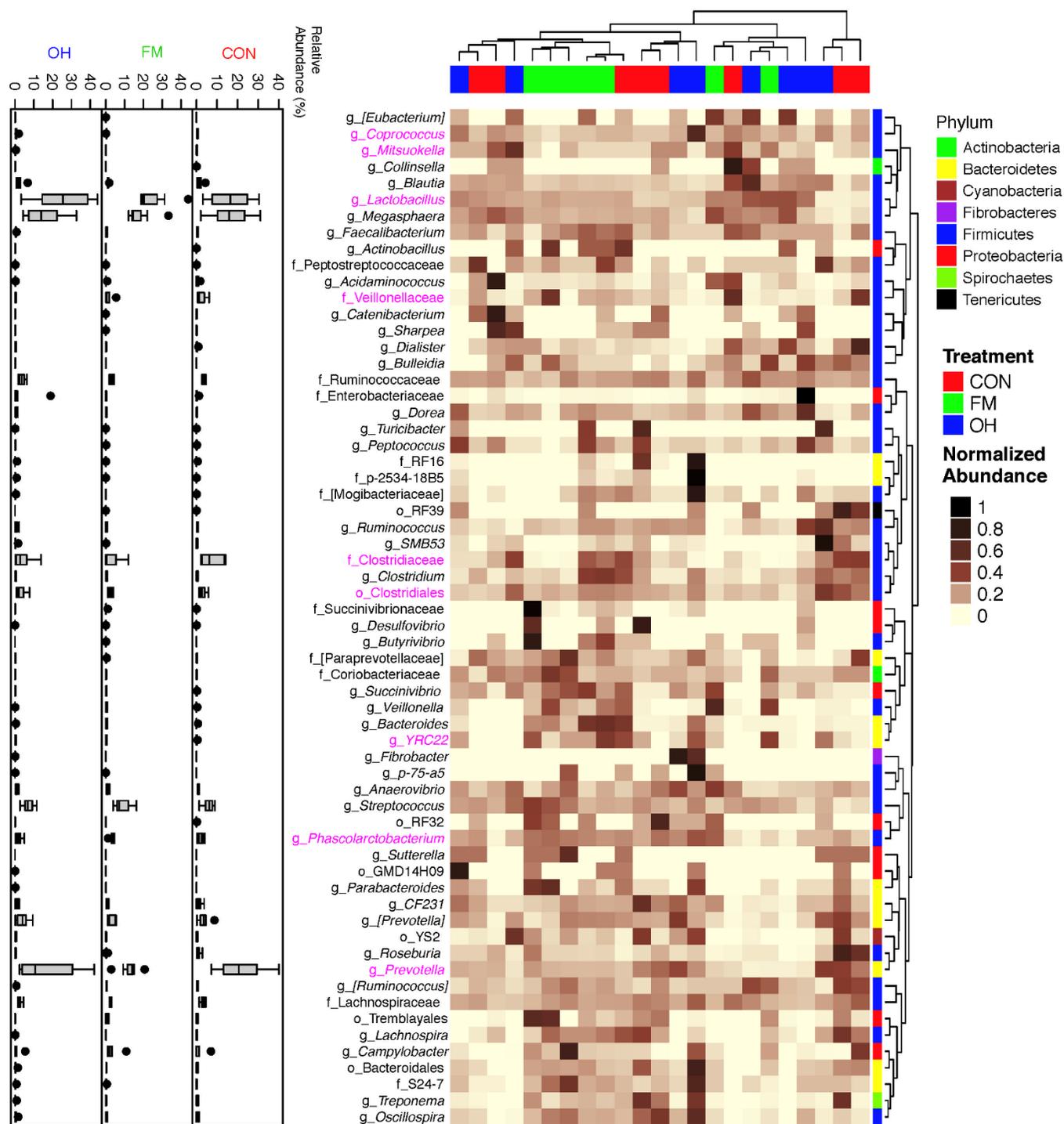
Figure 4.5. Cluster analysis of microbial communities in the ileal digesta of pigs fed control diet

(CON), flaxseed meal (FM) and oat hulls (OH) diets. The sample identifiers on the top branches

for diet treatments are coloured. Right branches are coloured to indicate the taxonomical assignment of the OTUs at the phylum level. Each row represents one bacterial taxa (relative abundance above 0.01%), and some taxa could only be classified to family (f), order (o), class (c), or phylum (p) level. The taxa names were labeled by the colour code (magenta) to indicate the abundance of the taxa significantly differed between dietary treatments (P < 0.05). The normalized relative abundance of bacterial taxa from illumina-sequenced 16S rRNA sequences in each sample is reflected by the colour of the scale (light yellow to black) on the heat map. The dendrogram on the top shows how the samples are clustered based on the Bray–Curtis dissimilarity measure, averaged by diet treatment. The dendrogram on the right shows clustering of bacterial taxa data based on the Spearman's rank correlation. The Box-Plots showed the relative abundances of bacterial taxa in ileal digesta microbiota among the three diet treatments.

Genes and Genomes (KEGG) functional pathways including transcription machinery and bacterial chemotaxis indicated more than 3.1 log-fold increase within the ileal mucosa of CON diet-supplemented pigs (**Fig 4.9B**). Functional pathways enriched within cecal digesta microbiota in FM diet-fed pigs were histidine metabolism, whereas cytoskeleton proteins were augmented in cecal digesta microbiota in CON diet-fed pigs (**Fig 4.10A**). As shown in **Figure 4.10B**, various functional pathways were enriched by more than 2.0 log-fold increase within ileal mucosa-associated microbiota across all treatments. Specifically, KEGG pathways including secretion, bacterial secretion and sulphur relay systems, protein kinases and biosynthesis and biodegradation of secondary metabolites, inorganic ion transport and metabolism of inositol phosphate, alpha-linoleic acid, and inorganic ion were enriched in OH diet-fed pigs. Furthermore, functional pathways including proximal tubule bicarbonate reclamation, benzoate and lysine degradation, as well as butanoate, arginine and proline, glyoxylate and dicarboxylate, ascorbate and aldarate, and phenylalanine metabolism were enriched in FM diet-fed pigs. Moreover, the most significantly enriched functional pathways in ileal mucosa of CON-fed pigs were starch and sucrose, cysteine and methionine, amino sugar and nucleotides sugar, galactose, nicotinate and nicotinamide, and tyrosine metabolism; peptidoglycan and glycosphingolipids biosynthesis, as well as glycolysis-gluconeogenesis, glycosyltransferases, antigen processing and presentation, proteasome, and carbohydrate digestion and absorption.

4.4.5. Correlations between specific intestinal microbiota, gut metabolites and blood lipids

Significant correlations were observed between selected taxa, with GI metabolites and lipid metabolism-related blood metabolites (**Supplementary Table 4.11-4.14**). In ileal digesta, positive correlations were observed between g. *Lactobacillus* and CA-CDCA (Rho = 0.427; P = 0.023), g. *Streptococcus* and fat digestibility (Rho = 0.417; P = 0.017). The presence of g. *Lactobacillus* was
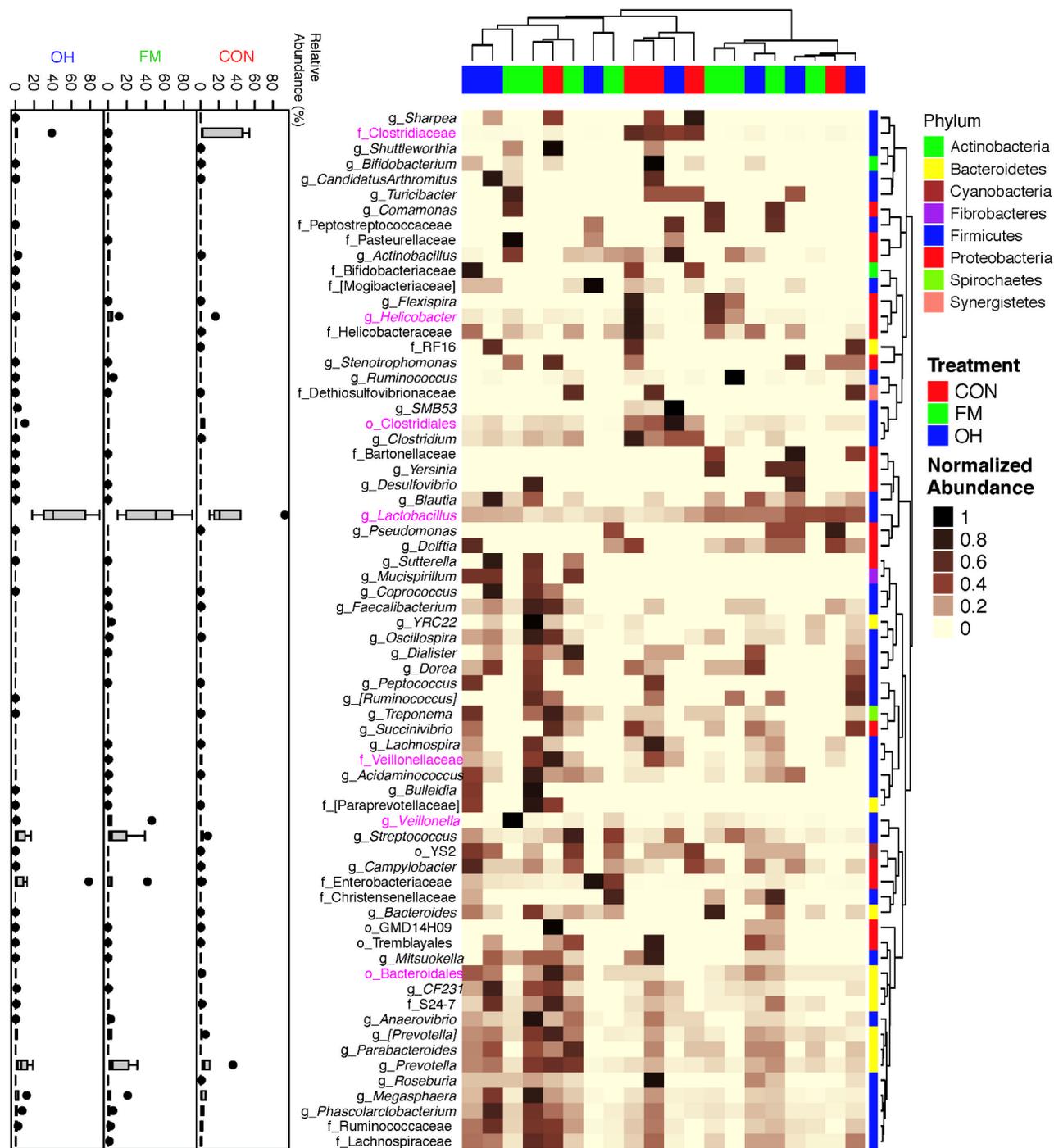
Figure 4.6. Cluster analysis of microbial communities in the cecal digesta of pigs fed control diet (CON), flaxseed meal (FM) and oat hulls (OH) diets. The sample identifiers on the top branches for diet treatments are coloured. Right branches are coloured to indicate the taxonomical

assignment of the OTUs at the phylum level. Each row represents one bacterial taxa (relative abundance above 0.01%), and some taxa could only be classified to family (f), order (o), class (c), or phylum (p) level. The taxa names were labeled by the colour code (magenta) to indicate the abundance of the taxa significantly differed between diet treatments (P < 0.05). The normalized relative abundance of bacterial taxa from illumina-sequenced 16S rRNA sequences in each sample is reflected by the colour of the scale (light yellow to black) on the heat map. The dendrogram on the top shows how the samples are clustered based on the Bray–Curtis dissimilarity measure, averaged by diet treatment. The dendrogram on the right shows clustering of bacterial taxa data based on the Spearman's rank correlation. The Box-Plots showed the relative abundances of bacterial taxa in cecal digesta microbiota among the three diet treatments.

Figure 4.7. Cluster analysis of microbial communities in the cecal digesta of pigs fed control diet (CON), flaxseed meal (FM) and oat hulls (OH) diets. The sample identifiers on the top branches for diet treatments are coloured. Right branches are coloured to indicate the taxonomical assignment of the OTUs at the phylum level. Each row represents one bacterial taxa (relative abundance above 0.01%), and some taxa could only be classified to family (f), order (o), class (c), or phylum (p) level. The taxa names were labeled by the colour code (magenta) to indicate the abundance of the taxa significantly differed between diet treatments ($P < 0.05$). The normalized relative abundance of bacterial taxa from illumina-sequenced 16S rRNA sequences in each sample is reflected by the colour of the scale (light yellow to black) on the heat map. The dendrogram on the top shows how the samples are clustered based on the Bray–Curtis dissimilarity measure, averaged by diet treatment. The dendrogram on the right shows clustering of bacterial taxa data based on the Spearman's rank correlation. The Box-Plots showed the relative abundances of bacterial taxa in cecal digesta microbiota among the three diet treatments.

negatively correlated with IDCA (Rho = -0.644; $P < 0.001$), whereas f. *Veillonellaceae* was positively correlated acetate (Rho = 0.534; $P = 0.002$). In ileal mucosa, g. *Lactobacillus* is positively correlated with propionate, LCA, DCA and IDCA; whereas g. *Veillonella* spp. is positively correlated with propionate, butyrate and valerate ($P < 0.05$). In cecal digesta, g. *Clostridium*, g. *Prevotella*, g. *Coprococcus*, and g. *Dorea* were negatively correlated with butyrate, but f. Coriobacteriaceae and g. *Acidiminococcus* were positively correlated with butyrate ($P < 0.05$).

4.5. DISCUSSION

The cross-talk between diet, gut microbiota, host's GI tract epithelium, GI metabolites and overlying mucus layer is complex and partly relies on amount and type of DF. As mentioned above, gut microbiota can modulate the pool and composition of both BA and SCFA, and in turn, these GI metabolites alter the richness and diversity of communal structure of intestinal microbiome (Ferrebee and Dawson, 2015; Nie et al., 2015). The observation that supplementation with 10% oat hulls did not affect feed intake was probably due to low SWC and WHC of the OH diet. A WHC of fibrous diets greater than 4.53 mL/g could induce a significant distension of the GIT with concomitant constraints on feed intake (Kyriazakis and Emmans. 1995; Ndou et al., 2013a). Considering the low bulk density of the OH diet, it was anticipated that feed intake will be depressed due to a greater gut fill effect (Kim et al., 2008). In contrast, supplementation with flaxseed meal reduced feed intake, which could be ascribed to the high WHC and SWC of the diet. Feedstuffs with high WHC and SWC induced satiety by absorbing more water, swelling and occupying space in the GIT (Kyriazakis and Emmans. 1995; Canibe and Bach-Knudsen, 2002; Ndou et al., 2013). Reduced intake may also be due to the fact that flaxseed meal is a rich source of soluble and mucilaginous NSP, suggesting that it increases digesta viscosity and delays GIT
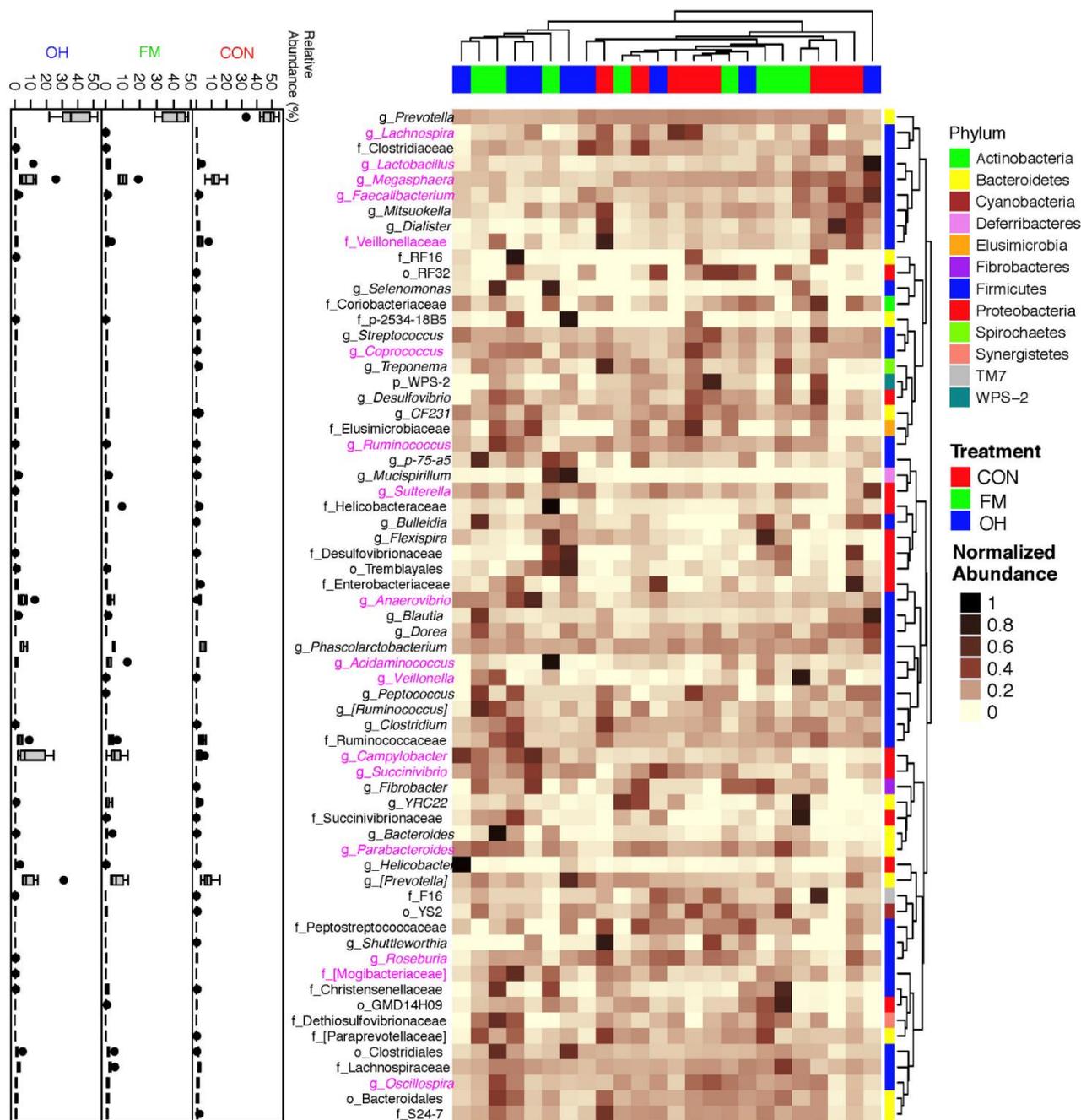
Figure 4.8. Figure 4.8. Cluster analysis of microbial communities in the cecal mucosa of pigs fed control diet (CON), flaxseed meal (FM) and oat hulls (OH) diets. The sample identifiers on the top branches for diet treatments are coloured. Right branches are coloured to indicate the taxonomical assignment of the OTUs at the phylum level. Each row represents one bacterial taxa

(relative abundance above 0.01%), and some taxa could only be classified to family (f), order (o), class (c), or phylum (p) level. The taxa names were labeled by the colour code (magenta) to indicate the abundance of the taxa significantly differed between diet treatments (P < 0.05). The normalized relative abundance of bacterial taxa from illumina-sequenced 16S rRNA sequences in each sample is reflected by the colour of the scale (light yellow to black) on the heat map. The dendrogram on the top shows how the samples are clustered based on the Bray–Curtis dissimilarity measure, averaged by diet treatment. The dendrogram on the right shows clustering of bacterial taxa data based on the Spearman's rank correlation. The Box-Plots showed the relative abundances of bacterial taxa in cecal mucosa microbiota among the three diet treatments.

A. Ileal digesta
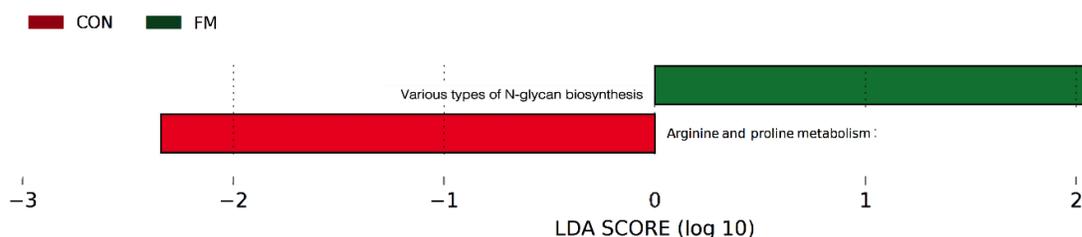
B. Ileal musosa
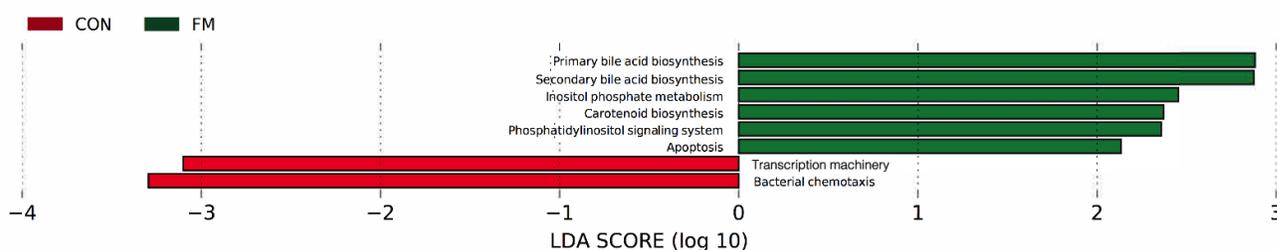
Figure 4.9. Figure 4.9. Predicted functional metagenomes of ileal digesta (A) and mucosa (B) microbiota of pigs fed control (CON) and flaxseed meal (FM) diets. Linear discriminant analysis (LDA) was performed to identify significant changes in the proportion of reconstructed functional pathways obtained from PICRUSt predictive algorithms at Kyoto Encyclopaedia of Genes and Genomes (KEGG; level 2 and 3). Analysis was performed using linear discriminant analysis of effect size (LEfSe), a metagenome analysis approach which performs the LDA following the Wilcoxon Mann-Whitney test to assess effect size of each differentially abundant variable. Colour code represents the class of treatment. Red indicates variables that were detected as significantly (Log LDA > 2.00) more abundant in CON whereas green indicates variables that were detected as significantly more abundant in FM diet-fed pigs).

Figure 4.10. Predicted functional metagenomes of in cecal digesta (A) and mucosa (B) microbiota of pigs fed the control (CON) and flaxseed meal (FM) diets. Linear discriminant analysis (LDA) was performed to identify significant changes in the proportion of reconstructed functional pathways obtained from PICRUSt predictive algorithms at Kyoto Encyclopaedia of Genes and Genomes (KEGG; level 2 and 3). Analysis was performed using linear discriminant analysis of effect size (LEfSe), a metagenome analysis approach which performs the LDA following the Wilcoxon Mann-Whitney test to assess effect size of each differentially abundant variable. Colour code represents the class of treatment. Red indicates variables that were detected as significantly (Log LDA > 2.00) more abundant in CON, whereas green indicates variables that were detected

as significantly more abundant in FM diet-fed pigs. Blue indicates variables that were detected as

significantly more abundant in OH-diet fed pigs.

emptying, thereby constraining feed intake (Bhatty, 1993; Jiménez-Moreno et al., 2009). Batterham et al. (1991) reported that decreased ADFI in growing pigs fed 300 g of flaxseed meal/kg of diet were attributed to reduced palatability. Consequently, the low ADFI observed in for the FM diet-fed pigs might account for their depressed weight gain. The finding that feed efficiency was reduced more in pigs fed the FM diet compared to those fed the control and OH diets supports the assertion that mucilaginous NSP with high SWC and WHC increase digesta viscosity which may prevent nutrient absorption (Takahashi et al., 2009). Although our observations on body weight gain and feed intake are contradictory to those reported by Eastwood et al. (2009), they agree with two earlier studies (Batterham et al., 1991; Bell and Keith, 1993), which reported that dietary inclusion of flaxseed meal beyond 100 g/kg of the diet had a negative impact on growth performance of pigs.

The greater concentration of acetate in the ileum and cecum of pigs fed the OH diet than those fed the FM diet was, however, unexpected, because it is well-established that insoluble fiber is less fermentable, especially in the small intestines than soluble fiber (Jiménez-Moreno et al., 2009). Considering that insoluble fiber from oat hulls is associated with gastric secretions, these observations also suggested that there could be other low molecular weight degradable digesta contents from endogenous secretions or non-fibrous components of the diet that are substrates for microflora. More work is needed to determine digestibility of dietary components. The finding that the concentration of propionic acid in the ileum increased with ingestion of flaxseed meal supports the data reported by Soder et al. (2012) and Lagkouvardos et al. (2015). These studies demonstrated that fermentation of flaxseed fibers yields a remarkably high proportion of propionic acid. Because g. *Lactobacillus* increase was associated with a lower abundance of g. *Faecalibacterium* also reported by Berggren et al. (1993), it can be speculated that the mechanism

by which the former suppresses the growth of the latter species is by competitive exclusion as well changes in enzymatic activities (Dierick and Decuypere, 1996). *Lactobacilli* and *streptococci* are major species in the pig intestine and can convert carbohydrates into lactic acid (Pluske et al., 2002) and then lactic acid can then be converted into propionate (Stanogias and Pearce, 1985). Interestingly, the concentration of butyrate in the cecum was higher in pigs fed the FM diet than OH diet-fed pigs. Thus, it seems highly likely that some cell wall polysaccharides, in particular the soluble NSP, present in flaxseed meal are stimulatory for the formation of butyrate presumably because these are rich in soluble proportions of xylose, arabinose, rhamnose and fucose that act as substrates for butyric acid producing bacteria (Ding et al., 2015). A rise in butyrate and nutrient concentration in the intestinal lumen increases VH or deeper crypts (Chen et al., 2014; Le et al., 2016), and is associated with an increased absorptive capacity of intestines and a healthy gut (Nyachoti et al., 2006). Supporting this phenomenon are findings in the present study that an increase in jejunal VH was observed in pigs which consumed the FM diet compared to those fed the CON and OH diets, suggesting an increase of ingesta in the lumen (Chen et al., 2014; Le et al., 2016; Vente-Spreeuwenberg et al., 2003).

Histological attributes of the intestines are modulated by DF, but their growth and development was critically associated with optimal growth performance in pigs (Heo et al., 2013; Jayaraman et al., 2016). Interestingly, the microbial metagenomics activities in the current study indicated that the KEGG functional pathways of apoptosis were elevated in ileal mucosa of pigs fed the FM diet and occurred at the same time with a decrease in the ileal VH and VCR in pigs fed the FM-diet. Apoptosis can be defined as a mechanism where excess or redundant cells are degenerated and removed during development and restricted tissue size is maintained as a normal and controlled process of an organism's growth or development (Otles and Ozgoz, 1985). The

increase in the apoptosis could have been a compensatory mechanism to open the way for replenishing mucosal cells following the damage which may be indirectly caused by the viscous characteristics of the NSP mucilage in flaxseed meal (Stanogias and Pearce, 1985). Butyrate had been shown to increase apoptosis in human colonic cell lines (Osles and Ozgoz, 2014). Reinforcing this idea is the observation in the present study that the KEGG metagenomics pathways of butyrate metabolism in ileal mucosa-associated microbiota were increased by more than 2.5 fold in pigs fed the FM diet. Moreover, there was a positive correlation between butyrate concentration and *Veillonella* spp., a well-known lactate-fermenting bacterium (Hegenholtz et al., 2013). The cross-feeding theory suggests that lactic acid can be utilized by *Veillonella* spp as a carbon source and converted into propionate and acetate (Hugenholtz et al., 2013). Thus, findings in the present study that respective increases in lactobacilli and streptococci were associated with increased Veillonellaceae families in ileal digesta and *Veillonella* spp in cecal mucosa of pigs that consumed flaxseed meal supports this phenomenon. Furthermore, the strong correlations observed between Veillonellaceae families and propionate in ileal digesta; g. *Veillonella* and propionate in ileal mucosa; and f. Veillonellaceae with acetate in cecal mucosa are further indicators of the presence of bacteria that do not ferment fiber but use fermentation products that are produced by others in the gut (Hugenholtz et al., 2013). Thus, the increased g. *Lactobacillus* in FM diet fed pigs supports the suggestions by Kiarie et al. (2007) that there are great opportunities for using flaxseed meal to modify intestinal microbial activity in a beneficial fashion.

Protein that escapes digestion in the upper gut can be fermented by microbial communities in the hindgut to produce branched-chain amino acids (Jha and Berrocosso, 2016). The proportions of p. Proteobacteria in cecal digesta were highest in FM, whereas p. Bacteroidetes colonized cecal mucosa of OH diets-fed pigs. Bacteroidetes and Proteobacteria phyla and in particular p.

Bacteroidetes have been linked with amino acid metabolism to produce branched-chain VFA (**BCVFA**), such as isovalerate and isobutyrate (Simpson and Campbell, 2015). Supporting these findings are the observations that similar trends were observed in the cecum and colon in which case pigs fed the FM diet tended to have the highest concentrations of valerate was in line with observations by Rasmussen et al. (1988) who postulated that valeric acid is exclusively a product of AA fermentation. Furthermore, this assertion is also in agreement with the observation that fat digestibility was depressed in pigs fed the FM diet which suggests that mucilaginous NSP proportions of flaxseed increase digesta viscosity, thereby impairing absorption of nutrients in the upper gut (Bhatty, 1993; Kiarie et al., 2007). Thus, the amount of substrate available for modulating hindgut microbial activity will be increased.

Although it is well-known that the host cannot metabolize bacterial protein or BCVFA (Simpson and Campbell, 2015), it is interesting to note that in this chapter functional pathways enriched with microbial activity in cecal digesta of FM diets-fed pigs included histidine metabolism. Moreover, functional pathways enriched with microbial activity in cecal mucosa of FM-fed pigs included arginine, proline, and phenylalanine metabolism and those enriched in OH diets-fed pigs included cysteine, methionine and tyrosine metabolism. In general, these observations suggest that deposition of the amount or composition of proteins that resist digestion in the upper gut is influenced by differences in the physicochemical properties of the DF sources. For example, due to its high WHC and SWC measured in this trial and its ability to increase digesta viscosity reported by Kiarie et al. (2007), flaxseed meal could depress protein digestibility thereby increasing the amino acid pool available for hindgut degradation. Supporting this postulation is the observation in the present study that lysine degradation is another functional pathway that was enriched in cecal mucosa-associated microbiota of pigs that consumed FM diets. A recently

published study of an extensive metagenomics approach has revealed that lysine can also be incorporated into bacterial biosynthetic pathway to produce butyrate (Vital et al., 2014). Conversely, oat hulls are classified as sources of insoluble DF and they could have depressed ileal amino acid digestibility by increasing digesta passage rate thereby increasing amount of amino acids that are available for hindgut microbial activity. The use of ileal-cannulated pigs would open ways for subsequent studies to test these postulations.

The observation that dietary inclusion of FM and OH depressed fat digestibility indicates that high DF content and WHC of the NSP may bind some of the BA and phospholipids present in the digesta, thereby reducing emulsification and absorption of dietary lipids (Ferrebee and Dawson, 2015). Another plausible explanation is that NSP from the fibrous ingredients are substrates for microbial activity that facilitates hydrolysis of bile salts (deconjugation) and reduce their solubility and emulsifying capacity (Ahn et al., 2003). Deconjugated BA are then bound to bacterial cells and DF, thereby increasing their excretion as well as that of dietary fat. Reinforcing this are the greater concentrations of pooled VFA and BA excreted by pigs fed FM and OH diets in the current study. Interestingly, apparent fat digestibility was reduced more in pigs fed flaxseed meal-enriched diet compared to those fed oat hulls-enriched diets. The differences in the extent to which fat digestibility was depressed, between FM and OH diets supports the assertion that variability in the monomeric compositions of the fibrous ingredients induce different physicochemical properties of digesta that play central roles in the development of the GIT and digestion processes (Montagne et al., 2003). Therefore, high viscosity of digesta which is likely to have been induced by soluble fiber in pigs fed the FM diet hampered diffusion of lipid micelles in the small intestine thereby further reducing lipid absorption (Bhatty, 1993; Kiarie et al., 2007).

Another potential contributing factor for the lower fat digestibility of the FM diet could be encapsulation of fat by the cell walls within the fiber matrices of flaxseed meal.

Apart from their role as detergents to facilitate digestion and absorption of fats in the GIT, BA also act as signaling molecules that regulate the FXR and in turn alter lipid, glucose and energy metabolism (Marcil et al., 2002; Ferrebee and Dawson, 2015; Nie et al., 2015). The similarity observed on the concentrations of ileal chenodeoxycholic and cholic acids in the FM and OH diets-fed pigs could be due to the dilution of BA by the non-digestible fibers. Although no diet-induced effects were observed on total serum triglycerides, differences in NSP composition between the fibrous ingredients could explain the extents to which serum cholesterol was reduced more in OH diet-fed pigs than in FM diet-fed pigs. There are two possible mechanisms that may explain serum cholesterol-lowering effects of soluble fiber from flaxseed meal and insoluble fiber from oat hulls. First, the hypocholesterolemic effects of soluble fibers could be due to their ability to increase digesta viscosity which might have reduced cholesterol and BA uptake. This increases BA and cholesterol excretion, alter hepatic cholesterol metabolism, and consequently reduce cholesterol concentration in blood (Santas et al., 2012). A second explanation is that oat hulls increased the insoluble fiber composition of the diet, which in turn reduces transit time of intraluminal contents of GIT (Jiménez-Moreno et al., 2009), thereby increasing cholesterol excretion. The same mechanism by which insoluble fiber increase digesta passage rate could also explain the reduction in fat digestibility observed in OH diets-fed pigs. Furthermore, oat hulls have a high degree of lignification (Bach Knudsen, 1997), which may act as a resin or a bile salt-sequestrating agent by reducing reabsorption of BA in small intestine (Fardet, 2010).

The contradictory observation that pigs fed diets supplemented with flaxseed meal had higher serum cholesterol than those fed oat hulls-containing diets, as well as elevated fecal

concentration of cholesterol and deconjugated BA suggests that there could be microbial activity that promoted production of secondary BA (Stevens and Hume, 1998; Ahn et al., 2003). This assertion is in agreement with the finding that bacterial communities in ileal digesta of pigs fed the FM differed with those in OH diets-fed pigs where members of the Firmicutes phyla were the most dominant followed by smaller relative abundance of Bacteroidetes and Proteobacteria. The observations also demonstrated that an increase in DF intake induced a shift in microbial composition due to the presence of substrate that promote growth of beneficial phyla and reduce the abundance of BA-tolerant species in ileal digesta (Turnbaugh et al., 2009; Devkota et al., 2012). Previous studies have also revealed that BA in intestinal digesta suppresses growth of p. Bacteroidetes and Actinobacteria, and consequently exert beneficial effects on p. Firmicutes, especially BA-7α-dehydroxylating species (Nie et al., 2015). Supporting these notions are positive correlations between bacterial order Clostridiales and family Clostridiaceae with secondary BA (lithocholic acid (LCA), deoxycholic acid (DCA), isodeoxycholic acid (IDCA), ursodeoxycholic acid (UDCA) observed in the current study supports the notion that g. *Clostridium* are the only members of the Firmicutes phylum that possess 7α/β-dehydroxylase and catalyze the hydrogenation reaction of primary BA to generate secondary BA (Midtvedt, 1974; Hayakawa, 1982; Doerner et al., 1997). Furthermore, a positive correlation between g. *Lactobacillus* and primary acids (CD-CDAC) and secondary IDCA observed in the current study agrees with the notion by Begley *et al*. (2005), and categorically indicates that gut commensal phyla tolerate BA by expressing bile salt hydrolase (BSH) activity.

The observations that order Clostridiales and Clostridiaceae families in ileal digesta, and g. *Clostridium* and Clostridiaceae families in cecal digesta were positively correlated with secondary BA, such as LCA, DCA, IDCA and UDCA are also consistent with reports by Ridlon *et al*. (2006;

2014). *Clostridium* and *Eubacterium* genera are members of Firmicutes phylum that convert primary BA into secondary forms through gut microbial 7-dehydroxylation and 7 α/β-epimerization (Wahlstroom et al., 2016). The observation that the Firmicutes phyla were the most dominant in ileal mucosa of pigs fed the control diet compared to that of pigs which consumed FM and OH diets was unexpected and is difficult to explain with variables measure in the current study. However, the positive correlation observed between g. *Streptococcus* and DCA in ileal mucosa is supported by Salvioli *et al*. (1982) who reported that administration of *Streptococcus* spp, bacteria that produce substances acting against c. Clostridia reduced cholesterol saturation and molar percentage of DCA in bile and consequently increased CA and DCA in feces. Moreover, flaxseed meal and oat hulls supplementation reduced the absorptive capacity of intestinal mucosa by decreasing VH, and indirectly promoted BA deconjugation (Mathlouthi et al., 2002; Archer et al., 1982; Gilliland and Speck, 1977). Metagenomic analyzes in previous studies have revealed that functional BSH activities are present in all major bacterial divisions in human gut including members of *Lactobacilli*, *Bifidobacteria*, *Clostridium* and Bacteroides (Ridlon et al., 2006; Mathlouthi et al., 2002; Archer et al., 1982; Jones et al., 2008). Interestingly, the microbial metagenomic analysis in the current study indicated that KEGG functional pathways of primary BA and secondary BA biosynthesis were enriched in ileal mucosal microbiota of FM diet-fed pigs. Thus, ingestion of flaxseed meal fiber could promote BA biotransformation by altering intestinal microbiota in a beneficial fashion in human nutrition. Conversely, this could have adverse effects in swine nutrition because BA formation and enterohepatic circulation (**EHC**) occurs at the expense of cholesterol and metabolizable energy needed for growth performance. In fact, EHC occurs in humans about six times a day (Wahlstroom et al., 2016) and BSH is enriched in gut microbiota compared to other microbial ecosystem (Jones et al., 2008). In this regard, EHC and

microbial species should be investigated in more detail to understand why performance is depressed in pigs that are fed nutritionally balanced high-fiber diets that are also supplemented with vegetable oil.

The VFA produced from DF fermentation could have contributed to hypocholesterolemic effects due to production of VFA, mainly ileal propionate in pigs fed FM diets, which has been reported to decrease endogenous synthesis of cholesterol in the liver (Vela´zquez et al., 2000; Yoon et al. 2006). The general observations that the concentrations of secondary BA increased in ileum, cecum and feces of pigs fed FM and OH diets, whereas that of primary BA decreased could be attributed to that a portion of these BA are re-channeled back to the liver through the EHC system (Graffner et al., 2016). However, an increase in BA excretion could be due to bioconversion of primary BA in secondary forms and re-absorption into EHC is interrupted by increase in DF in flaxseed meal and oat hulls. Supporting these observations are previous studies which have also shown that approximately 95% of total BA are reabsorbed into the hepatic system (Graffner et al., 2016). Moreover, hindgut microbiota has been reported to facilitate biotransformation of primary BA into secondary forms through gut microbial 7α-dehydroxylation and/or 7α/β-epimerization (Stevens and Hume, 1998; Ahn et al., 2003; Wahlstrom et al., 2016). The observation that a decrease in weight gain in pigs fed FM diet coincided with an increase in concentration of secondary BA and reduction in fat digestibility concurs with findings by Mathlouthi et al. (2002). These studies suggests that increase in DF reduce bile acids capacity for fat solubilization and emulsification, resulting in decrease in growth performance. The treatment-induced differences observed in BA concentrations from one intestinal region to the other suggests that the flaxseed and oat hulls induce variable effects on EHC of BA either by altering gut microbiota composition or changes in digesta bulking properties.

The ratios of coprostanol: cholesterol and that of total NS: cholesterol can be used as markers to assess the effect of feeding DF and its fermentability on lipid metabolism (Santas et al., 2012). Based on results in the current study, the increase in NS excretion, in particular, ileal coprostanol, cholesterol and bioconversion of cholesterol to secondary NS namely cholestanol, sitosterol and stigmasterol in the cecum appears to be one of the main mechanisms responsible for lower serum cholesterol observed in pigs that were fed the FM diet compared to those fed the OH diet. These findings indicate that DF may enhance the breakdown of cholesterol, leading to increases in secondary metabolites excreted in the form of secondary BA and NS bound to NSP matrices. Thus, bioconversion of coprostanol and NS increased in cecum contents and feces of pigs fed diets enriched with DF.

In conclusion, insoluble DF reduced serum cholesterol more than soluble DF and the former depressed growth performance by reducing feed intake. The hypocholesteremic effects of both soluble and insoluble DF are ascribed to their ability to act as immediate substrates for intestinal fermentation which in turn decrease fat digestibility and cholesterol absorption by reducing bile acid reabsorption. The intake of either soluble DF from flaxseed meal or insoluble DF from oat hulls modulates associations between microbiota and metabolites as well as their predicted metagenomic functions. Fat excretion in pigs fed diets enriched with both soluble and insoluble DF could be due to microbial fat or inefficient digestion due to deconjugation of BA by increased bacterial activity in the GIT. Although total triglycerides were not affected, feeding diets containing soluble or insoluble DF induced variable effects on histological attributes of small intestines and profiles of volatile fatty acids, bile acids and neutral sterols, and gastrointestinal microbiota depending on intestinal segment.

LIMITATIONS AND IMPLICATIONS OF CHAPTER 4

One limitation proposed from Chapter 4 is that the design of the study and variables measured could not allow us to pinpoint whether fat excretion in pigs fed the high-fiber diets was due to microbial fat or inefficient digestion and malabsorption of dietary fat due to deconjugation of BA by increased bacterial activity in the GIT. Another limitation is that the VFA detected in different segments in experiment in Chapter 4 are not accurate indicators of fermentability but are net values after production and absorption and only represent a small fraction of total production from fiber fermentation. The VFA and BA concentrations reported in Chapter 4 were measured once during slaughter and were not normalized to account for changes in the flows of gastrointestinal contents. Thus, these values lack precisions and were also influenced by the time of slaughter which was not uniform among all pigs. The other limitation is that the slaughter model used in Chapter 4 could not allow us to sample enough digesta needed to analyze fermentability of DF and flows of in the ileum and entire GIT.

The implications raised are that more work was needed to determine the effects of DF source on intestinal and fecal flows of FA in pigs. This stimulated the need to design experiment in Chapter 5 to facilitate the assessment of variables that could allow us to identify the actual sources and flows of FA and normalized concentrations of BA. Thus, the ileal cannulated pig model was used in Chapter 5 and pigs were fed diets with an indigestible marker so that more digesta sample can be repeatedly collected over a long period of time to determine FA flows and normalized BA as well as digestibility of other dietary components. In Chapter 5, the ileal cannulated pig model (*in vivo*) was then integrated with an *in vitro* technique to predict the production and absorption of VFA.

CHAPTER FIVE

MANUSCRIPT 2

Flaxseed meal and oat hulls supplementation modulates dietary fiber digestibility, and flows of fatty acids and bile acids, and predicted production and absorption of hindgut volatile fatty acids in growing pigs[1]

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[1]Materials used in this chapter were used to prepare two companion papers that were published in the Journal of Animal Science as follows:

S.P. Ndou, E. Kiarie, and C.M. Nyachoti. J. Anim. Sci. doi:10.1093/jas/sky399. (**Paper 3**)

S. P. Ndou, E. Kiarie, N. Ames and C. M. Nyachoti. J. Anim. Sci. doi:10.1093/jas/sky398. (**Paper 4**)

Authors' contributions

S.P.N. and C.M.N. designed the study. S.P.N conducted the animal study, the statistical analysis and wrote the manuscript. E.K., N.A., and C.M.N. critically reviewed the manuscript. C.M.N. was the principal investigator who supervised all aspects of the study.

5.1. ABSTRACT

The present study investigated the effects of dietary addition of soluble fiber from flaxseed meal and insoluble fiber from oat hulls on production and absorption of hindgut volatile fatty acids (VFA), digestibility of dietary fiber (DF) and fatty acids (FA), and flows of FA and bile acids (BA) in the ileum and feces. Twelve ileal cannulated barrows were individually housed and offered corn-soybean meal-based diets without (control), or with flaxseed meal (FM) or oat hulls (OH) in a 2-period cross-over design ($n = 8$). The production and absorption of hindgut VFA was greater ($P < 0.05$) for the FM diet compared to the control and OH diets. The quantity of digested energy in the upper gut was reduced ($P < 0.05$) more in pigs fed flaxseed meal compared to oat hulls-fed pigs. The consumption of the FM diet increased ($P < 0.05$) the quantity of digested energy, energy produced and absorbed from VFA in the hindgut, and the percentage contribution of VFA from fermentation to total tract digestible energy, compared to the control and OH diets. Pigs fed the control diet had greater ($P < 0.05$) apparent ileal digestibility (AID) of saturated FA (SFA) and insoluble DF and apparent total tract digestibility (ATTD) of SFA than pigs offered the OH and FM diets. The AID of total FA and monounsaturated FA in FM diet-fed pigs was lower ($P < 0.05$) compared to those fed the control and OH diets. The ATTD of CP, neutral detergent fiber, insoluble and total DF was lower ($P < 0.05$) in pigs fed the OH diet than in pigs that consumed the control and FM diets. In the terminal ileum, pigs fed OH and FM diets excreted more ($P < 0.05$) primary BA and all secondary BA (except lithocholic acid) compared to control diet-fed pigs. The intestinal flows of lithocholic acid in FM diet-fed pigs were higher ($P < 0.05$) than in pigs offered the control diet. Pigs fed FM and OH diets excreted more ($P = 0.001$) fecal ursodeoxycholic and total bile acids compared to pigs that consumed the control diet. The ileal flows of eicosapentaenoic and erucic acids in pigs fed the FM and OH diets were greater ($P < 0.05$) than in

pigs fed the control diet. Fecal flows of all SFA, and palmitoleic, palmitelaidic, oleic, nervonic, linoleic, eicosapentaenoic, erucic, docosatetraenoic and osbond acids were greater ($P < 0.05$) in OH diet-fed pigs compared to pigs fed other diets. In conclusion, soluble fiber from flaxseed meal reduced ileal digested energy at a lesser extent than insoluble fiber from oat hulls. Dietary addition of flaxseed meal caused a corresponding increase in the contribution of energy from hindgut VFA fermentation to total tract digestible energy, compared with oat hulls. Consumption of high-fiber diets reduced FA digestibility, increased gastrointestinal flows of FA and excretion of BA, and induced variable effects on digestibility of DF components and fecal flows of unsaturated FA.

Key words: fibrous co-products, hindgut fermentation, dietary fiber, pigs.

5.2. INTRODUCTION

Supplementation with agro-industrial co-products in swine diets increase the dietary fiber (**DF**) content. The general assumption is that performance of pigs will not decline if pigs are fed nutritionally-balanced high-fiber diets (Chapter 2). However, previous studies have demonstrated poor fat digestion and performance in growing pigs that are fed nutritionally-balanced high-fiber diets that contain co-products and supplemental fat (Bakker et al., 1996; Gutierrez et al., 2013). Moreover, our findings in Chapter 4 indicated that both soluble and insoluble fiber reduced fecal fat digestibility and soluble fiber depressed growth performance. These discrepancies suggest that processes contributing to these responses are complex and remain to be elucidated. Moreover, the increase in DF content cause a reduction in digestible energy in the upper gut (Bach Knudsen and Hansen, 1991). Conversely, incremental levels of DF induce a corresponding increase in energy contribution from volatile fatty acids (**VFA**) produced from hindgut fermentation (Anguita et al.,

2006; Iyayi and Adeola, 2015). The role played by DF solubility on amount of energy contributed from gastrointestinal fermentation warrants further investigations.

The effect of practical sources of soluble or insoluble DF on hindgut VFA production and absorption and flows of fatty acids (**FA**) and bile acids (**BA**) will require further investigation to better understand and develop strategies to improve utilization of fibrous co-products. Therefore, the first objective of this experiment was to use a combined *in vivo-in vitro* fermentation methodology to investigate the effects of soluble DF from flaxseed meal and insoluble DF from oat hulls on hindgut VFA production and absorption in growing pigs fed diets that were supplemented with added fat. The second objective of the current experiment was to determine the effect of adding soluble and insoluble DF on digestibility of DF and FA, and flows of FA and BA in the ileum and feces of growing pigs.

5.3. MATERIALS AND METHODS

5.3.1. Diets, Pigs, Experimental Design and Sample Collection (In vivo assay)

The experimental procedures and use of animals in this study were approved by the Animal Care Committee of the University of Manitoba and pigs were cared for according to the guidelines of the Canadian Council on Animal Care (CCAC, 2009). The diets included; a corn-soybean meal-based diet (control diet), a 12% flaxseed meal-containing diet (**FM**), and a diet containing 10% oat hulls (**OH**) (**Table 5.1**). The diets were similar to those reported in Chapter 4. A total of twelve Genesis crossbred barrows [(Yorkshire-Landrace dam) × Duroc sire] with an initial BW of 35.1 ± 0.44 (mean ± SEM) kg were obtained from University of Manitoba's Glenlea Swine Research Unit (Winnipeg, MB, Canada). Pigs were housed individually in pens. The experimental room temperature was maintained at 22.0 ± 2.2 ºC (mean ± SD) with a 14 h light-10 h dark cycle.

Table 5.1. Compositions of the control, flaxseed meal and oat hulls diets (as fed)[1]

Item	Control	FM	OH
		Diet[1]	
Ingredient composition (%)			
Corn	64.53	57.40	52.99
Oat Hulls	-	-	10.00
Flaxseed meal	-	12.00	-
Soybean meal, 44% CP	31.00	25.49	31.50
Vegetable oil	1.35	2.05	2.39
Limestone	0.683	0.667	0.640
Monocalcium phosphate	0.752	0.637	0.750
Salt	0.35	0.35	0.35
Vitamin-mineral premix[2]	1.00	1.00	1.00
L-Lysine HCl	-	0.086	0.010
DL-Methionine	0.031	0.010	0.060
Threonine	-	0.012	0.010
Titanium dioxide	0.30	0.30	0.30
Analyzed compositions[3]			
Crude protein (N $\times$ 6.25), %	19.20	19.30	19.36
Gross energy, kcal/kg	3,900	3,995	3,980
Acid detergent fiber	3.7	5.0	7.6
Nitrogen detergent fiber	9.3	18.2	18.7
Soluble dietary fiber	4.7	6.9	4.5
Insoluble dietary fiber	10.5	17.6	21.1
Total dietary fiber	15.2	24.9	25.5

[1]FM = flaxseed meal-containing diet; OH = oat hulls-containing diet.

[2]Provided the following nutrients per kg of air-dry diet: 8,250 IU retinol (vitamin A); 200 IU cholecalciferol (vitamin D$_3$); 40 UI α-tocopherol (vitamin E); 4 mg vitamin K; 1.5 mg vitamin B$_1$; 7 mg vitamin B$_2$; 2.5 mg vitamin B$_6$; 25 μg vitamin B$_{12}$; 14 mg calcium pantothenate; 2 mg folic acid; 21 mg niacin (vitamin B$_3$); and 200 μg biotin (vitamin B$_7$). Minerals: 15 mg Cu (as copper sulphate); 0.4 mg iodine (as potassium iodine); 120 mg iron (as ferrous sulphate); 20 mg Mn (as manganese oxide); 0.3 mg Se (as sodium selenite); 110 mg Zn (as zinc oxide).

throughout the study. Following a 7-d adaptation period to the experimental room, each pig was surgically equipped with a simple T-cannula at the terminal ileum as described by Nyachoti et al., (2002). After surgery, the cannulated pigs were allowed a recovery period of 10 d. After the recovery period, pigs were assigned to the three diets in a 2-period cross-over design. In each period, 4 pigs were offered one of the three treatments to give 8 replicates per diet ($n = 8$). Pigs were weighed at the beginning of each experimental period and offered a daily feed amount equivalent to supply 2.8 times the maintenance energy requirement as recommended in NRC (2012). Daily feed allowances were offered in two equal meals at 0800 and 1600 h. Each experimental period lasted 16 d; where 12 d were for adaptation to the diets, followed by collection of fecal samples on the 13th and 14th day and collection of ileal digesta contents on Day 15 and 16.

Using the rectal palpation technique, fecal samples were collected into plastic bags, subsampled and sealed into Eppendorf tubes and immediately stored at -80 °C for determining fecal VFA concentrations. Another subsample of feces was immediately stored at -20 °C for determining apparent total tract digestibility (**ATTD**) of dietary components, flows FA and BA. Digesta samples were collected by attaching empty plastic bags on the cannula and immediately subsampled, sealed into Eppendorf tubes and store at -80 °C for determining the concentration of VFA in ileal digesta contents. Another subsample was immediately stored at -80 °C to provide a substrate for the *in vitro* fermentation assay. Immediately after collecting samples for VFA analyzes, plastic bags contained 10% formic acid were also attached and changed every 30 minutes unless filled with digesta. Digesta samples collected in 10% formic acid were immediately stored at -20 °C for determining ileal digestibility of dietary components and the flows of FA and BA. At the end of each day of collection, all samples were pooled for each pig, day of collection and period. The samples were mixed in a mechanical blender, freeze-dried and stored at -80 °C.

5.3.2. In vitro fermentation assay

Fermentation of ileal digesta was carried out using fecal inoculum. The collection and preparation of fecal inoculum was performed according to modifications of the method described by Montoya et al. (2016). All procedures were performed under a constant flow of CO_2. Fresh fecal samples were collected, sealed, and placed into pre-warmed (38 ^{o}C) plastic bags that were filled with CO_2 and a sterile anaerobic medium (0.1 M-phosphate buffer at pH 7) to give final slurry of [1:5, or 200 g of feces/L (w/v)]. After mixing of the slurry for 60 s, the fecal slurries were filtered through a double layer of sterile cheesecloth to extract fecal inoculum into a pre-warmed (38 ^{o}C) vacuum flask flushed with CO_2.

The *in vitro* fermentation of freeze-dried ileal digesta substrate was conducted using fecal inoculum as illustrated in the schematic flow diagram in **Figure 5.1.** To each Nalgene bottle containing 1.0 ± 0.1 g dry matter (**DM**) of the freeze-dried ileal digesta (substrate) or empty (Blank incubation) and 100 mL of salt medium, 100 ml of the inoculum mixture were added. The Nalgene bottle were flushed with CO_2, and immediately capped. Therefore, there were five replicate bottles per ileal digesta substrate; one bottle was used for determining VFA concentrations after fermentation, the second and third bottles were used to determine DM fermentability or disappearance. The fourth and fifth bottles were used as blanks for correcting VFA and DM fermentability associated with the fecal digesta slurry before (0 h) and after fermentation (48 h), respectively. After inoculation, the bottles with ileal digesta substrate were placed into pre-warmed (37°C) incubators for 48 h, respectively. After incubation, the bottles were vented and cooled in ice water. Terminal pH was recorded and then the bottles were centrifuged at 14,000rpm, 4°C for 10 min. The supernatant was carefully extracted, transferred to an Eppendorf tube and
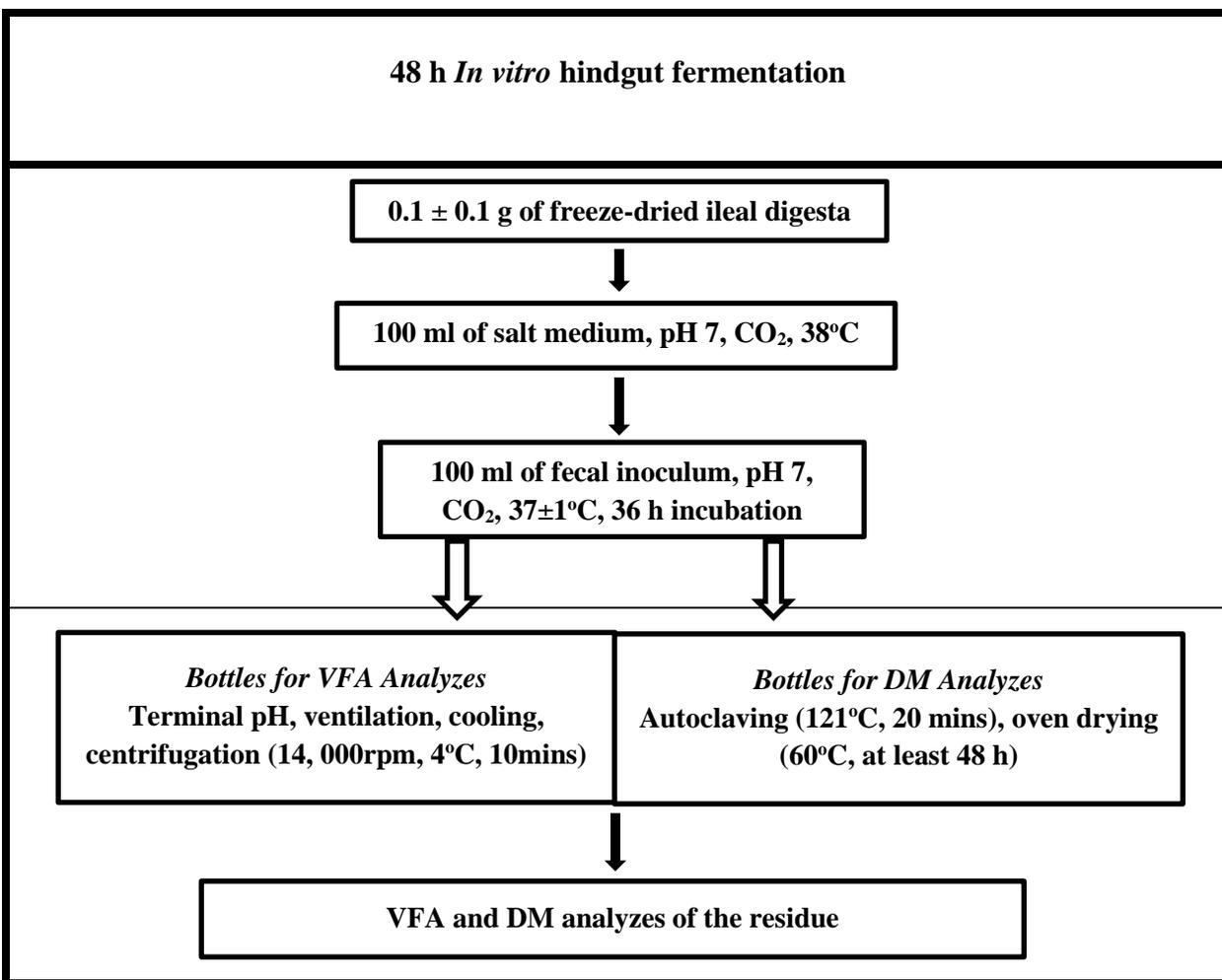
Figure 5. 1. In vitro fermentation of freeze-dried ileal digesta using fecal inoculum.

stored at -80°C until analyzed for VFA. The remaining three bottles were placed in an autoclave (121°C for 20 mins) to completely inactivate microbial activity. The DM of the unfermented residue was determined in the three remaining bottles by drying them in a fanned oven at 60°C for 48 h until a constant weight of undigested DM was attained.

5.3.3. Laboratory Analyzes

Diets, freeze-dried ileal contents and feces were ground through a 1-mm screen in a laboratory mill (Thomas Wiley Mill Model 4, Thomas Scientific, Swedesboro, NJ). The DM, gross energy (**GE**), crude protein (**CP**), neutral detergent fiber (**NDF**), acid detergent fiber (**ADF**), and titanium contents of the diets, ileal digesta and fecal samples were determined as described in Chapter 4.

The experimental diets, ileal digesta and feces were also analyzed for FA content following the methodology described by Folch et al. (1957) using a 2:1 chloroform: methanol mixture (**Table 5.2**). The extracted FA were methylated to FA methyl esters using modifications based on methods described by Metcalfe and Schmitz (1961). The soluble (**SDF**), insoluble (**IDF**) and total (**TDF**) dietary fiber contents were analyzed in all diets, ileal digesta and fecal samples according to method 991.43 of AOAC (2012) using the Ankom^{TDF} Dietary Fiber Analyzer (Ankom Technology). The concentrations of BA in freeze-dried ileal digesta and fecal contents were extracted according to procedures described in Chapter 4.

5.3.4. Calculations and Statistical analyzes

The determined AID and ATTD were calculated as follows:

Determined AID or ATTD (%) = (1 − ((Nutrient$_{F/I}$ ÷ Nutrient$_D$) × (T$_D$ ÷ T$_{F/I}$))) × 100,

Table 5.2. Fatty acids (FA) profile of ingredients and experimental diets

Item	Ingredients				Diet[1]		
	Corn	Soybean	Flaxseed meal	Oat hulls	CON	FM	OH
Saturated fatty acids (SFA)							
Lauric (C12:0)	0.01	0.00	0.01	0.00	0.01	0.01	0.01
Myristic (C14:0)	0.04	0.07	0.07	1.20	0.07	0.06	0.08
Pentadecyclic (C15:0)	0.07	0.18	0.06	0.39	0.07	0.06	0.07
Palmitic acid (C16:0)	12.23	15.72	7.15	31.84	12.68	10.71	12.01
Margaric (C17:0)	0.09	0.20	0.06	0.26	0.11	0.10	0.11
Stearic (C18:0)	1.75	4.10	2.09	3.45	2.85	2.94	3.15
Arachidic (C20:0)	0.00	0.24	0.19	0.97	0.36	0.31	0.35
Behenic (C22:0)	0.18	0.36	0.15	0.81	0.24	0.23	0.27
Lignoceric (C24:0)	0.24	0.26	0.00	0.00	0.17	0.00	0.00
$\sum$SFA	15.06	21.14	9.95	39.48	16.57	14.59	16.22
Monounsaturated fatty acids (MUFA)							
Palmitoleic (C16:1n-7)	0.12	0.09	0.09	0.41	0.11	0.10	0.11
Palmitelaidic (C16:1t)	0.05	0.01	0.03	0.02	0.03	0.03	0.02
Oleic (C18:1n-9)	25.50	9.04	11.86	0.99	22.54	21.89	22.42
Vaccenic (C18:1n-7)	0.48	1.39	0.68	33.59	0.74	0.78	0.89
Gadoleic (C20:1)	0.35	0.15	0.14	1.58	0.27	0.23	0.25
$\sum$MUFA	26.51	10.61	12.81	37.17	23.69	23.04	23.70
Polyunsaturated fatty acids (PUFA)							
Linoleic (C18:2)	56.77	57.32	14.10	21.21	54.99	45.84	54.07
Linolenic (C18:3n-6)	0.00	0.00	0.00	0.00	0.00	0.01	0.01
α-Linolenic (C18:3n-3)	1.61	10.90	62.97	2.14	4.73	16.46	5.95
Eicosadienoic (C20:2)	0.02	0.03	0.08	0.00	0.03	0.04	0.03
Eicosatrienoic (C20:3n-3)	0.00	0.00	0.08	0.00	0.00	0.01	0.00
Eicosapentaenoic(20:5n-3)	0.03	0.00	0.00	0.00	0.00	0.01	0.02
Erucic (C22:1n-9)	0.00	0.00	0.01	0.57	0.00	0.01	0.01
Nervonic (C24:1n-9)	0.00	0.00	0.17	0.32	0.00	0.17	0.16
$\sum$PUFA	58.43	68.25	77.24	23.35	59.74	62.37	60.07
$\sum$FA	-	-	-	-	4.56	6.02	6.05
Calculated IV[2], g/100g	125	136	200	45	127	142	129

[1]FM = flaxseed meal-containing diet; OH = oat hulls-containing diet.

[2]IV = iodine value.

Determined hindgut disappearance$_{in\ vivo}$ (%) = $(1 - ((DM_F \div DM_{I/C}) \times (T_{I/C} \div T_{F/C}))) \times 100$,

where Nutrient$_{F/I}$ are the contents of dietary components (g/kg DM) in the feces ($_F$) or ileal ($_I$) digesta, respectively; Nutrient$_D$ is the content of each nutrient (g/kg DM) in the diet; T_D is the titanium dioxide (g/kg DM) in the diet; $T_{F/I}$ are the concentrations of titanium dioxide (g/kg DM) in feces (F) or ileal (I) digesta, respectively; DM_D is the content of DM (g/kg DM) in the diet; $DM_{F/I}$ are the contents of DM (g/kg DM) in the feces or ileal digesta, respectively (Montoya et al., 2016).

The concentrations of VFA in the terminal ileal digesta and the feces were normalized for the food DM intake (DMI) as described by Montoya et al. (2016) using the following equation:

Normalized VFA concentration (mmol/kg DMI) = VFA concentration (mmol/kg DM) × $(T_D/T_{F/I})$.

Predicted digestibility of DM and VFA produced in the hindgut were determined after *in vitro* fermentation of ileal digesta with fecal inoculum, respectively, and calculated using the following equations:

Predicted hindgut disappearance$_{in\ vitro}$ (%) = $(DM_b - (DM_a - (DM_{blank\ initial} + DM_{blank\ final})/2)))/DM_b \times 100$,

VFA produced by fermentation (mmol/kg DM incubated) = $(VFA_{sample} - ((VFA_{blank\ initial} + VFA_{blank\ final})/2))$ / sample weight (g DM) × 100,

where DM_b is the DM (mg) of ileal digesta before *in vitro* fermentation, DM_a is the DM (mg) of ileal digesta after *in vitro* fermentation. $DM_{blank\ initial}$, $DM_{blank\ final}$, $VFA_{blank\ initial}$ and $VFA_{blank\ final}$ are the DM (mg) and the VFA (mmol) in the blank bottle (which contained inoculum but no ileal digesta) before (initial) and after (final) *in vitro* fermentation, respectively (Cole et al., 2013).

The predicted ATTD of DM and the VFA production and absorption in the hindgut were calculated based on combining results for *in vivo* ileal digesta flows (ileal cannulated pig) with *in vitro* concentrations (hindgut fermentation). The *in vivo* measurements represented digestion in the upper gut, and *in vitro* values represented fermentation in the hindgut. The $PAD_{I/F}$ of DM and predicted hindgut VFA production were calculated as follows:

$$\text{Predicted ATTD}\,(\%) = (DM_D - (((100 - DM\ \text{fermentability}_{in\ vitro})/100) \times DM\ \text{flow}_I))/OM_D \times 100,$$

$$\text{Predicted hindgut VFA production (mmol/kg DMI)} = \text{VFA produced by fermentation (mmol/kg ileal digesta DM incubated)} \times DM\ \text{flow}_I\ \text{(kg DM/kg DMI)},$$

where DM_D (g/kg DM) is the DM content in the diet, DM fermentability*in vitro* (%) is the DM fermentability determined from incubation of ileal digesta, and DM flow$_I$ (g/kg DMI) is the ileal flow of DM. The values of VFA entering the hindgut (is the ileal normalized VFA concentration) and the amounts of VFA produced in the hindgut (i.e. *in vitro* predicted hindgut VFA production) were used to estimate the amounts of VFA absorbed in the hindgut based on the following equation:

Amount of VFA absorbed from the hindgut (mmol/kg DMI) = *in vitro* predicted hindgut VFA production (mmol/kg DMI) + ileal VFA concentration (mmol/kg DMI) – fecal VFA concentration (mmol/kg DMI),

Extent of VFA absorbed in the hindgut (%) = (amount of VFA absorbed from the hindgut (mmol/kg DMI) / (*in vitro* predicted hindgut VFA production (mmol/kg DMI) + ileal VFA concentration (mmol/kg DMI))) × 100.

The ileal and total tract digestible energy was calculated as follows: Ileal and total tract digestible energy = Determined AID/ATTD × Dietary GE content. The amount of energy in the hindgut was determined by subtracting ileal digestible energy from total tract digestible energy. The energy produced from VFA were determined by adding the equivalent energy for each VFA absorbed in the hindgut. The equivalent energy for acetic, propionic, butyric and valeric acids were 208, 364, 520 and 676 kcal/mol (Weast, 1977). The percentage contribution of energy from VFA to total available energy was determined as follows:

Contribution of available energy from hindgut fermentation (%) = (energy absorbed from hindgut VFA (kcal/kg DM feed) / (energy digested in the ileum (kcal/kg DM feed) + energy absorbed from hindgut VFA(kcal/kg DM feed))) × 100 (Iyayi and Adeola, 2015).

Data were analyzed using a generalized linear mixed model procedure of SAS (SAS, Institute, Inc., Cary, NC). Comparisons of means were performed using the Tukey-Kramer honestly significance difference test. Significant differences among means were declared at an α of $P \leq 0.05$, and trends declared for P values between 0.05 and 0.10 were discussed.

5.4. RESULTS

5.4.1. Gastrointestinal concentrations of VFA (in vivo)

Pigs fed diets supplemented with flaxseed meal and oat hulls had greater ($P < 0.05$) concentration of ileal acetic acid than those offered the control diet (**Table 5.3**). The concentration of propionic acid was greater ($P < 0.037$) in the ileum of pigs fed the FM diet compared to those fed the control and OH diets. There were no ($P > 0.10$) dietary effects observed on the concentration of butyric and valeric acids in the terminal ileum. The concentrations of acetic and valeric acids were greater ($P < 0.05$) in feces of pigs fed the FM and OH diets than for pigs that consumed the control diet. The concentration of propionic acid in feces of pigs fed the control and FM diets were lower ($P < 0.05$) than in pigs fed the OH diet.

There were no significant dietary effects observed on the concentration of butyric acid in feces. The concentrations of acetic and valeric acid were greater ($P < 0.05$) in fecal contents than in ileal digesta contents among all diets. Although there was no ($P > 0.10$) difference between the ileal and fecal propionic acid in OH diet-fed pigs, pigs fed the control and FM diets had greater ($P < 0.05$) concentrations of propionic acid in feces than in ileal digesta effluent. Similarly, no significant differences were observed between ileal and fecal concentrations of butyric acid for control diet-fed pigs. The ileal concentration of butyrate was lower ($P < 0.05$) compared to the fecal concentration for pigs fed diets containing flaxseed meal and oat hulls.

5.4.2. Production and Absorption of Hindgut VFA (in vivo-in vitro techniques)

The quantity of acetic, propionic and butyric acids produced by *in vitro* fermentation was higher ($P < 0.05$) for the diet containing flaxseed meal compared to the control and OH diets (**Table 5.4**). The quantity of valeric acid produced during *in vitro* fermentation was higher ($P <$

Table 5.3. Normalized ileal and fecal concentrations of VFA determined in pigs fed the control, flaxseed meal and oat hulls diets[1] (n = 8)

Item	Diet[1]			SEM	P^2
	Control	FM	OH		
Determined ileal VFA concentrations (mmol/kg DM intake) (*in vivo*)[3]					
Acetic	2.27[b]	6.29[a]	6.87[a]	0.526	<0.001
Propionic	0.03[b]	1.52[a]	0.11[b]	0.438	0.037
Butyric	1.33	1.86	2.13	0.310	0.171
Valeric	0.07	0.10	0.09	0.017	0.336
Determined fecal VFA concentration (mmol/kg DM intake) (*in vivo*)[4]					
Acetic	7.96[b]	11.42[a]	13.32[a]	0.788	<0.001
Propionic	4.75[b]	5.73[b]	7.02[a]	0.395	0.001
Butyric	3.31	3.53	4.44	0.674	0.281
Valeric	0.52[b]	1.21[a]	1.14[a]	0.175	0.019
Statistical comparison of within diet ileal and fecal VFA concentrations[5]					
P_{Acetic}	<0.001	0.012	0.036		
SEM	0.779	1.861	1.456		
$P_{Propionic}$	0.011	0.024	0.163		
SEM	1.683	0.615	0.580		
$P_{Butyric}$	0.156	0.032	0.045		
SEM	1.123	0.612	0.578		
$P_{Valeric}$	0.042	0.047	0.039		
SEM	0.127	0.229	0.263		

[1]FM = flaxseed meal-containing diet; OH = oat hulls-containing diet.

[2a,b,c]Mean values within a row with unlike superscripts differ ($P < 0.05$). Mean values with their pooled standard errors (n = 8 per treatment).

[3]The ileal concentrations of VFA were determined directly from the digesta contents that were collected through the ileal cannula.

[4]The VFA concentrations were determined from feces that were collected directly through anus.

[5]P_{Acetic}, $P_{propionic}$, $P_{butyric}$, $P_{valeric}$ are the P-values for comparing ileal and fecal acetic, propionic, butyric and valeric acids, respectively.

0.05) for the FM diet than for the control and OH diets. The predicted quantity of acetic and propionic acids produced in the hindgut were greatest ($P < 0.05$) in pigs offered the FM diet, followed by those fed the OH diet and then those fed the control diet. The predicted amounts of butyric and valeric acids produced in the hindgut were greater ($P < 0.05$) in pigs that consumed the FM diet than those fed the control and OH diets. The predicted amounts of acetic, butyric and propionic acids absorbed from the hindgut were highest ($P < 0.05$) for the FM diet, followed by the OH diet, and lowest in the control diet. The predicted quantities of valeric acid absorbed in the hindgut were greater ($P < 0.05$) for the FM diet compared to the control and OH diets. The predicted apparent absorption of acetic acid in the hindgut was highest ($P < 0.05$) in pigs fed the FM diet, followed by the control diet-fed pigs and lowest in those fed the OH diet. The predicted apparent absorption of butyric acid in the hindgut was lowest ($P < 0.05$) in pigs fed the control diet, followed by the OH diet-fed pigs and highest in those fed the FM diet. The predicted apparent absorption of hindgut propionic acid in FM diet-fed pigs were higher ($P < 0.05$) compared to those that consumed the control and OH diets. The predicted apparent absorption of valeric acid in the hindgut of pigs fed the OH diet was lower ($P < 0.05$) compared to those that were offered the control and FM diets.

5.4.3. Digestibility and flows of fatty and bile acids

The AID of SFA and IDF and ATTD of MUFA were greater ($P < 0.05$) for pigs fed the FM and OH diets than for pigs fed the control diet (**Table 5.5**). There were no significant dietary effects observed on AID of CP, NDF and SDF, and ATTD of SDF. The AID of MUFA and TFA and ATTD of TFA in FM diet-fed pigs was lower than in pigs fed OH and control diets ($P < 0.01$). The AID of ADF and ATTD of PUFA were greater ($P < 0.01$) in pigs that consumed the control and OH diets than those fed the FM diet. A tendency was observed in which the AID of TDF ($P =$

Table 5.4. Predicted production and absorption of VFA in the hindgut (in vivo-in vitro methodology) of pigs fed the control, flaxseed meal and oat hulls diets[1] (n = 8)

Item	Control	FM	OH	SEM	P^2
		Diet[1]			
Hindgut VFA produced by *in vitro* fermentation methodology (mmol/kg DM incubated)[3]					
Acetic	2438[c]	4342[a]	2787[b]	79.9	<0.001
Propionic	954[c]	1875[a]	1438[b]	84.1	<0.001
Butyric	202[c]	795[a]	416[b]	15.4	0.034
Valeric	211[b]	604[a]	220[b]	14.9	0.008
Predicted hindgut VFA production (mmol/kg DM intake) (*in vivo-in vitro technique*)[4]					
Acetic	346[c]	759[a]	416[b]	18.0	0.041
Butyric	29[b]	142[a]	61[b]	16.1	0.025
Propionic	135[c]	332[a]	211[b]	12.8	<0.001
Valeric	30[b]	107[a]	35[b]	3.0	0.038
Predicted VFA absorbed from the hindgut (mmol/kg DM intake) (*in vivo-in vitro technique*)[5]					
Acetic	340[c]	751[a]	414[b]	14.7	0.004
Butyric	27[c]	140[a]	60[b]	4.2	<0.001
Propionic	135[c]	330[a]	199[b]	11.6	0.028
Valeric	29[b]	105[a]	32[b]	2.9	0.013
Predicted apparent VFA absorption in the hindgut (%) (*in vivo-in vitro technique*)[6]					
Acetic	97.7[b]	98.4[a]	96.9[c]	0.23	<0.001
Butyric	89.8[c]	97.1[a]	92.9[b]	0.94	0.012
Propionic	96.5[b]	98.2[a]	96.7[b]	0.22	<0.001
Valeric	98.4[a]	98.8[a]	96.6[b]	0.45	0.003

[1]FM = flaxseed meal-containing diet; OH = oat hulls-containing diet.

[2][a,b,c]Mean values within a row with unlike superscripts differ ($P < 0.05$). Mean values with their pooled standard errors (n = 8 per treatment).

[3]The hindgut production of VFA in pigs was determined after *in vitro* fermentation of freeze-dried ileal digesta with fecal inoculum for 36 h at 37°C.

[4]The predicted hindgut production of VFA in pigs was estimated based on the VFA produced after *in vitro* incubation of ileal digesta with a fecal inoculum corrected for the ileal flow of DM.

[5]The quantity of VFA absorbed in the hindgut was obtained after adding the VFA entering (ileal concentrations) and produced (estimated based on *in vitro* incubation of ileal digesta) in the hindgut, and then subtracting the excreted VFA (fecal concentrations).

[6]The apparent absorption in the pig hindgut was calculated based on the ratio between the predicted amount of VFA absorbed from the hindgut and the sum of the VFA entering (ileal concentrations) and produced (predicted based on an *in vitro* assay) in the hindgut.

0.082) in pigs fed the control diet was greater than in that were offered the OH diet. The ATTD of SFA was greater ($P = 0.005$) in pigs fed the OH diet than in pigs that consumed the control and FM diets. The ATTD of IDF ($P < 0.01$) in pigs fed OH diet was lower compared to those fed the control and FM diets. The ATTD of ADF ($P = 0.068$) in pigs fed the control and FM diets tended to be greater than in those offered the OH diet. The ATTD of CP was lower ($P < 0.001$) in pigs fed the FM and OH diets, respectively, than in pigs fed the control diet. The AID of NDF and TDF was greater ($P = 0.001$) in pigs fed the FM diet than in those fed the control and OH diets.

The ileal flows of capric, linoleic, linolenic, eicosadienoic, eicosatrienoic, arachidonic and docosatetraenoic acids were the same ($P > 0.10$) in pigs fed all the diets (**Table 5.6**). The flows of lauric, myristic, margaric, stearic, behenic, lignoceric, palmitoleic, eicosapentanoic and erucic acids in the terminal ileum of pigs fed FM and OH diets were greater ($P < 0.05$) compared to that in pigs fed the control diet. The ileal flows of pentadecyclic ($P = 0.076$), arachidic ($P = 0.089$) and gadoleic ($P = 0.078$) acids in OH diet-fed pigs tended to be greater than in pigs that consumed the control diet. The ileal flow of palmitic acid were greatest ($P = 0.032$) in pigs offered OH diet, followed by those fed the FM diet and then those fed the control diet.

The flows of palmitelaidic and oleic acids were greater ($P < 0.05$) in the terminal ileum of pigs that consumed the FM diet than those fed the control diet. The flows of vaccenic and α-linolenic acids in the terminal ileum of pigs fed the OH and control diets were lower ($P < 0.05$) than in those fed the FM diet. Pigs fed the FM diet tended to have higher ($P = 0.089$) ileal flows of nervonic acid compared to pigs that consumed the control and OH diets. The fecal flow of capric, vaccenic and arachidonic acids were greater ($P < 0.05$) in pigs fed the OH diet than in those fed the control diet (**Table 5.7**). The flows of lauric, pentadecyclic, palmitic, margaric, stearic, lignoceric, palmitoleic, palmitelaidic, oleic, nervonic, linoleic, eicosatrienoic, eicosapentaenoic,

Table 5.5. Apparent ileal and total tract digestibility (%) of dietary components in growing pigs fed the control, flaxseed meal and oat hulls diets[1] (n = 8)

Item	Diet[1]			SEM	*P*
	Control	FM	OH		
Apparent ileal digestibility					
DM	72.4[a]	59.9[b]	58.0[b]	3.10	0.008
CP	78.9	71.0	75.9	2.64	0.140
SFA	77.4[a]	62.1[b]	66.3[b]	3.24	0.001
MUFA	70.4[a]	34.1[c]	55.2[b]	6.99	0.007
PUFA	76.4[a]	64.0[b]	67.8[b]	2.91	0.047
Total FA	89.7[a]	80.1[c]	84.1[b]	1.13	0.017
ADF	4.4[a]	-23.4[b]	11.9[a]	8.61	0.004
NDF	32.8	42.6	34.9	4.42	0.323
Soluble dietary fiber	68.9	65.9	61.0	5.11	0.219
Insoluble dietary fiber	45.5[a]	29.2[b]	27.3[b]	3.10	0.001
Total dietary fiber	48.3[A]	35.5[AB]	28.2[B]	6.37	0.082
Apparent total tract digestibility					
DM	90.6[a]	86.3[b]	75.3[c]	1.55	0.001
CP	90.1[a]	86.1[b]	83.2[c]	0.97	<0.001
Total FA	89.6[a]	72.4[c]	77.2[b]	0.58	<0.001
SFA	67.5[c]	72.6[b]	78.4[a]	1.04	0.005
MUFA	83.0[a]	70.0[b]	75.9[b]	0.22	0.002
PUFA	78.4[a]	57.1[c]	67.2[b]	0.29	0.017
ADF	48.2[A]	42.8[A]	18.7[B]	9.46	0.068
NDF	67.3[b]	80.5[a]	52.6[c]	3.74	<0.001
Soluble dietary fiber	95.8	97.1	95.5	1.17	0.636
Insoluble dietary fiber	74.9[a]	65.4[a]	31.6[b]	4.47	<0.001
Total dietary fiber	67.3[b]	80.5[a]	52.6[c]	3.52	<0.001

[1]FM = flaxseed meal-containing diet; OH = oat hulls-containing diet.

[a,b,c]Mean values within a row with unlike superscripts differ ($P < 0.05$).

docosatetraenoic and osbond acids in feces of OH diet-fed pigs were greater ($P < 0.05$) than in pigs fed the control and FM diets. The fecal flows of myristic, arachidic, behenic and erucic acids were greater ($P = 0.01$) in pigs fed the OH diet, followed by the FM diet-fed pigs and lowest in those fed the control diet. Dietary inclusion of flaxseed meal and oat hulls did not affect ($P > 0.10$) the fecal flows of gadoleic, linolenic and eicosadienoic acids in all pigs. The concentration of chenodeoxycholic + cholic acids, deoxycholic, isodeoxycholic and ursodeoxycholic acids in the terminal ileum of pigs fed the FM and OH diets was higher ($P < 0.05$) than in control diet-fed pigs (**Table 5.8**). The concentration of ileal lithocholic acid in pigs fed the FM diet were higher ($P < 0.05$) than in pigs offered the control diet. The ileal concentrations of secondary and total BA in pigs fed the control diet were lower ($P < 0.01$) compared to those that consumed FM and OH diets. In fecal contents, there were no differences ($P > 0.10$) in the concentration of chenodeoxycholic acid + cholic, deoxycholic and isodeoxycholic acids in pigs fed all the diets. The concentration of lithocholic acid excreted in feces of pigs fed the control and OH diets were lower ($P < 0.001$) than in pigs fed the FM diet. Pigs fed the FM and OH diets excreted more ($P = 0.001$) fecal ursodeoxycholic, secondary and total bile acids compared to those that consumed the control diets.

5.4.4. Digestibility and disappearance of dry matter and energy

The determined AID of DM was greater ($P < 0.05$) for pigs fed the FM and OH diets than for pigs fed the control diet (**Table 5.9**). The determined ATTD of DM ($P < 0.05$) in pigs fed the OH diet was lower compared to those fed the control and FM diets. The predicted ATTD of DM in FM diet-fed pigs was lower ($P < 0.05$) than in pigs fed the control and OH diets. The determined hindgut disappearance of DM was greater ($P < 0.05$) in pigs that consumed the FM diet compared to those that fed the OH and control diets, respectively. A tendency was observed in which the

Table 5.6. Ileal fatty acid flows in growing pigs fed control, flaxseed meal and oat hulls diets[1] (n = 8)

Item	Control	FM	OH	SEM	P
	Diet[1]				
Saturated fatty acids					
Capric (C10:0)	0.3	0.7	0.1	0.31	0.300
Lauric (C12:0)	3.7[b]	10.3[a]	8.2[a]	1.43	0.014
Myristic (C14:0)	22.5[b]	41.6[a]	47.0[a]	6.7	0.043
Pentadecyclic (C15:0)	19.2[B]	31.9[AB]	39.9[A]	6.11	0.076
Palmitic acid (C16:0)	2838[c]	5208[b]	4787[a]	620	0.032
Margaric (C17:0)	36.5[b]	52.4[a]	59.3[a]	4.41	0.005
Stearic (C18:0)	769[b]	1220[a]	1091[ab]	111	0.041
Arachidic (C20:0)	96.2[B]	159[AB]	166[A]	23.22	0.089
Behenic (C22:0)	63.4[b]	126[a]	115[a]	15.67	0.029
Lignoceric (C24:0)	71.4[b]	153.3[a]	138.2[a]	19.22	0.011
Monounsaturated fatty acids					
Palmitoleic (C16:1n-7)	36.9[b]	52.8[a]	61.1[a]	4.91	0.004
Palmitelaidic (C16:1t)	14.2[b]	29.1[a]	21.3[ab]	4.48	0.013
Oleic (C18:1n-9)	6171[b]	9080[a]	8275[ab]	1165	0.007
Vaccenic (C18:1n-7)	-3.7[b]	106[a]	4.2[b]	25.71	0.013
Gadoleic (C20:1)	68.1[B]	110[AB]	135[A]	20.99	0.078
Nervonic (C24:1n-9)	-0.1	3.0	0.1	1.03	0.098
Polyunsaturated fatty acids					
Linoleic (C18:2)	87.6	5831	2531	1869	0.105
Linolenic (C18:3n-6)	10756	14084	16356	2779	0.377
α-Linolenic (C18:3n-3)	521[b]	1975[a]	814[b]	271.0	0.003
Eicosadienoic (C20:2)	11.1	16.9	13.1	2.37	0.224
Eicosatrienoic (C20:3n-3)	-0.2	0.1	1.7	1.03	0.359
Arachidonic (C20:4n-6)	9.00	14.0	14.4	2.67	0.259
Eicosapentaenoic (C20:5n-3)	18.9[b]	40.7[a]	57.1[a]	8.27	0.012
Erucic (C22:1n-9)	5.3[b]	10.3[a]	11.7[a]	1.51	0.016
Docosatetraenoic (C22:4n-6)	18.0	4.6	2.7	10.86	0.534

[1]FM = Flaxseed meal-containing diet; OH = Oat hulls-containing diet.

[a,b,c]Mean values within a row with unlike superscripts differ ($P < 0.05$).

Table 5.7. Fecal fatty acid flows in growing pigs fed control, flaxseed meal and oat hulls diets[1](n = 8)

Item	Diet[1]			SEM	*P*
	Control	FM	OH		
Saturated fatty acids					
Capric (C10:0)	0.4[b]	0.1[ab]	1.2[a]	0.19	0.022
Lauric (C12:0)	5.3[b]	9.8[b]	15.8[a]	1.48	<0.001
Myristic (C14:0)	73[c]	138[b]	219[a]	14.95	<0.001
Pentadecyclic (C15:0)	167[b]	271[b]	481[a]	36.12	<0.001
Palmitic acid (C16:0)	1351[b]	1850[b]	3704[a]	242.11	<0.001
Margaric (C17:0)	98[b]	144[b]	270[a]	21.42	<0.001
Stearic (C18:0)	5059[b]	7252[b]	13081[a]	754	<0.001
Arachidic (C20:0)	97[c]	146[b]	228[a]	13.53	<0.001
Behenic (C22:0)	36.9[c]	58.8[b]	95.2[a]	5.46	<0.001
Lignoceric (C24:0)	43.6[b]	62.0[b]	113.8[a]	6.82	<0.001
Monounsaturated fatty acids					
Palmitoleic (C16:1*n*-7)	1.4[b]	2.2[b]	3.8[a]	0.35	<0.001
Palmitelaidic (C16:1t)	2.4[b]	3.7[b]	5.6[a]	0.60	0.004
Oleic (C18:1*n*-9)	1101[b]	1615[b]	3119[a]	235.26	<0.001
Vaccenic (C18:1*n*-7)	101[b]	229[ab]	375[a]	54.56	0.010
Gadoleic (C20:1)	11.1	9.4	18.1	3.87	0.278
Nervonic (C24:1n-9)	2.0[b]	3.2[b]	6.5[a]	0.63	<0.001
Polyunsaturated fatty acids					
Linoleic (C18:2)	193[b]	338[b]	544[a]	50.81	<0.001
Linolenic (C18:3*n*-6)	-0.2	7.3	0.4	3.75	0.298
α-Linolenic (C18:3*n*-3)	24.6[b]	89.2[a]	42.2[b]	6.82	<0.001
Eicosadienoic (C20:2)	1.5	0.8	1.8	0.59	0.463
Eicosatrienoic (20:3*n*-3)	0.2[b]	0.4[b]	1.9[a]	0.46	0.039
Arachidonic (C20:4*n*-6)	1.3[b]	1.9[ab]	2.8[a]	0.30	0.014
Eicosapentaenoic (C20:5*n*-3)	1.3[b]	2.1[b]	4.1[a]	0.47	0.001
Erucic (C22:1*n*-9)	2.4[c]	5.7[b]	9.3[a]	0.99	<0.001
Docosatetraenoic (C22:4*n*-6)	60[b]	86[b]	131[a]	13.56	0.007
Osbond (C22:5n-6)	16.7[b]	13.3[b]	39.1[a]	6.15	0.015

[1]FM = Flaxseed meal-containing diet; OH = Oat hulls-containing diet.

[a,b,c]Mean values within a row with unlike superscripts differ ($P < 0.05$).

predicted hindgut DM disappearance in pigs fed diets supplemented with flaxseed meal and oat hulls was greater ($P = 0.06$) than in pigs fed the control diet.

The quantity of digested energy in the stomach and small intestines was reduced ($P < 0.05$) more in pigs fed the OH diet than in pigs fed the FM diet. The quantity of digested energy was higher ($P < 0.05$) in pigs fed the FM diet compared to pigs fed the control and OH diets. The amount of energy produced from VFA by fermentation was lower ($P < 0.05$) in pigs fed the OH and control diets, respectively, when compared with those fed the FM diet. Energy excreted in fecal VFA of pigs fed the OH diets was higher ($P < 0.05$) than in control diet-fed pigs and that excreted in that consumed the FM diet was intermediate. The energy absorbed from hindgut VFA was higher ($P < 0.05$) in pigs fed the FM diet compared to other diets. The contribution of VFA from fermentation to the total tract digestible energy was greatest in FM diet-fed pigs.

5.5. DISCUSSION

Different DF sources are fermented at different rates and the solubility of fiber is important in determining the nutrient flows and quantity of VFA produced or absorbed in the GIT. The increases observed in the normalized concentrations of ileal or fecal acetic and propionic acids or that of fecal valeric acid in pigs fed the FM and OH diets in this study indicates that the addition of fiber-rich ingredients stimulate gastrointestinal fermentation (Chapter 4). The greater normalized concentrations of fecal VFA than ileal digesta concentrations supports the notion that microbiota composition and activities are more pronounced in the hindgut compared to the upper gastrointestinal segments (Jarwoski and Stein, 2016). Supporting this suggestion are the observations in Chapter 4 that species diversity and richness were pronounced more in the cecum compared to the ileum. Although significant differences between ileal digesta and fecal normalized VFA concentrations were noticed, it is important to note that these concentrations reflect a net

Table 5.8. Normalized concentrations (mg/g DMI) of bile acids in ileal and fecal contents of growing pigs fed control, flaxseed meal and oat hulls diets[1] (n = 8)

Item	Control	FM	OH	SEM	P
		Diet[1]			
Terminal ileum					
Chenodeoxycholic + cholic acid	11.8[b]	23.0[a]	22.6[a]	1.59	<0.001
Deoxycholic acid	2.3[b]	4.4[a]	4.0[a]	0.42	0.032
Isodeoxycholic acid	0.3[b]	0.5[a]	0.5[a]	0.05	0.002
Lithocholic acid	0.2[b]	0.4[a]	0.4[ab]	0.06	0.039
Ursodeoxycholic	1.7[b]	3.6[a]	4.1[a]	0.34	<0.001
Secondary bile acids[2]	4.9[b]	8.9[a]	8.9[a]	0.78	0.002
Total bile acids[3]	16.8[b]	31.9[a]	31.5[a]	2.26	<0.001
Feces					
Chenodeoxycholic + cholic acid	0.03	0.05	0.08	0.02	0.150
Deoxycholic acid	0.1	0. 1	0.3	0.06	0.120
Isodeoxycholic acid	0.03	0.1	0.04	0.01	0.605
Lithocholic acid	0.3[b]	0.8[a]	0.3[b]	0.05	<0.001
Ursodeoxycholic acid	0.6[b]	1.7[a]	1.4[a]	0.19	0.001
Secondary bile acids[2]	1.1[b]	2.1[a]	2.0[a]	0.27	0.001
Total bile acids[3]	1.1[b]	2.7[a]	2.1[a]	0.26	0.001

[1]FM = Flaxseed meal-containing diet; OH = Oat hulls-containing diet.

[2]Calculated as: deoxycholic + isodeoxycholic + lithocholic + ursodeoxycholic acid.

[3]Calculated as: chenodeoxycholic + cholic + deoxycholic + isodeoxycholic + lithocholic + ursodeoxycholic acid.

[a,b,c]Mean values within a row with unlike superscripts differ ($P < 0.05$).

balance of production and absorption (Montoya et al., 2016). Thus, VFA concentrations are more accurate measures of unabsorbed VFA than VFA production per se and according to our assumption in Chapter 4 using these values to describe VFA absorption can be misleading (Montoya et al., 2016).

Findings from this study are supported by reports by McNeil et al. (1978), Cummings and Macfarlane (1991), Topping and Clifton (2001) and Montoya et al. (2016), all whom endorsed that gastrointestinal flows and concentrations of VFA are not accurate descriptors of VFA production. The higher quantity of acetic, propionic and butyric acids produced by *in vitro* fermentation observed for diets supplemented with flaxseed meal and oat hulls diets, compared to the control diet, may be attributed to the presence of fermentable fiber fractions that acted as substrate for microbial activity. As hypothesized, the differences in the solubility or composition of DF or NSP components between flaxseed meal and oat hulls, led to production of greater amounts of acetic, propionic and butyric acids by *in vitro* fermentation in FM diet-fed pigs compared to OH diet-fed pigs (Chapter 4). Soluble DF is fermented faster and more extensively by gastrointestinal microbial communities than insoluble DF (Bach Knudsen, 1997; Jha et al., 2011). Moreover, it is well-established that flaxseed meal supplementation promotes propionic acid production (Soder et al., 2012; Lagkouvardos et al., 2015) by altering intestinal BA profiles that shape microbiota compositions (Chapter 4). Reinforcing this idea is the observation in Chapter 4 that that respective increases in *lactobacilli* and *streptococci* ssp. promotes cross-feeding thereby creating room for members of *Veillonellaceae* families to increase propionic acid production in pigs fed flaxseed meal-containing diets. In the study described in Chapter 4, we also observed that the presence of *Veillonellaceae* is also strongly associated with acetic acid content in the cecum of pigs fed flaxseed meal-containing diets.

Table 5.9. Determined and predicted digestibility of dry matter (DM), digested, excreted, absorbed, and available energy in gastrointestinal segments of growing pigs fed the control, flaxseed meal and oat hulls diets[1] (n = 8)

Item	Diet[1]			SEM	P[2]
	Control	FM	OH		
Determined and predicted DM digestibility[3]					
Determined AID, % (*in vivo*)	72.4[a]	59.9[b]	58.0[b]	3.10	0.008
Determined ATTD, % (*in vivo*)	90.6[a]	86.3[b]	83.2[c]	1.55	0.001
Predicted ATTD, % (*in vivo-in vitro*)	91.2[a]	88.6[a]	80.5[b]	1.05	0.002
Determined and predicted hindgut DM disappearance					
Determined disappearance, % (*in vivo*)	29.3[c]	39.1[a]	35.2[b]	1.12	0.035
Predicted disappearance, % (*in vivo-in vitro*)	20.1[B]	33.1[A]	28.3[A]	3.06	0.060
Digested energy, kcal/kg DM feed					
Stomach and small intestines	3178.4[a]	2805.7[b]	2497.7[c]	60.68	<0.001
Cecum and Colon	385.9[b]	873.2[a]	386.9[b]	47.73	0.001
Energy produced from VFA	156.3[c]	427.8[a]	215.9[b]	8.68	<0.001
In vitro production from ileal digesta contents, kcal/kg DM feed					
Fecal excretion	5.45[b]	7.04[ab]	8.32[a]	0.687	0.023
Quantity absorbed	154.9[c]	417.9[a]	212.2[b]	7.075	<0.001
Available energy from fermentation[5], %	4.5[c]	14.5[a]	7.8[b]	0.426	0.011
Comparison of determined and predicted DM digestibility and disappearance					
P_{ATTD}	0.013	0.082	0.011		
SEM	5.67	7.12	5.45		
$P_{\text{Hindgut disappearance}}$	0.841	0.101	0.213		
SEM	7.16	1.73	4.24		

[1]FM = Flaxseed meal-containing diet; OH = Oat hulls-containing diet.

[2] [a,b,c]Mean values within a row with unlike superscripts differ ($P < 0.05$). [A,B,C]Mean values within a row with unlike superscripts tended to differ ($0.05 < P < 0.10$).

[3]AID = Apparent ileal digestibility; ATTD = Apparent total tract digestibility; VFA = volatile fatty acids.; SEM Standard error of means.

The higher production of valeric acid during *in vitro* fermentation observed in the present study exclusively indicates a great extent of protein fermentation (Rasmussen et al., 1988). Supporting this notion are the depressed values of protein digestibility observed in pigs fed flaxseed meal compared to those fed oat hulls and increases in digesta viscosity in pigs fed flaxseed reported by Kiarie et al. (2007). The mucilaginous NSP fractions in flaxseed increase digesta viscosity, and consequently impairing nutrient absorption in the small intestines (Bhatty 1993; Kiarie et al., 2007). This implies that there is an increase in the ileal flow of AA in pigs fed the FM diet that are available for promoting hindgut valeric acid production compared to the control and OH diets. As a consequence, insoluble DF components from oat hulls are less fermentable compared to soluble DF components from flaxseed meal. The increase in production of valeric acid during *in vitro* concurs with findings from Chapter 4 that flaxseed meal supplementation tended to increase cecal and colonic valeric concentrations compared to oat hulls.

The higher determined and predicted hindgut butyric acid production in FM diets than other diets can be ascribed to the presence of polysaccharides composed of xylose, arabinose, rhamnose, and fucose that act as substrates for butyric acid–producing bacteria (Ding et al., 2015). It is important to note that there was a similar trend between hindgut VFA produced by *in vitro* fermentation and predicted hindgut VFA production among all diets, except for butyrate acid in OH diets. In this regard, the amount of butyric acid produced by *in vitro* fermentation was greater for the OH diet compared to the control diet, but the predicted hindgut butyric acid was similar for the two diets. This phenomenon is difficult to explain using variables measured in this experiment. Furthermore, this paradox is exacerbated by our previous observations reported in Chapter 4 that well-known butyric acid-producing Firmicutes in the intestines of FM diet-fed pigs were underpopulated compared to the control and OH diets-fed pigs. Jha et al. (2011) cautioned that

some differences in the end-products of fermentation within certain DF sources cannot be explained. This could be ascribed to the interaction between the compositions of the substrate, inoculum and the *in vitro* model itself (Jha et al., 2011). Another reason could be that the *in vivo-in vitro* methodology does not account for changes that occur in microbiota compositions and passage rate that assumed to be equal for all diets.

Immediately after fermentation, an unknown portion of VFA is utilized by microbes, another is utilized by colonocytes or absorbed into the blood stream and then the remainder is excreted. As illustrated ('?' in **Figure 5.2**) the *in vivo-in vitro* model does not account for VFA production and absorption in the upper gastrointestinal segments. However, although it takes into consideration the net result of VFA production and absorption in the upper gut, it is assumed that the quantities of VFA produced and absorbed are relatively low (Montoya et al., 2016). The higher estimates of the quantities of absorbed VFA in high-fiber diets may be attributed to the increase in the VFA production. Furthermore, the differences in the quantities of VFA absorbed between FM and OH diets suggest further strengthens the notion proposed in Chapter 4 that flaxseed meal and oat hulls induce variable effects on the GIT development and VFA absorption capacity. Our data also indicates that the predicted VFA absorption in the hindgut is also influenced by the DF source and inclusion level. The predicted extents of VFA absorption in the present study ranged between 89.8 and 98.8%. These values are in accordance with a plethora of findings that at least 90 and 95% of the produced VFA are rapidly absorbed by colonocytes in pigs and humans, respectively (Topping and Clifton, 2001; Montoya et al., 2016).

The observation that addition of flaxseed meal and oat hulls in pig diets reduced the determined AID and ATTD of DM was probably due to the presence of DF which physically reduced the enzymatic digestion of nutrients in the upper gut and microbial degradation of

intestinal contents that escape digestion by endogenous enzymes. Interestingly, the determined ATTD of DM was reduced more in pigs fed oat hulls compared to those fed flaxseed meal. The differences in the extent to which the determined ATTD of DM was depressed between FM and OH diets support the assertion that variability in the monomeric compositions of DF components in these co-products. The fibrous ingredients could have induced variable effects in the development of the GIT (Chapter 4) and digestion processes (Montagne et al., 2003).

The greater AID of ADF in the ileum of pigs fed the OH diet than those fed the FM diet was, however, unexpected, because it is well-established that insoluble DF is less fermentable, especially in the small intestines than soluble fiber (Jiménez-Moreno et al., 2009). Considering that insoluble fiber from oat hulls is associated with gastric secretions, these observations also suggest that there could be other low molecular weight degradable digesta contents from endogenous secretions or non-fibrous components of the diet that are substrates for microflora. It is also well-established that DF fractions have different fermentability capacities depending on the source of DF and the region of the GIT (Canibe and Bach-Knudsen, 2002; Ndou et al., 2015; Jaworski and Stein, 2017). However, the increase in fermentability of ADF in pigs fed the OH diet explains the finding observed in Chapter 4 that the production of acetate was greater in pigs supplemented with oat hulls compared to pigs fed flaxseed meal.

Although there were no dietary effects on ileal digestibility of CP, the observation that addition of flaxseed meal and oat hulls reduced AID of MUFA, SFA, PUFA and TFA intake may be ascribed to the fact that the presence of DF could have induced sub-optimal absorption of added lipids predisposed by encapsulation of FA within the DF matrices or by deconjugation of BA. The high DF content and WHC of non-starch polysaccharides in flaxseed meal and oat hulls may also bind some of the BA present in the digesta contents, thereby reducing emulsification and
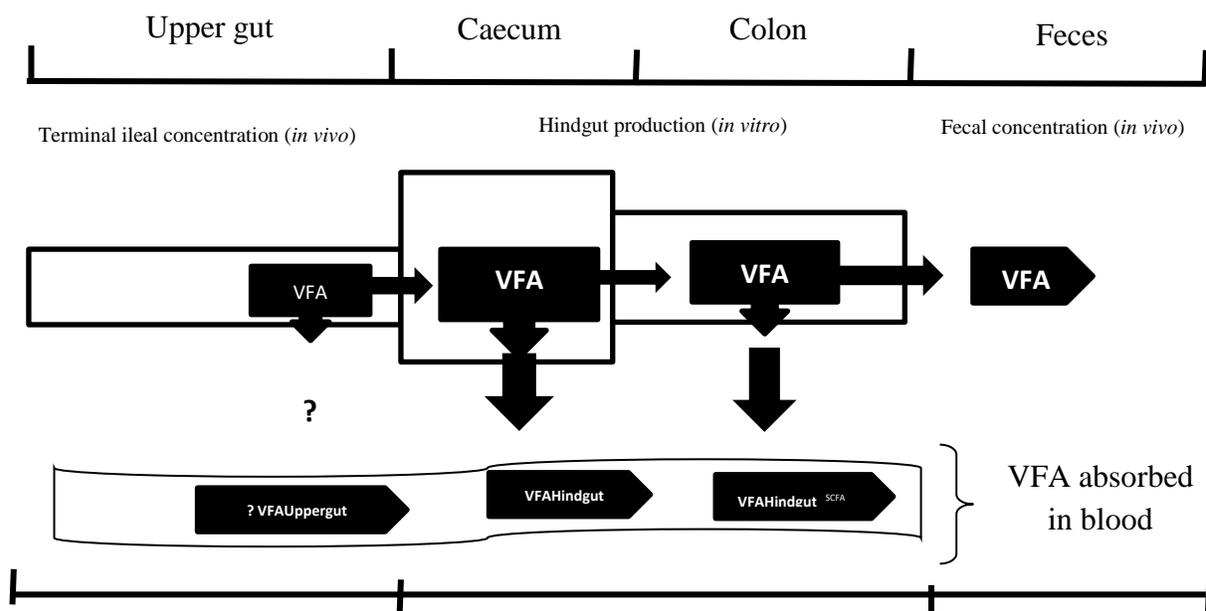
Figure 5.2. Principle of the in vivo-in vitro methodology to determine production and absorption of VFA in the caecum and colon. The '?' represents the VFA absorbed in the foregut, which were not measured in the current study (modifications of schematic diagram reported by Montoya et al. (2016).

absorption of dietary lipids (Ferrebee and Dawson, 2015). Supporting these postulations are the increase in the ileal flows of BA, FA and the low digestibility values or fermentability of DF fractions in the upper gastrointestinal tract. Moreover, the observation that flaxseed meal supplementation reduced the digestibility of TFA and fermentability of ADF more than oat hulls further supports the postulation raised in Chapter 4 that these DF sources behave differently during transit in the GIT. This implies that, as a well-known rich source of soluble and mucilaginous NSP with high swelling and water holding capacity, flaxseed meal might have increased digesta viscosity, thereby preventing nutrient absorption (Bhatty, 1993; Takahashi et al., 2009; Kiarie et al., 2007). These findings also concur with our observation in the previous experiment in Chapter 4 that feed efficiency was reduced more in pigs fed the FM diet compared to those fed the control and OH diets. Conversely, oat hulls increased the insoluble DF content, which in turn reduces transit time of intraluminal contents of small intestines (Jiménez-Moreno et al., 2009), thereby reducing FA absorption observed in OH diets-fed pigs. However, we did not measure passage rate in the current study.

Another plausible explanation for the reduction in digestibility of TFA in pigs fed flaxseed meal and oat hulls is that DF from these co-products increase the substrate for microbial activity which facilitates deconjugation of BA and reduce their ability to solubilize and emulsify dietary lipids (Ahn et al., 2003). The deconjugated BA are then bound to bacterial cells and DF, thereby increasing their excretion as well as that of lipids. Reinforcing this are the greater ileal and fecal flows of BA and FA in pigs fed FM and OH diets compared to those fed the control diet in the current study. Therefore, high viscosity of digesta which is likely to have been induced by soluble fiber in pigs fed the FM diet hampered diffusion of lipid micelles in the small intestine thereby further reducing FA absorption (Bhatty, 1993; Kiarie et al., 2007). Additionally, insoluble DF from

oat hulls could have indirectly reduced transit time of intraluminal contents of GIT (Jiménez-Moreno et al., 2009), thereby reducing digestibility of nutrients in the stomach and small intestines.

A similar trend was observed between AID and ATTD of TFA in which case pigs fed the FM diet had the lowest digestibility values than those fed the OH diets was in line with findings in Chapter 4 that fecal digestibility of crude fat was lower in pigs fed oat hulls compared to pigs fed flaxseed meal. However, it is intriguing that when compared with the control diet, the ATTD of TFA was lower than the AID of TFA among the FM and OH diets. By extrapolation, with reference to the control diet, the AID of TFA in FM and OH diets were reduced by 9.6 and 5.7%, respectively. In the feces, digestibility of TFA was reduced by 17.3 and 10.5% in FM and OH diets, respectively. At the same time, the quantity of BA excreted in the terminal ileum and feces was more than 50% among FM and OH diets. The values are in agreement with peculiar findings from previous research that total tract digestibility of fat was more negative (-54%) than ileal fat digestibility (-19%) in pigs (Graham et al., 1986). Moreover, more fascinating observations which also coincided with our findings revealed that fermentation increased fecal fat excretion by 47% and fecal BA by 35% compared with the control group in humans (Judd and Truswell, 1981). Therefore, it is highly likely that the decrease in digestibility of FA reported in this experiment are attributable to not only deconjugation of BA but also to the microbial production of FA in the hindgut of pigs fed FM and OH diets. Supporting this assertion are the increased values of fecal flows than that of ileal flows of FA in pigs fed the FM and OH diets which confirm that microbial activity are a major contributory factor in elevating fecal fat in pigs fed high-fiber diets. The contribution of endogenous secretions of FA from the host to the gastrointestinal flows of FA should also be given a careful consideration.

Apart from their role as detergents to facilitate digestion and absorption of fats in the GIT, BA are not only detergents that emulsify fats but are signaling molecules that also regulate the farnesoid X receptor (FXR) and in turn alter lipid, glucose and energy metabolism (Marcil et al., 2002; Ferrebee and Dawson, 2015; Nie et al., 2015). The increase in the flows of chenodeoxycholic and cholic acids in the terminal ileum of FM and OH diets-fed pigs could be ascribed to the dilution of these primary BA by the non-digestible fibers (Chapter 4). Although no diet-induced effects were observed on the fecal flow of primary BA, deoxycholic acid and isodeoxycholic acids, differences in NSP composition between the flaxseed meal and oat hulls could explain why lithocholic acid was excreted more in pigs fed oat hulls compared to those that consumed flaxseed meal. This observation is in agreement with findings in the previous experiment that hypocholesterolemia was distinctively pronounced more in OH diet-fed pigs than in FM diet-fed pigs (Chapter 4). Furthermore, oat hulls are rich sources of insoluble DF that are highly lignified (Bach Knudsen, 1997), and lignin acts as resins or a bile salt-sequestrating agents by reducing reabsorption of gastrointestinal BA (Fardet, 2010). The lack of dietary effects in the flow of deoxycholic and isodeoxycholic acids in feces cannot be explained by measurements in the present experiment. However, similarities in chenodeoxycholic and cholic acids among all diets could be ascribed to that almost 95% of are rechanneled back to the liver through the enterohepatic circulatory system (Graffner et al., 2016). Another plausible explanation which is supported by findings from our previous study (Ndou et al., 2017) is the observation that fecal flows of ursodeoxycholic acids increased and this is can be ascribed to microbial activity that promoted production of secondary BA (Stevens and Hume, 1998; Ahn et al., 2003). Mechanisms by which primary BA are transformed into their secondary forms are through gut microbial 7α-

dihydroxylation and 7α/β-epimerization (Stevens and Hume, 1998; Ahn et al., 2003; Wahlstrom et al., 2016).

A plethora of treatment-induced differences were observed on the ileal flows of pentadecyclic, palmitelaidic, vaccenic, gadoleic, nervonic, and α-linolenic acids and almost all the fecal flows of FA except gadoleic, linoleic and eicosadienoic acids between FM and OH diets-fed pigs. The differences in the flows of these gastrointestinal FA observations suggests that soluble and insoluble DF induce variable effects on the ability of gut microbiota to alter changes in FA profiles in the GIT. The increase in the ileal flow of n-3 FA such as α-Linolenic and eicosapentaenoic acids observed in the current study is in agreement with findings reported by Martínez-Ramírez et al. (2013) and indicates a likelihood of activity of the Δ6- and Δ5-desaturases and chain-elongases in the upper sections of the GIT of the pig. The increase in the ileal and fecal flows of PUFA such as eicosadienoic, eicosatrienoic, arachidonic, erucic, docosatetraenoic and osbond that were virtually absent in the diets can also suggest that microbial fermentation in different regions of the GIT contributes to the proportion of unsaturated FA in the digesta of non-ruminant animals (Martínez-Ramírez et al. 2013). Therefore, the presence of long-chain unsaturated FA in gastrointestinal contents can be used as reliable indicators to assess the effects of feeding DF and its fermentability on lipid metabolism (Santas et al., 2012). These findings also indicate that endogenous secretions of FA from the host into gastrointestinal contents might have contributed to the ileal and fecal flows of these FA.

The increase in DF content resulted in the reduction in ileal digestible energy in FM and OH diets. The quantity of ileal digested energy was, however, reduced more in pigs fed the OH diet compared with those fed the FM diet indicating that different DF sources influence nutrient utilization depending on their physicochemical properties (Ndou et al. 2013ab; Ndou et al., 2015).

Conversely, the increased digested energy in the cecum and colon of pigs fed the FM diet may have been due to an increase in the flow of soluble DF and other dietary components as a consequent of increased in digesta viscosity. Furthermore, the similarities in the digested energy in the cecum and colon between pigs fed the control diet and the OH diet was, however, unexpected because oat hulls increased DF flow in the hindgut. The observation that addition of flaxseed meal increased hindgut energy production concurs with results of Iyayi and Adeola (2015) and confirms that VFA produced from hindgut fermentation contribute to metabolizable energy. The observation that the quantity of energy digested and produced from VFA in different sections of the GIT was variable among the diets also supports the postulation by Ndou et al. (2013b) that fibrous ingredients behave differently in different segments of the GIT depending on their bulking properties.

The percentage contribution of VFA produced from fermentation in the cecum and colon to the sum of digestible energy in the entire GIT was 14.5% in FM diet-fed pigs and greater than 4.5 and 7.8%, respectively for the control and OH diets, respectively. Despite the similarities in the DF level in pigs fed flaxseed and oat hulls, the differences in the solubility of these fibrous feedstuffs and their constituent DF fractions are responsible for differences in the percentage contributions. It is also intriguing to note that although the DF content of high-fiber diets in the current study was greater than 11.0% (TDF) in Christensen et al. (1999) and similar to 24% (TDF) in Anguita et al. (2006) and 14.7% (NDF) in Iyayi and Adeola (2015) but the percentage contributions of energy from hindgut VFA fermentation in the current study were lower than in these studies. The lower values reported in the present study than those of the above authors can be ascribed to the differences in the physicochemical properties that influence fermentability. However, these observations collectively agree with the report by Jensen (2001) that the energy

produced from fermentation by gastrointestinal microbiota contributes between 2.4 and 29.5% of the total energy available to pigs.

It is also of interest to note that the ATTD of the sum of FA in pigs fed the FM diet in the current experiment was lower than ATTD of crude fat observed in previous experiment in Chapter 4, for a similar diet. A similar trend was also reported for other diets by Jorgensen et al. (1992, 2000), Duran-Montage et al. (2007) and Martinez-Ramirez et al. (2013) and these findings including ours indicated that other fat-soluble components might have lower digestibility than FA. Another reason might be related to the difference in the methodology used to quantify the lipid content of fecal samples. This suggests that FA extraction might have been more efficient than the crude fat extraction. More work is needed to compare the effects of different methods of lipid extraction (including ether extract or crude fat with hexane, acid-hydrolyzed fat extract or fatty acids extraction using menthol and chloroform mixtures).

In conclusion, dietary supplementation with flaxseed meal reduced ileal digested energy at a lesser extent than oat hulls. Addition of flaxseed meal in pig diets caused a corresponding increase in hindgut digested energy and energy from VFA production in the hindgut compared with oat hulls. Addition of flaxseed meal and oat hulls in pig diets reduced FA digestibility, increased GI flows of FA and excretion of BA. Dietary supplementation with flaxseed meal and oat hulls induced variable effects on digestibility of DF fractions and fecal flows of unsaturated FA. Future studies are needed to estimate absorption capacity and gene expression of transporters VFA absorption and metabolism. Different fiber sources are fermented at different rates and the solubility of DF is important in determining fermentability of DF and the quantity of VFA produced and absorbed.

LIMITATIONS AND IMPLICATIONS OF CHAPTER 5

First limitation observed in Chapter 5 was that although ileal and cecal cannulated pig model assisted us to determine the diet-induced effects on the flow of FA and BA in the terminal ileum as well as in the feces, it is difficult to identify the actual source of lipids excreted either in the terminal ileum or feces. Therefore, in Chapter 6 we introduced the ileal- and cecal-cannulated pig model so that we can assess the pigs' responses to DF and lipid supplementation in different compartments of the hindgut (cecum and colon).

Another major limitation of concern is that the fibrous co-products used in Chapter 4 and 5 contained lipids in the form of free fat and triacylglycerols (neutral fats) and phospholipids embedded within their fiber matrices. Furthermore, the differences in the lipid compositions among these fibrous co-products inevitably resulted in variation in the inclusion level supplemental fat and FA profiles of the diets in Chapter 5. Besides this, the corn and soybean meal used in diets fed to pigs in Chapter 4 and 5 contained DF and it was difficult to balance their ratios across all diets. The presence of lipid residues within flaxseed and oat hulls and that of DF fractions from corn and soybean meal are likely to have imposed some confounding effects on responses to the interaction between fat and DF observed in Chapter 4 and 5. Thus, another unique aspect of Chapter 6 is that ileal- and cecal-cannulated pigs were fed diets containing pectin and cellulose as the only sources of soluble and insoluble DF, respectively, to replace flaxseed meal and oat hulls, respectively. In this regard, we postulated that the use of purified cellulose and pectin as well-characterized source of lipid-free DF in Chapter 6 will open way for investigating the interaction between dietary fiber and lipid saturation by eliminating the confounding effects that could have been induced by the fiber matrices-bound lipids in flaxseed and oat hulls used in Chapter 4 and 5. At the same time, beef tallow or corn oil were used as the only sources of dietary lipids and

compared in Chapter 6 to investigate if the variation in FA profile or FA saturation influence FA absorption and DF fermentability as postulated in Chapter 5.

Together with hemicellulose, pectin and cellulose are the main constituents of plant cell wall polysaccharides that represent the major DF components within most co-products including flaxseed meal and oat hulls. Thus, the use of these two fibrous co-products would also provide a closer to practical dietary intervention that would be feasible for understanding the fundamental principle behind the role played by added fat during the interaction between DF and added lipid sources. This eliminates the confounding effects that may be imposed by lipids that are within the plant cell walls.

In Chapter 4 we observed that a diversity in the structural and functional microbiota composition among the ileum and cecum as well as between digesta contents and mucosa within each of these segments. Furthermore, previous studies using *in vitro* assays also illustrated that source of inoculum influences fermentability (Coles et al., 2005; Awati et al., 2006; Pecka-Kielb et al., 2016). The major limitation of the *in vivo-in vitro* techniques used in Chapter 5 is that we used fecal inoculum to ferment ileal digesta to imitate hindgut fermentation and assuming that digesta contents and microbial communities in the cecum and colon are uniform. However, it is well-established that DF fractions and fermentability differ from cecum to colon (Jarwoski and Stein, 2016) and the microbiota composition between the cecum and colon are also different. Therefore, the values of predicted hindgut VFA production and absorbtion reported in Chapter 5 using ileal digesta and fecal inoculum may not represent the best predictions of the quantity of VFA produced or absorbed exclusively in the cecum or colon and may lead to misleading conclusions. Thus, as illustrated in Figure 6.1, the ileal and cecal cannulated pig model used in Chapter 6 opened a way for collecting cecal digesta that was used to inoculate freeze-dried ileal

digesta to simulate fermentation in the cecum in the *in vivo-in vitro* fermentation technique. This model also create an opportunity to collect cecal digesta that was used as a subtrate for *in vitro* fermentation using fecal inoculum to simulate fermentation in the colon. The use of the ileal- and cecal-cannulated pig model in Chapter 6 also made it possible for different aspects of digestibility of dietary components, DF fermentability and FA flows to be investigated by sampling feces, and ileal and cecal digesta separately over a period of 12 h.

CHAPTER SIX

MANUSCRIPT 3

Interactive effects of dietary fiber and lipid type on fiber digestibility, gastrointestinal fatty acids flows, and predicted production and absorption of volatile fatty acids in the cecum and colon of growing pigs[1]

Author's contributions

The authors' contributions are as follows: S.P.N., E.K., C.F.M.dL. (Deceased) and C.M.N. designed the study. S.P.N conducted the animal study, the statistical analysis and wrote the manuscripts. E.K., M.C.W., N.A., and C.M.N. critically reviewed the manuscripts. C.M.N. was the principal investigator who supervised all aspects of the study. All the authors reviewed and approved the manuscripts.

6.1. ABSTRACT

The present study investigated the interactive effects of dietary fiber (DF) and lipid types on fermentability of DF and digestibility, and flows of fatty acids (FA) and production and absorption of volatile fatty acids (VFA) in the cecum and colon of growing pigs. Eight ileal and cecal cannulated Yorkshire barrows were fed either pectin- or cellulose-containing diets that were supplemented with either corn oil or beef tallow. Pigs were allocated diets in two indepentent Youden Latin squares with a 2×2 factorial arrangement of treatments, to give eight replicates per treatment. Ileal and cecal digesta, and feces were collected, analyzed for VFA content, freeze-dried and subsampled for DF and FA analysis. The other subsample was subjected to *in vitro* fermentation using inoculum from fresh cecal digesta or feces. The predicted amounts of VFA produced and absorbed in the cecum and colon were higher ($P < 0.05$) in pigs fed soluble fiber compared to those offered insoluble fiber. Supplementation with beef tallow depressed the determined ileal and predicted cecal fermentability of organic matter (OM) and acetic and propionic acids production ($P < 0.001$) in the colon more in pigs fed soluble fiber compared to insoluble fiber-fed pigs. There was an interaction ($P < 0.05$) between DF and lipid type on apparent ileal (AID) and total tract (ATTD) digestibility of DM, AID, apparent cecal digestibility (ACD) and ATTD of insoluble and total DF. In this regard, AID of DM, saturated and polyunsaturated fatty acids, and AID of soluble, insoluble and total DF in pectin fed with beef tallow showed higher ($P < 0.05$) fermentability than when fed with corn oil. The ACD of soluble DF was greater ($P < 0.001$) in corn oil-fed pigs compared with beef tallow-fed pigs. The ACD and ATTD of insoluble and total DF, and faecal flows of capric, palmitelaidic, palmitoleic and dihomo-γ-linolenic acids in pectin-containing diets were greater ($P < 0.05$) than in cellulose-containing diets. In conclusion, the predicted quantity of VFA produced and absorbed, and fermentability of OM in the cecum and

colon is greater in pigs fed soluble fiber from pectin diets compared with those fed on insoluble fiber from cellulose, and beef tallow supplementation depress fermentation. The interaction between DF solubility and the degree of saturation of dietary FA modulates digestibility of lipids, FA flows and fermentability of DF fractions but differs for soluble and insoluble fiber sources, saturated and unsaturated fatty acids and varies in different gastrointestinal segments.

Keywords: cellulose, hindgut fermentation, short chain fatty acids, pectin, pigs.

6.2. INTRODUCTION

Low-cost agro-industrial co-products are increasingly being used in pig diets resulting in increased dietary fiber (**DF**) content. High-fiber diets are supplemented with lipids to meet the energy requirement for a nutritionally-balanced diet for pigs. A common assumption is that growth performance in pigs fed nutritionally-balanced high-fiber diets that are supplemented with added lipids will not decline. However, previous studies including our observation in pigs fed soluble DF-rich diets in Chapter 4 have reported poor performance and fat digestibility in pigs fed fibrous diets based on co-products plus supplementary fat (Bakker, 1996; O'Doherty et al., 2002; Ball et al., 2010).

Fat digestibility was reduced and intestinal FA and BA flows were increased in pigs fed fibrous co-products in Chapter 5, yet diets were formulated to be nutritionally-balanced. This paradoxical relationship could be ascribed to overestimation of the caloric values of added lipids or imply that understanding of the interaction between DF solubility and lipid types in pigs is far from complete. Furthermore, agro-industrial co-products used elsewhere including those used in Chapter 4 and 5 may contain triacylglycerols (neutral fats) and phospholipids embedded within their fiber matrices. The fiber-bound lipids resulted in different dietary fat content and FA profiles

of diets used in studies in Chapters 4 and 5. This could have inevitably imposed confounding effects that makes it difficult to understand the role played by added fats. Thus, for this experiment we suggested that the use of purified sources of DF such as cellulose and pectin, that are lipid-free and well-characterized models for insoluble DF (**IDF**) and soluble DF (**SDF**) (Bach Knudsen, 1997; 2001; Gutierrez et al., 2013), respectively, to replace flaxseed meal and oats hulls in Chapter 4 and 5. The common sources of supplementary lipids in pig diets include animal fats such as beef tallow, that are rich in saturated fatty acids (**SFA**) or plant oils such as corn oil, that are rich in unsaturated fatty acids (**UFA**) (Ravindran et al., 2016). Therefore, beef tallow and corn oil were used in this study to test if dietary FA profiles influenced DF fermentability and FA utilization. Data on the effects of dietary supplementation with either saturated or unsaturated fatty acids on the quantity of volatile fatty acids (**VFA**) produced or absorbed during hindgut fermentation of either soluble or insoluble dietary fiber is scantily documented. From the literature in ruminants it is well known that a high dietary fat content can inhibit fermentation by gut microflora or reduce the microflora population (Mallet and Rowland, 1988; Brooks et al., 1954). This is one aspect of pig nutrition that has not been systematically studied and will require further clarification to better understand and develop strategies to improve utilization of fibrous feedstuffs in swine.

Thus, the first objective was to use a combined *in vivo-in vitro* fermentation methodology to predict production and absorption of VFA and fermentability of organic matter in the cecum and colon of growing pigs fed two sources of dietary fiber (pectin or cellulose), and two types of lipids (corn oil or beef tallow) in a factorial arrangement of treatments. The second objective was to determine the interactive effects of DF type and source of dietary lipids on FA flows, and digestibility of dietary components in the GIT segments using ileal and cecal cannulated pigs that were fed the aforementioned diets.

6.3. MATERIALS AND METHODS

6.3.1. Diets, Pigs, Experimental Design and Sample Collection (In vivo assay)

The experimental procedures and use of pigs in this trial were approved by the University of Guelph Animal Care Committee and all procedures were conducted according to the guidelines of the Canadian Council on Animal Care (**CCAC**, 2009). A total of eight Yorkshire barrows (25.3 (SD 1.67) kg mean body weight) were housed individually in pens (1.2 x 1.8 m). The temperature in the room was maintained at 22 (SD 2.3)ºC with a 14 h light-10 h dark cycle. After a 7 d adaptation period to experimental room, pigs were surgically equipped with 2 simple T-cannulas. Before surgeries, pigs were injected with a Primix (Carprofen (2mg/kg BW; 50mg/mL), Excede (0.06/kg BW), Bupivicaine (2 mL/25 kg BW), and Buprenorphine (0.01 mg/kg BW; 0.3 mg/mL) to prevent pain and infections. The first cannula was inserted at the terminal ileum as described in Chapter 5 and the second cannula was placed in the cecum approximately 5 cm proximal to the cecocolic junction. During and after the surgery and recovery period, welfare-related assessments such as body temperature, feeding and general behaviour were conducted and recorded for each pigs. After surgery, pigs were allowed a recovery period of 10 d. During the recovery period, the cannulated pigs were given Metacam (15mg/mL) Oral Suspension for Swine to control inflammation and pain. The areas around the cannulas were cleaned twice a day with an antiseptic soap and a zinc oxide cream was applied to prevent pain and infection. Each pen was equiped with welfare enrichment toys and chains to improve the welfare conditions of the pigs. All pigs were healthy.

Four casein-cornstarch-based diets were formulated to contain 150 g/kg of either cellulose (Cellulose powder BH65 FCC; Cambrian Chemicals, Inc., Oakville, ON) or pectin (Citrus pectin, GENU® pectin type VIS, CP Kelco, Grossenbrode, Germany) and either 62 g/kg of corn oil or

63.7 g/kg of beef tallow. Either corn oil or beef tallow were supplemented in each diet, as the only sources of dietary FA. Thus, giving a 2×2 factorial arrangement of treatments. Diets met specifications for growing pigs (NRC, 2012) (**Table 6.1**). Titanium dioxide was included (3.0 g/kg) as an indigestible marker in all diets.

The eight pigs were used in two blocks, four pigs per block. In each block, pigs were randomly allocated to the experimental diets in a Youden square design with four treatments (diets), three columns (experimental periods) and 4 rows (pigs), to give 6 observations per treatment diet. Pigs were weighed at the beginning of each experimental period and offered a daily feed ration equivalent to supply 2.8 times the estimated requirement for maintenance energy (i.e., 197 kcal ME/BW$^{0.6}$ kg; NRC, 2012). Daily feed ration was offered in two equal meals at 0800 and 2000 h. Pigs were offered feed that was mixed water [1:2, or 500 g of feed/L (wt/v)] to improve palatability. All pigs finished their daily feed allowance throughout the experiment.

Each experimental period lasted 15 d; 9 d for acclimatization to the experimental diets, followed by 2 d for faecal, 2 d for cecal contents and 2 d for ileal digesta collection. Using the grab sample technique, fresh faecal samples were collected into plastic bags and immediately frozen at -20°C. Another sub-sample of fresh feces was collected into 50-mL Eppendorf tube and stored at -80°C for determining VFA concentration. Ileal and cecal digesta were collected from each pig by attaching a sterile 500-mL plastic bags to the cannula barrel. Digesta samples were collected over a 12-h period between 0800 and 2000 h. A subsample of digesta collected from each pig during the 1st, 5th and 8th h post-prandial was transfered into 50-mL Eppendorf tube and immediately frozen for VFA determination. The remaining sub-sample was immediately frozen and stored as substrate for *in vitro* fermentation assays. However, throughout the sample collection period, plastic bags were filled with 10 mL of 10 % formic acid. Formic acid was added in the plastic bags

Table 6.1. Ingredient and analyzed compositions (%) of the experimental diets (as-fed basis)

Item	Diet[1]			
	CC	CB	PC	PB
Ingredients, %				
Corn-starch	35.0	34.8	35.0	34.8
Casein	13.5	13.5	13.5	13.5
Cellulose	15.0	15.0	-	-
Pectin	-	-	15.0	15.0
Sucrose	26.7	26.7	26.7	26.7
Beef tallow	-	6.37	-	6.37
Corn oil	6.20	-	6.20	-
Limestone	0.65	0.65	0.65	0.65
Monocalcium phosphate	1.50	1.50	1.50	1.50
Salt	0.35	0.35	0.35	0.35
[2]Vitamin-mineral premix	0.50	0.50	0.50	0.50
Lysine	0.085	0.085	0.085	0.085
DL-Methionine	0.156	0.156	0.156	0.156
Threonine	0.105	0.105	0.105	0.105
Titanium dioxide	0.30	0.30	0.30	0.30
Analyzed compositions, %				
Dry matter	93.1	93.1	93.1	93.1
Crude protein	12.5	12.5	12.7	12.6
Calcium	0.69	0.69	0.69	0.68
Phosphorus	0.46	0.46	0.46	0.46
Fat content	6.50	6.71	6.29	6.75
Total fatty acids	7.28	7.33	7.26	7.31
NSP (mg/g)				
Galactose	-	-	3.23	3.13
Glucose	110	109	25.0	24.6
Mannose	6.04	6.09	-	-
Xylose	17.6	17.7	-	-
Uronic acids	0.39	0.41	70.9	71.6
Total NSP	134	134	99.1	98.8
Soluble dietary fiber	0.42	0.43	10.10	9.90
Insoluble dietary fiber	15.60	15.60	3.17	3.24
Total dietary fiber	16.02	16.03	13.35	13.15

[1]CC, cellulose and corn oil-containing diet; CB, cellulose and beef tallow-containing diet; PC, pectin and corn oil-containing diet; PB, Pectin and beef tallow-containing diet.

[2]Vitamin-mineral premix provided the following nutrients (per kg of air-dry diet): Vitamins: A, 0.60 mg, D_3 0.01 mg, E, 40 mg, K, 2 mg, B_1, 1.5 mg, B_2, 7 mg, B_6, 2.5 mg, B_{12}, 25 µg, calcium pantothenate, 14 mg, folic acid, 1 mg, niacin, 21 mg, biotin, 70 µg. Minerals: Cu, 10 mg (as copper sulphate), iodine, 0.4 mg (as potassium iodine), iron, 120 mg (as ferrous sulphate), Mn, 10 mg (as manganous oxide), Se, 0.3 mg (as sodium selenite), Zn, 110 mg (as zinc oxide).

and samples were immediately frozen to minimize further fermentation and volatilization of VFA. After collection, digesta samples for determination of digestibility of dietary components and substrate for *in vitro* fermentation assays were thawed to semi-solid state, pooled within pig and collection period, freeze-dried and stored at -20°C.

6.3.2. In vitro fermentation assay

Fermentation of ileal and cecal digesta was carried out using cecal and faecal inoculum, respectively. The collection and preparations of fecal and cecal inoculum were performed according to modifications of the method proposed by Montoya et al. (2016). All procedures were performed under anaerobic conditions under a constant flow of CO_2. Fresh faecal or cecal samples were collected, sealed, and placed into pre-warmed (38°C) plastic bags that were filled with carbon dioxide and a sterile anaerobic medium (0.1 M-phosphate buffer at pH 7) to give final slurry of [1:5, or 200 g of feces or cecal digesta/L (w/v)]. After mixing of the slurry for 60s, the faecal or cecal slurries were filtered through a double layer of sterile cheesecloth to extract faecal or cecal digesta inoculum into a pre-warmed (38°C) vacuum flask flushed with carbon dioxide.

The *in vitro* fermentation of freeze-dried ileal and cecal digesta substrate was conducted using cecal and faecal inoculum as illustrated in the schematic flow diagram in **Figure 6.1**. To each Nalgene bottle containing 1.0 ± 0.1 g DM of the freeze-dried ileal or cecal digesta (substrate) or empty (Blank incubation) and 100 mL of salt medium, 100 ml of the inoculum mixture were added. The Nalgene bottle were flushed with CO_2, and immediately capped. Therefore, there were five replicate bottles per ileal/cecal digesta substrate; one bottle was used for determining VFA concentrations after fermentation, the second and third bottles were used to determine OM fermentability. The fourth and fifth bottles were used as blanks for correcting VFA and OM fermentability associated with the faecal or cecal digesta slurry before (0 h) and after fermentation

(12 or 24 h), respectively. After inocuation, the bottles with ileal and cecal digesta substrate were placed into pre-warmed (37°C) incubators for 12 and 24 h, respectively. After incubation, the bottles were vented and cooled in ice water. Terminal pH was recorded and then the bottles were centrifuged at 14,000rpm, 4°C for 10 min. The supernatant was carefully extracted, transferred to an Eppendorf tube and stored at -80°C until analyzed for VFA. The remaining three bottles were placed in an autoclave (121°C for 20 mins) to completely inactivate microbial activity and remove volatile end products of OM fermentation. The DM of the unfermented residue was determined in the three remaining bottles by drying them in a fanned oven at 60°C for 48 h until a constant weight of undigested OM was attained.

6.3.3. Laboratory Analyzes

The diets, feces, ileal and cecal digesta were analyzed in duplicates for DM, OM and ash following standard procedures (AOAC, 1990), and titanium as described by Lomer et al. (2000). The diets were also analyzed for NSP, CP, GE and mineral contents as described in Chapter 4. The FA and DF compositions of the diets, digesta and feces were analyzed following procedures described in Chapter 5. The dried residue of the *in vitro* fermentation from the three bottles were analyzed for OM contents (AOCS, 1998). The concentrations of VFA in digesta, feces and supernatants obtained from the *in vitro* fermentation were analyzed in duplicates according to procedures described in Chapter 4.

6.3.4. Calculations and Statistical analyzes

The iodine value (**IV**) and iodine value of the product (**IVP**) of the experimental diets was calculated as follow:
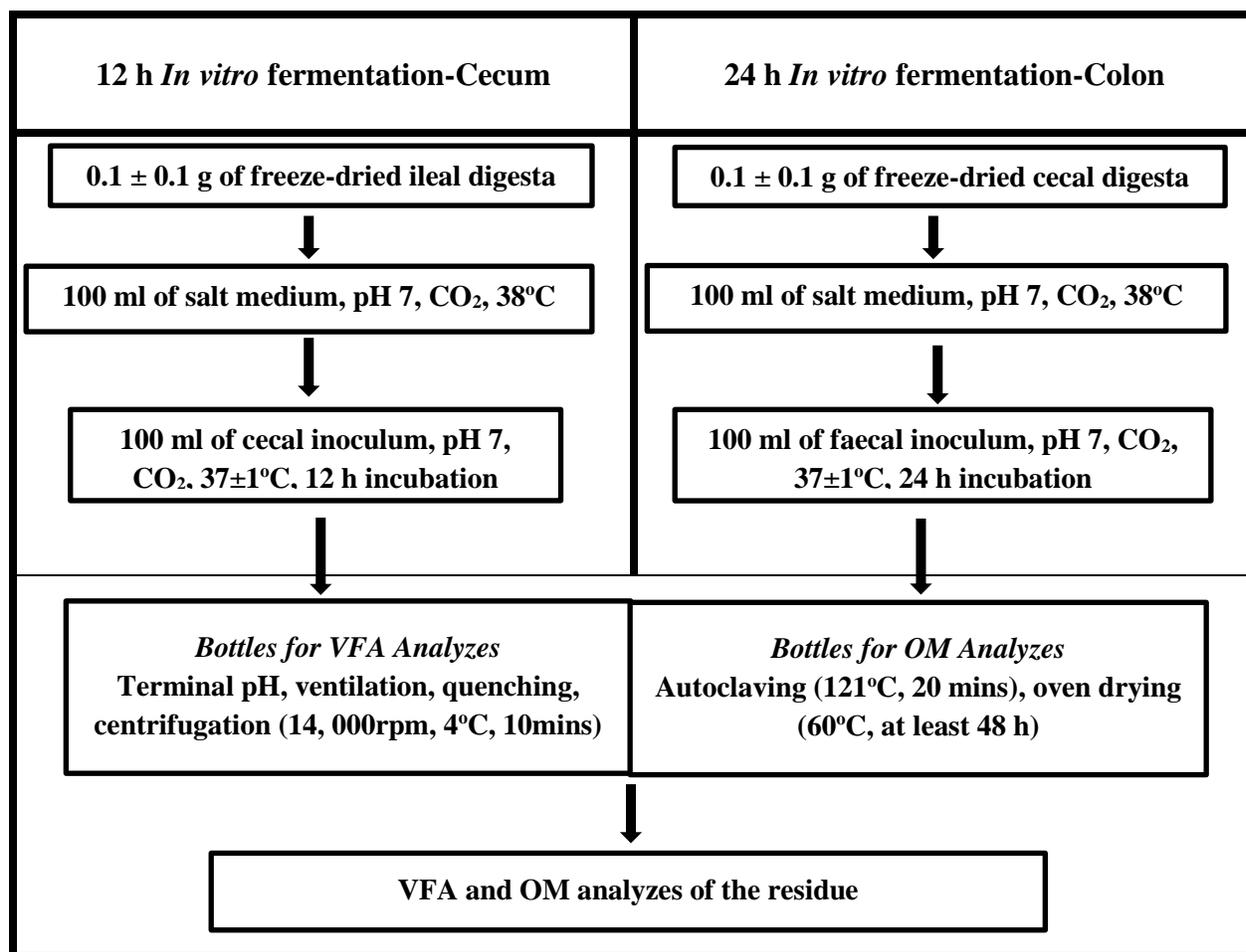
Figure 6. 1. *In vitro* fermentation of freeze-dried ileal and cecal digesta using cecal and feces

inoculum, respectively.

IV = ([C16:1] × 0.95) + ([C18:1] × 0.86) + ([C18:2] × 1.732) + ([C18:3] × 2.616) + ([C20:1] × 0.785) + ([C22:1] × 0.723), in which the brackets indicate concentration (percentage) of fatty acid (Adopted from AOCS, 1998).

IVP = (IV of dietary lipids) × (% dietary lipid) × 0.10 (Adopted from Madsen et al. (1992).

The calculations for the *in vivo* and *in vitro* assay were adopted from Montoya et al. (2016).

The apparent ileal, cecal and total tract digestibilities and fermentability in the cecum and colon were calculated as follows:

Apparent digestibility (%) = $(1 - ((OM_{F/C/I} \div OM_D) \times (T_D \div T_{F/C/I}))) \times 100$,

Cecum or Colon fermentability $_{in\ vivo}$ (%) = $(1 - ((OM_{F/C} \div OM_{I/C}) \times (T_{I/C} \div T_{F/C}))) \times 100$,

where OM_D is the content of OM (g/kg DM) in the diet; $OM_{F/C/I}$ are the contents of OM (g/kg DM) in the feces, cecal or ileal digesta, respectively; T_D is the titanium dioxide (g/kg DM) in the diet; and $T_{F/C/I}$ are the titanium dioxide (g/kg DM) in feces, cecal or ileal digesta, respectively.

The ileal, cecal and faecal flow of fatty acids in the stomach and small intestines, cecum or colon was calculated using the following equation:

Flow$_{nutrient}$ (g/kg DMI) = Nutrient$_{F/C/I}$ × $(T_D \div T_{F/C/I})$,

where Flow$_{nutrient}$ is the flow of dietary components; Nutrient$_{I/C/F}$ is the concentration of each dietary component in feces (F), ileal (I) or cecal (C) digesta, respectively.

The concentrations of VFA in the terminal ileal digesta, cecal digesta and the feces were normalized for the food DM intake (DMI) using the following equation:

Table 6.2. Fatty acid profile (g/100 g of total fatty acids) of experimental diets

Item[‡]	Diet[*]			
	CC	CB	PC	PB
Caprylic (C8:0)	0.01	0.01	0.01	0.01
Capric (C10:0)	0.05	0.07	0.06	0.08
Lauric (C12:0)	0.09	0.20	0.09	0.20
Myristic (C14:0)	0.36	1.49	0.40	1.52
Myristoleic (C14:1)	0.02	0.18	0.02	0.16
Pentadecyclic (C15:0)	0.06	0.20	0.07	0.20
Palmitic acid (C16:0)	12.95	20.58	12.89	20.89
Palmitelaidic (C16:1t)	0.05	0.23	0.05	0.25
Palmitoleic (C16:1n-7)	0.10	2.28	0.14	2.27
Margaric (C17:0)	0.10	0.43	0.10	0.44
Stearic (C18:0)	1.84	11.07	1.89	11.48
Oleic (C18:1n-9)	26.79	41.86	26.69	41.51
Vaccenic (C18:1n-7)	0.35	1.90	0.51	1.74
Linoleic (C18:2)	55.45	15.85	55.29	15.54
Linolenic (C18:3n-6)	0.00	0.04	0.00	0.04
α-Linolenic (C18:3n-3)	0.83	1.99	0.83	2.01
Arachidic (C20:0)	0.39	0.26	0.38	0.27
Gadoleic (C20:1)	0.29	0.59	0.34	0.62
Eicosadienoic (C20:2)	0.02	0.22	0.02	0.23
Dihomo-γ-linolenic (C20:3n-6)	0.00	0.05	0.00	0.06
Eicosatrienoic (C20:3n-3)	0.01	0.05	0.01	0.05
Arachidonic (C20:4n-6)	0.00	0.19	0.00	0.17
Eicosapentaenoic (C20:5n-3)	0.01	0.01	0.02	0.01
Behenic (C22:0)	0.11	0.09	0.09	0.09
Erucic (C22:1n-9)	0.01	0.01	0.00	0.01
Docosatetraenoic (C22:4n-6)	0.13	0.04	0.12	0.04
Osbond (C22:5n-6)	0.01	0.02	0.01	0.02
Docosapentaenoic (C22:5n-3)	0.00	0.04	0.00	0.03
Docosahexaenoic (C22:6n-3)	0.00	0.01	0.00	0.01
Lignoceric (C24:0)	0.13	0.03	0.12	0.04
Nervonic (C24:1n-9)	0.00	0.04	0.00	0.03
$\sum$SFA	16.08	34.41	16.09	35.23
$\sum$UFA	83.92	65.59	83.91	64.77
$\sum$MUFA	27.59	47.09	27.74	46.57
$\sum$PUFA	56.33	18.50	56.17	18.20
[‡]Calculated IV, g/100g	122	71	121	71
[§]Calculated IVP, g/100g	88	52	89	52

*CC, cellulose and corn oil-containing diet; CB, cellulose and beef tallow-containing diet; PC, pectin and corn oil-containing diet; PB, Pectin and beef tallow-containing diet.

‡$\sum$SFA = total saturated fatty acids; $\sum$UFA = total unsaturated fatty acids; $\sum$MUFA = total monounsaturated fatty acids; $\sum$PUFA = total polyunsaturated fatty acids; IV = iodine value; IVP = iodine value of the product.

Normalized VFA concentration (mmol/kg DMI) = VFA concentration (mmol/kg DM) $\times$ $(T_D/T_{F/C/I})$.

Predicted fermentability of OM and VFA produced in the cecum and colon were determined after *in vitro* fermentation of ileal and cecal digesta with cecal and faecal inoculum, respectively, and calculated using the following equations:

Cecum or colon fermentability$_{in\ vitro}$ (%) = $(OM_b - (OM_a - (OM_{blank\ initial} + OM_{blank\ final})/2)))/OM_b \times 100$,

VFA produced by fermentation (mmol/kg DM incubated) = $(VFA_{sample} - ((VFA_{blank\ initial} + VFA_{blank\ final})/2))$ / sample weight (g DM) $\times 100$,

where OM_b is the OM (mg) of ileal or cecal digesta before *in vitro* fermentation, OM_a is the OM (mg) of ileal or cecal digesta after *in vitro* fermentation. $OM_{blank\ initial}$, $OM_{blank\ final}$, $VFA_{blank\ initial}$ and $VFA_{blank\ final}$ are the OM (mg) and the VFA (mmol) in the blank bottle (which contained inoculum but no ileal or cecal digesta) before (initial) and after (final) *in vitro* fermentation, respectively (Coles et al., 2013).

The predicted apparent ileal, cecal and total tract digestibility ($PAD_{I/C/F}$) of OM and the VFA production and absorption in the cecum or colon were calculated based on combining results for *in vivo* ileal or cecal digesta flows (ileo-cecal cannulated pig) with *in vitro* concentrations (cecum or colon fermentation). The *in vivo* measurements represented digestion in the upper gut,

and *in vitro* values represented fermentation in the cecum or colon. The $PAD_{I/C/F}$ of OM and predicted cecal or colonic VFA production were calculated as follows:

$PAD_{I/C/F}$ (%) = $(OM_D − (((100 − OM_c$ fermentability$_{in\ vitro})/100) × OM$ flow$_{I/C}))/OM_D ×$ 100;

Predicted cecum or colon VFA production (mmol/kg DMI) = VFA produced by fermentation (mmol/kg ileal or cecal digesta DM incubated) × DM flow$_{I/C}$ (kg DM/kg DMI);

where OM_D (g/kg DM) is the OM in the diet, OM_c fermentability$_{in\ vitro}$ (%) is the OM fermentability determined from incubation of either ileal or cecal digesta, and OM flow$_{I/C}$ (g/kg DMI) is either the ileal or cecal flow of OM.The values of VFA entering the cecum or colon (is the ileal or cecal normalized VFA concentration) and the amounts of VFA produced in the cecum or colon (i.e. *in vitro* predicted cecal or colonic VFA production) were used to estimate the amounts of VFA absorbed in the cecum or colon based on the following equation:

Amount of VFA absorbed from the cecum or colon (mmol/kg DMI) = *in vitro* predicted cecal or colonic VFA production (mmol/kg DMI) + ileal or cecal VFA concentration (mmol/kg DMI) – cecal or faecal VFA concentration (mmol/kg DMI),

Extent of VFA absorbed in the cecum or colon (%) = (amount of VFA absorbed from the cecum or colon (mmol/kg DMI) / (*in vitro* predicted cecal or colonic VFA production (mmol/kg DMI) + ileal VFA concentration (mmol/kg DMI))) × 100.

Statistical analyzes were performed using SAS (version 9.4, 2009; SAS Inst. Inc., Cary, NC). Data were analyzed as a completely randomized design with 2×2 factorial treatment arrangements using the GLIMMIX procedure. The models accounted for the main effects of dietary fiber type and lipid type and associated two-way interactions as fixed factors. The pig, block and period were random variable. Treatment effects were determined with orthogonal contrasts for a 2×2 factorial arrangement. The pig (nested with period) was the experimental unit for all the response criteria. The GLM procedure was used to compare ileal, cecal and faecal VFA concentrations for each diet. Comparisons of means were performed using the Tukey-Kramer honestly significance difference test. A two-independent samples t test procedure was performed to compare the determined and predicted digestibilities and fermentabilities for each experimental diet. Significant differences among means were declared at $P < 0.05$, and trends declared for P values between 0.05 and 0.10 were discussed.

6.4. RESULTS

6.4.1. Gastrointestinal concentrations of VFA (in vivo)

An interaction between dietary fibre and lipid type was observed on the concentration of caecal acetic acid which decreased ($P < 0.001$; **Table 6.3**) with addition of beef tallow in pigs fed cellulose-containing diets but had no effect in pectin-containing diets-fed pigs. The lipid source had no effect on the concentrations of all the SCFA in the intestinal segments but the main effects of dietary fibre were observed in which the concentrations of butyric and valeric acids in the ileum, and that of acetic, butyric and propionic acids in feaces were higher ($P < 0.05$) in pigs that consumed pectin-containing diets compared with those fed diets containing cellulose.

There were no differences ($P > 0.05$) in the concentrations of individual SCFA in the ileum, caecum and faeces for each diet, with the exception of butyric acid ($P_{butyric} < 0.05$) across all diets

and acetic acid ($P_{acetic} < 0.05$) for diets enriched with corn oil that had lower faecal and caecal concentrations compared to ileal concentrations.

6.4.2. Production and absorption of SCFA in the caecum (**in vivo-in vitro**)

There were interactions between dietary fibre and lipid type on the quantity of individual SCFA produced during *in vitro* fermentation ($P < 0.01$, **Table 6.4**) and predicted amount of SCFA produced ($P < 0.001$) in the caecum. In this context, the addition of beef tallow decreased the quantity of acetic acid produced during *in vitro* fermentation and the predicted caecal acetic, propionic and valeric acids ($P < 0.001$) in diets containing pectin but did not have effects in cellulose-containing diets. An interaction of dietary fibre and lipid type was observed in which the addition of beef tallow increased the production of butyric and propionic acids during *in vitro* fermentation and the predicted butyric acid production in cellulose-containing diets but decreased in pectin-containing diets ($P < 0.001$). The interaction of dietary fibre and lipid type was observed in which addition of beef tallow decreased the quantity of valeric acid produced during *in vitro* fermentation ($P < 0.001$) in diets containing pectin but did not have any effect in cellulose-containing diets.

There was a significant interaction between dietary fibre and lipid type on the predicted quantity of SCFA absorbed and apparent absorption of acetic and propionic acid in the caecum (**Table 6.4**). In this case, the predicted quantities of acetic, butyric and valeric acids decreased with the addition of beef tallow in diets containing pectin, but did not have any effect in diets containing cellulose ($P < 0.001$). There were no interactions ($P > 0.10$) between dietary fibre and lipid type on the predicted quantities of propionic acid and the predicted apparent acetic, butyric, propionic and valeric acids in the caecum. The main effects of dietary fibre were such that diets containing pectin had greater ($P < 0.05$) predicted propionic acid absorbed and the predicted apparent absorption of acetic, butyric and propionic acids compared to diets containing cellulose. Moreover, the main effects of dietary fibre were observed in which case diets containing cellulose and beef tallow had the least ($P < 0.05$) predicted apparent valeric acid absorption compared with other diets.

Table 6.3. Ileal, cecal and faecal concentrations of VFA in ileal- and cecal-cannulated pigs fed the experimental diets[1]

Item	Diet[2]				SEM	P[3]		
	CC	CB	PC	PB		Fiber	Lipid	F × L
Determined ileal VFA concentrations (mmol/kg DM intake) (*in vivo*)[4]								
Acetic	10.41[X]	8.86	17.78[X]	18.09	2.002	<0.001	0.592	0.651
Butyric	1.25[X]	0.90[X]	2.83[X]	2.29[X]	0.747	0.032	0.468	0.142
Propionic	2.86	2.11	5.11	4.23	0.907	0.37	0.391	0.149
Valeric	0.55	0.55	0.87	0.99	0.273	0.035	0.726	0.172
Determined cecal VFA concentrations (mmol/kg DM intake) (*in vivo*)[5]								
Acetic	6.70[cY]	8.04[b]	14.45[aY]	15.11[a]	0.431	<0.001	0.156	<0.001
Butyric	0.25[Y]	0.20[Y]	0.32[Y]	0.36[Y]	0.072	0.139	0.921	0.421
Propionic	3.60	3.83	4.34	4.01	0.851	0.468	0.939	0.857
Valeric	0.34	0.79	0.50	0.53	0.299	0.885	0.461	0.794
Determined faecal VFA concentrations (mmol/kg DM intake) (*in vivo*)[6]								
Acetic	6.64[Y]	6.35	9.37[Z]	10.38	0.880	0.006	0.826	0.477
Butyric	0.32[Y]	0.15[Y]	0.48[Y]	0.62[Y]	0.158	0.045	0.883	0.174
Propionic	2.08	2.27	3.73	2.61	0.467	0.042	0.314	0.076
Valeric	0.61	0.37	0.61	0.79	0.165	0.233	0.851	0.445
Statistical analysis for comparing ileal, cecal and faecal VFA concentrations[7]								
P_{Acetic}	0.037	0.086	0.022	0.123				
SEM	1.23	0.96	1.02	1.86				
$P_{Butyric}$	0.023	0.036	0.047	0.031				

SEM	0.235	0.351	0.654	1.43
$P_{\text{Propionic}}$	0.465	0.887	0.356	0.612
SEM	1.77	2.12	1.36	1.23
P_{Valeric}	0.638	0.456	0.796	0.094
SEM	0.32	0.14	0.22	0.37

[1]Mean values with their pooled standard errors (n = 6 per treatment).

[2]CC, cellulose and corn oil-containing diet; CB, cellulose and beef tallow-containing diet; PC, pectin and corn oil-containing diet; PB, Pectin and beef tallow-containing diet.

[3]Contrast statements: Fiber = Cellulose vs. Pectin; Lipid = Corn oil vs. Beef tallow; F×L = Interaction of dietary fiber and lipid type.

[a,b,c]Mean values within a row with unlike superscripts differ ($P < 0.05$).

[4]The ileal concentrations of VFA were determined directly from the frozen digesta contents that were collected through the ileal cannula.

[5]Cecal concentrations were determined directly from the frozen samples of digesta collected through the cecal cannula.

[6]The faecal concentrations were determined from the samples of feces that were collected directly through anal palpation.

[7][X,Y,Z]Mean values, for either acetic, butyric, propionic or valeric acid, within a column with unlike superscripts differ ($P < 0.05$).

6.4.3. Production and absorption of SCFA in the colon

There was an interaction ($P < 0.01$; **Table 6.5**) of dietary fibre and lipid type on the amount of individual SCFA produced during in vitro fermentation and predicted quantity of each SCFA produced in the colon. In this regard, the addition of beef tallow decreased the quantity of acetic acid produced during in vitro fermentation and predicted acetic acid production in pigs fed pectin and cellulose diets. An interaction of dietary fibre and lipid type was observed in which addition of beef tallow decreased the quantity of butyric acid produced by in vitro fermentation and predicted butyric acid production in cellulose-containing diets but did not have effects in pectin-containing diets ($P < 0.01$). An interaction between dietary fibre and lipid types decreased the quantity of propionic acid produced during in vitro fermentation in pectin-containing diets but did not have effects in cellulose-containing diets ($P < 0.001$). There were no significant interaction effects of dietary fibre and lipid type on valeric acid production but the main effects of dietary fibre type decreased ($P < 0.001$) the quantity of valeric acid produced by in vitro fermentation and the predicted valeric acid production in cellulose-containing diets compared with pectin-containing diets.

There was a significant interaction between dietary fibre and lipid type on the predicted quantity of butyric and propionic acid absorbed, and the apparent absorption of butyric and propionic acid, except acetic and valeric acid in the colon (**Table 6.5**). In this case, the predicted quantities of butyric acid decreased with the addition of beef tallow in diets containing cellulose, but did not have any effect in diets containing cellulose ($P < 0.001$). The interactive effects of dietary fibre and lipid types were observed in which addition of beef tallow decreased the predicted quantity of propionic acid absorbed in diets containing pectin but did not have effects in diets containing cellulose. An interaction ($P < 0.01$) of dietary fibre and lipid type in which the addition

of beef tallow decreased the predicted apparent butyric and propionic acid absorption in diets containing cellulose but did not have effects in pectin containing diets. The main effects of dietary fibre were such that diets containing pectin had greater ($P < 0.05$) predicted acetic and valeric acid absorption and compared to diets containing cellulose.

6.4.4. Determined (in vivo) and predicted (in vivo-in vitro) digestibilities and fermentabilities

There was an interaction ($P < 0.001$; **Table 6.6**) between dietary fibre and lipid type in which the addition of beef tallow increased the determined total tract digestibility of OM in diets containing pectin but did not have effects in cellulose-containing diets. There were no significant interactions between dietary fibre and lipid types on the apparent digestibility of OM in the ileum and caecum, and predicted apparent caecal and total tract digestibility of OM. The main effects of dietary fibre increased the determined apparent ileal and caecal digestibilities of OM and the predicted apparent caecal digestibility of OM ($P < 0.001$) in pectin-containing diets compared with cellulose-containing diets.

For each diet, there were no differences between the determined and predicted apparent caecal ($P_{Caecum} > 0.10$; **Table 6.6**) digestibilities of OM. In contrast, the predicted total tract digestibilities of OM were greater ($P_{Total\ tract} < 0.05$) compared to the determined total tract digestibilities of OM in cellulose-containing diets but not in pectin-containing diets.

There were significant interactions (**Table 6.7**) between dietary fibre and lipid type in which the addition of beef tallow decreased the determined fermentability of OM in the ileum and predicted fermentability of OM in the caecum in pectin-containing diets but did not have effects in diets containing cellulose. There were no interactive effects ($P > 0.10$) between dietary fibre and lipid types on the determined caecal and colonic and predicted colonic fermentabilities of OM. The main effects of dietary fibre were observed in which the determined caecal and colonic and predicted colonic fermentabilities of OM were lower ($P < 0.001$) in cellulose-containing diets compared with pectin-containing diets.

Within each diet, the determined fermentability of OM in the caecum were greater ($P_{Caecum} < 0.05$; **Table 6.7**) than the predicted fermentability of OM. The determined fermentability of OM in the colon were higher ($P_{Colon} < 0.05$) compared with its predicted counterpart for diets containing

Table 6.4. Predicted production and absorption of VFA in the cecum (in vivo-in vitro assay) of pigs fed the experimental diets[1]

Item	Diet[2]				SEM	P[3]		
	CC	CB	PC	PB		Fiber	Lipid	F × L
Cecal VFA produced by fermentation (mmol/kg DM incubated) (*in vitro technique*)[4]								
Acetic	832[c]	903[c]	3008[a]	2701[b]	22.94	<0.001	0.595	<0.001
Butyric	77[d]	106[c]	323[a]	252[b]	5.21	<0.001	0.429	<0.001
Propionic	330[d]	381[c]	1200[a]	1154[b]	9.36	<0.001	0.976	<0.001
Valeric	42[c]	42[c]	88[b]	91[a]	1.30	0.001	0.878	<0.001
Predicted cecal VFA production (mmol/kg DM intake) (*in vivo-in vitro technique*)[5]								
Acetic	135[c]	133[c]	483[a]	446[b]	4.01	<0.001	0.596	<0.001
Butyric	12[d]	16[c]	52[a]	43[b]	0.83	<0.001	0.633	<0.001
Propionic	56[c]	56[c]	193[a]	124[b]	1.69	<0.001	0.972	<0.001
Valeric	7[c]	6[c]	78[a]	70[b]	1.48	<0.001	0.695	<0.001
Predicted VFA absorbed from the cecum (mmol/kg DM intake) (*in vivo-in vitro technique*)[6]								
Acetic	137[c]	133[c]	485[a]	449[b]	3.94	<0.001	0.581	<0.001
Butyric	12[c]	14[c]	51[a]	42[b]	0.84	<0.001	0.564	0.003
Propionic	55	54	193	192	16.72	<0.001	0.798	0.123
Valeric	8[c]	6[c]	79[a]	71[b]	1.47	<0.001	0.677	0.022
Predicted apparent VFA absorption in the cecum (%) (*in vivo-in vitro technique*)[7]								
Acetic	93.8	93.3	97.0	96.4	0.88	0.002	0.535	0.017
Butyric	90.5	90.7	96.8	94.9	2.34	0.024	0.682	0.129
Propionic	90.3	90.1	97.6	98.0	2.73	0.343	0.964	0.624
Valeric	94.7	89.5	99.2	98.9	3.22	0.045	0.406	0.153

[1]Mean values with their pooled standard errors (n = 6 per treatment).

[2]CC, cellulose and corn oil-containing diet; CB, cellulose and beef tallow-containing diet; PC, pectin and corn oil-containing diet; PB, Pectin and beef tallow-containing diet.

[3]Contrast statements: Fiber = Cellulose vs. Pectin; Lipid = Corn oil vs. Beef tallow; F×L = Interaction of dietary fiber and lipid type.

[a,b,c]Mean values within a row with unlike superscripts differ ($P < 0.05$).

[4]The cecal production of VFA in pigs was determined after *in vitro* fermentation of ileal digesta collected from pigs fed the experimental diets with pig cecal inoculum for 12 h at 37°C.

[5]The predicted cecal production of VFA in pigs was estimated based on the VFA produced after *in vitro* incubation of ileal digesta with a cecal inoculum corrected for the ileal flow of DM.

[6]The quantity of VFA absorbed in the cecum was obtained after summing the VFA entering (ileal concentrations) and produced (estimated based on *in vitro* incubation of ileal digesta) in the cecum, and then subtracting the excreted VFA (cecal concentrations).

[7]The apparent absorption in the pig cecum was calculated based on the ratio between the predicted amount of VFA absorbed from the cecum and the sum of the VFA entering (ileal concentrations) and produced (predicted based on an *in vitro* assay) in the cecum.

Table 6.5. Predicted production and absorption of VFA in the colon (in vivo-in vitro assay) of pigs fed the experimental diets[1]

| Item | Diet[2] | | | | SEM | P[3] | | |
	CC	CB	PC	PB		Fiber	Lipid	F × L
Colon VFA produced by fermentation (mmol/kg DM incubated) (*in vitro technique*)[4]								
Acetic acid	688[c]	617[d]	2232[a]	1964[b]	3.52	<0.001	<0.001	<0.001
Butyric acid	129[b]	104[c]	284[a]	294[a]	1.08	<0.001	<0.001	0.001
Propionic acid	267[c]	178[c]	883[a]	594[b]	5.47	<0.001	<0.001	<0.001
Valeric acid	42	42	88	91	1.30	0.037	0.878	0.103
Predicted colon VFA production (mmol/kg DM intake) (*in vivo-in vitro technique*)[5]								
Acetic acid	107[c]	94[d]	315[a]	306[b]	1.91	<0.001	0.565	<0.001
Butyric acid	20[b]	6[c]	40[a]	46[a]	4.23	<0.001	0.149	<0.001
Propionic acid	42[c]	27[c]	140[a]	79[b]	11.85	<0.001	0.001	<0.001
Valeric acid	6	6	12	15	2.45	0.012	0.505	0.232
Predicted VFA absorbed from the colon (mmol/kg DM intake) (*in vivo-in vitro technique*)[6]								
Acetic acid	109	96	320	311	18.88	<0.001	0.555	0.532
Butyric acid	20[b]	7[c]	40[a]	46[a]	4.45	<0.001	0.170	<0.001
Propionic acid	43[c]	29[c]	141[a]	80[b]	11.85	<0.001	<0.001	<0.001
Valeric acid	6	7	12	15	2.57	0.001	0.415	0.459
Predicted apparent VFA absorption in the colon (%) (*in vivo-in vitro technique*)[7]								
Acetic acid	94.3	93.4	97.0	96.6	1.34	0.098	0.765	0.645
Butyric acid	92.8[a]	75.1[b]	95.6[a]	95.1[a]	2.89	0.052	0.001	0.034
Propionic acid	95.4[a]	92.5[b]	97.3[a]	96.5[a]	0.92	0.004	0.014	0.001
Valeric acid	89.2	91.1	95.5	90.6	4.29	0.469	0.716	0.710

[1]Mean values with their pooled standard errors (n = 6 per treatment).

[2]CC, cellulose and corn oil-containing diet; CB, cellulose and beef tallow-containing diet; PC, pectin and corn oil-containing diet; PB, Pectin and beef tallow-containing diet.

[3]Contrast statements: Fiber = Cellulose vs. Pectin; Lipid = Corn oil vs. Beef tallow; F×L = Interaction of dietary fiber and lipid type.

[a,b,c]Mean values within a row with unlike superscripts differ ($P < 0.05$).

[4]The colonic production of VFA in pigs was determined after *in vitro* fermentation of cecal digesta collected from pigs fed the experimental diets with pig faecal inoculum for 24 h at 37°C.

[5]The predicted colonic production of VFA in pigs was estimated based on the VFA produced after *in vitro* incubation of cecal digesta with a faecal inoculum corrected for the cecal flow of DM.

[6]The quantity of VFA absorbed in the colon was obtained after summing the VFA entering (cecal concentrations) and produced (estimated based on *in vitro* incubation of cecal digesta) in the colon, and then subtracting the excreted VFA (faecal concentrations).

[7]The apparent absorption in the pig colon was calculated based on the ratio between the predicted amount of VFA absorbed from the colon and the sum of the VFA entering (cecal concentrations) and produced (predicted based on an *in vitro* assay) in the colon.

cellulose, whereas there were no within-diet differences ($P_{Colon} > 0.05$) for diets containing pectin, irrispective of dietary lipid source.

There were interactions ($P < 0.05$, **Table 6.8**) between DF solubility and lipid type on AID of DM, SFA, MUFA, PUFA and TFA, and on AID of SDF, IDF and TDF. In this regard, the AID of DM, SFA and PUFA was greater ($P < 0.05$) in the pectin and beef tallow-containing diet compared with the other diets. The AID of MUFA was greater ($P < 0.05$) in cellulose and corn oil- and pectin and beef tallow-containing diets than in both cellulose and beef tallow- and pectin and corn oil-containing diets. The AID of TFA in diets containing cellulose were greater ($P < 0.05$) compared to pectin-containing diet. There were no significant interactive effects of DF and lipid type on AID of N and starch, but main effects of DF were observed on AID of starch in which cellulose-containing diets had greater ($P < 0.05$) AID of starch compared with pectin-containing diets.

The pectin and beef tallow-containing diet had higher ($P < 0.01$) AID of SDF than cellulose-containing diets or pectin and corn oil-containing diet. The AID of IDF and TDF in diets containing cellulose were lower ($P < 0.05$) compared to pectin-containing diets. There was an interaction ($P < 0.05$; **Table 6.8**) between dietary fiber and lipid type on ACD of SFA, MUFA, TFA, and on ACD of SDF, IDF and TDF. In this case, the cellulose and beef tallow-containing diet had greater ($P < 0.01$) ACD of SFA compared to the other diets.

Table 6.6. Determined and predicted apparent digestibility of organic matter in gastrointestinal segments of ileal-and -cecal cannulated pigs fed the experimental diets[1]

Item	CC	CB	PC	PB	SEM	Fiber	Lipid	F × L
	Diet[2]					P[3]		
Determined apparent digestibility (%) (*in vivo*)[4]								
Ileum	43.6	45.3	62.7	61.3	0.124	<0.001	0.109	0.518
Cecum	57.6	55.9	82.6	84.3	0.235	0.01	0.719	0.123
Total tract	67.3[cY]	66.8[cY]	90.8[b]	93.9[a]	0.63	<0.001	<0.001	<0.001
Predicted apparent digestibility (%) (*in vivo-in vitro methodology*)[4]								
Cecum	64.5	63.6	92.9	93.0	0.96	0.365	0.149	0.485
Total tract	87.5[X]	86.4[X]	95.2	95.8	0.131	0.041	0.361	0.395
Statistical analysis for comparing the determined and predicted apparent digestibilities within each diet[6]								
P_{Cecum}	0.386	0.635	0.236	0.112				
SEM	0.63	0.73	5.36	4.65				
$P_{Total tract}$	0.032	<0.001	0.865	0.365				
SEM	1.79	0.69	0.46	0.37				

[1]Mean values with their pooled standard errors (n = 6 per treatment).

[2]CC, cellulose and corn oil-containing diet; CB, cellulose and beef tallow-containing diet; PC, pectin and corn oil-containing diet; PB, Pectin and beef tallow-containing diet.

[3]Contrast statements: Fiber = Cellulose vs. Pectin; Lipid = Corn oil vs. Beef tallow; F×L = Interaction of dietary fiber and lipid type.

[a,b,c]Mean values within a row with unlike superscripts differ ($P < 0.05$).

[4]The *in vivo* digestibilities were determined directly in ileal- and cecal- cannulated pigs.

[5]The predicted ceacal and total tract digestibilities in pigs were computed based on the ileal and cecal organic matter digestibility estimated in pigs and the predicted cecal and colon fermentability of organic matter in the cecum and colon of pigs based on an *in vitro* fermentation assay using pig cecal and faecal inoculum.

[6] [X,Y]Mean values within a column with unlike superscripts differ ($P < 0.05$).

Table 6.7. Determined and predicted fermentability of organic matter in gastrointestinal segments of ileal- and cecal-cannulated pigs fed the experimental diets[1]

	Diet[2]				SEM	P[3]		
Item	CC	CB	PC	PB		Fiber	Lipid	F × L
Determined fermentability (%) (*in vivo*)[4]								
Ileum	22.4[c]	19.2[c]	65.1[a]	60.5[b]	0.138	<0.001	<0.001	<0.001
Cecum	31.1[X]	32.6[X]	70.2[X]	73.9[X]	0.215	<0.001	0.291	0.374
Colon	34.6[X]	36.3[X]	88.3	88.4	0.536	<0.001	0.348	0.112
Predicted fermentability (%) (*in vitro*)[5]								
Cecum	16.4[cY]	17.5[cY]	59.4[aY]	55.8[bY]	0.169	<0.001	<0.001	<0.001
Colon	24.8[Y]	22.3[Y]	81.7	83.4	0.436	<0.001	0.492	0.667
Statistical analysis for comparing the determined and predicted fermentability[6]								
P_{Cecum}	0.023	<0.01	0.043	0.038				
SEM	3.26	4.32	5.12	2.53				
P_{Colon}	0.023	0.011	0.361	0.056				
SEM	2.86	1.23	6.36	4.44				

[1]Mean values with their pooled standard errors (n = 6 per treatment).

[2]CC, cellulose and corn oil-containing diet; CB, cellulose and beef tallow-containing diet; PC, pectin and corn oil-containing diet; PB, pectin and beef tallow-containing diet.

[3]Contrast statements: Fiber = Cellulose vs. Pectin; Lipid = Corn oil vs. Beef tallow; F×L = Interaction of dietary fiber and lipid type.

[a,b,c]Mean values within a row with unlike superscripts differ ($P < 0.05$).

[4]The *in vivo* fermentability was determined in pigs as the difference between ileal, cecal and faecal flows of organic matter.

[5]The predicted fermentability in cecum and colon in pigs were respectively determined after *in vitro* fermentation of ileal and cecal digesta with pig cecal and faecal inoculum, respectively.

[6][X,Y]Mean values within a column with unlike superscripts differ ($P < 0.05$).

The ACD of MUFA in the diets containing cellulose and corn oil or that containing pectin and beef tallow was greater ($P < 0.01$) than in the diet containing cellulose and beef tallow as well as in the diet containing pectin and corn oil. The ACD of TFA in cellulose and corn oil-containing diet was greater ($P < 0.01$) than in pectin-containing diets or cellulose and beef tallow-containing diet. There were no interactions ($P > 0.05$) between dietary fiber and lipid type on ACD of DM, N, starch and PUFA. However, the main effects of dietary fiber were such that diets containing pectin had greater ($P < 0.05$) ACD of DM compared to diets containing cellulose. Moreover, main effects of lipid type were observed on ACD of PUFA in which case cellulose and beef tallow-containing diet had greater ($P < 0.05$) ACD of PUFA compared to the other diets.

The cellulose and corn oil-containing diet had greater ($P < 0.01$) ACD of SDF compared to the other diets, and the pectin and beef tallow-containing diet had lower ACD of SDF than pectin and corn oil-containing diet. The ACD of IDF ($P < 0.05$) and TDF ($P < 0.001$) in cellulose-containing diets was lower than that in pectin-containing diets. An interaction ($P < 0.05$; **Table 6.8**) of dietary fiber and lipid type was observed on ATTD of DM, PUFA and TFA, and on ATTD of IDF and TDF but not ($P > 0.05$) on ATTD of N, starch, SFA and MUFA, and ATTD of SDF. In this regard, the ATTD of DM in cellulose and corn oil-containing diet was greater ($P < 0.001$) compared to all other experimental diets (**Table 6.8**). The ACD of PUFA in cellulose and corn oil- and pectin and beef tallow-containing diets was greater ($P < 0.05$) than in cellulose and beef tallow- and pectin and corn oil-containing diets. The pectin-containing diets had lower ($P < 0.001$) ATTD of TFA compared with cellulose-containing diets.

The ATTD of IDF and TDF was greater ($P < 0.05$) in pectin-containing diets compared with cellulose-containing diets. However, main effects of DF observed indicated that the ATTD of SDF in cellulose-containing diets was greater ($P < 0.05$) compared to pectin containing diets.

Table 6.8. Apparent ileal, cecal, and total tract digestibility (%) and fermentability (%) of dietary components by growing pigs fed the experimental diets[1]

Item[4]	Diet[2]				SEM	P[3]		
	CC	CB	PC	PB		Fiber	Lipid	F×L
Apparent ileal digestibility								
DM	52.6[b]	52.0[b]	56.0[b]	61.7[a]	2.26	<0.001	0.348	0.025
N	94.8	90.5	90.7	95.1	2.07	0.856	0.429	0.146
Starch	94.0	94.3	90.2	91.9	2.17	0.041	0.726	0.301
SFA	70.7[c]	75.0[b]	72.3[c]	80.6[a]	1.07	<0.001	0.546	0.031
MUFA	90.7[a]	87.3[b]	86.5[b]	91.9[a]	1.24	0.071	0.078	0.001
PUFA	80.7[c]	85.3[b]	79.1[c]	90.6[a]	1.57	<0.001	0.111	<0.001
TFA	95.8[a]	96.0[a]	89.4[b]	85.1[c]	1.23	0.047	0.246	0.028
Apparent ileal fermentability								
SDF	77.3[b]	76.4[b]	70.5[c]	88.7[a]	0.94	0.028	0.878	0.001
IDF	16.2[c]	11.2[c]	34.6[b]	38.1[a]	2.11	<0.001	0.976	0.014
TDF	33.4[c]	33.6[c]	60.6[b]	67.4[a]	1.04	<0.001	0.058	0.035
Apparent cecal digestibility								
DM	57. 6[B]	57.0[B]	58.9[A]	60.0[A]	1.10	0.035	0.511	0.063
N	96.8	90.5	90.7	94.9	2.27	0.071	0.633	0.211
Starch	93.7	92.4	93.3	94.4	2.85	0.064	0.062	0.689
SFA	78.7[c]	92.4[a]	89.3[b]	79.1[c]	0.41	0.021	0.912	0.001
MUFA	93.7[a]	82.7[b]	84.6[b]	94.4[a]	2.04	<0.001	0.878	0.001
PUFA	89.0[B]	95.1[A]	88.1[B]	89.1[B]	4.65	0.054	0.012	0.073
TFA	94.8[a]	72.7[c]	88.5[b]	87.1[b]	1.57	0.001	0.311	0.041
Apparent cecal fermentability								
SDF	84.2[a]	81.5[b]	74.6[c]	67.4[d]	2.68	<0.001	0.675	0.001
IDF	25.3[b]	30.0[b]	73.9[a]	77.1[a]	14.18	0.048	0.076	0.048
TDF	52.6[b]	54.5[b]	71.8[a]	75.8[a]	3.04	<0.001	0.078	0.001

	CC	CB	PC	PB	SEM	Fiber	Lipid	F×L
Apparent total tract digestibility								
DM	72.3[a]	68.4[b]	74.2[a]	71.8[a]	1.38	<0.001	0.581	<0.001
N	95.6	93.0	92.2	95.1	5.73	0.384	0.564	0.373
Starch	95.6	93.1	93.7	95.9	6.72	0.248	0.932	0.674
SFA	80.5	74.2	76.1	80.7	4.44	0.086	0.976	0.606
MUFA	90.6	93.1	93.7	91.9	4.05	0.396	0.878	0.151
PUFA	90.5[a]	84.2[b]	86.1[b]	90.6[a]	1.12	<0.001	0.076	0.041
TFA	94.6[a]	94.3[a]	86.9[b]	85.1[b]	3.57	<0.001	0.976	<0.001
Apparent total tract fermentability								
SDF	92.5	92.7	90.8	88.7	2.34	0.024	0.682	0.129
IDF	47.6[b]	55.0[b]	82.7[a]	87.1[a]	2.73	0.003	0.964	0.024
TDF	59.1[b]	55.0[b]	92.6[a]	90.4[a]	3.22	0.045	0.406	0.015

[1]Mean values with their pooled standard errors (n = 6 per treatment).

[2]CC, cellulose and corn oil-containing diet; CB, cellulose and beef tallow-containing diet; PC, pectin and corn oil-containing diet; PB, Pectin and beef tallow-containing diet.

[3]Contrast statements: Fiber = Cellulose vs. Pectin; Lipid = Corn oil vs. Beef tallow; F×L = Interaction of dietary fiber and lipid type.

[a,b,c]Mean values within a row with unlike superscripts differ ($P < 0.05$).

[4]DM, dry matter; N, nitrogen; SFA, saturated fatty acids; UFA, unsaturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, total polyunsaturated fatty acids; TFA, total fatty acids; SDF, soluble dietary fiber; IDF, insoluble dietary fiber; TDF, total dietary fiber.

6.4.5. Ileal, cecal and fecal flow of fatty acids

There were interactions ($P < 0.05$, **Table 6.9**) between dietary fiber and lipid type on C8:0, C15:0, C16:1t, C16:1n-7, C17:0, C18:0, C18:1n-9, C18:1n-7, C18:2, C18:3n-6, C20:0, C20:0, C20:3n-3 and C22:0. In this regard, the ileal flow of 8:0 was greater ($P < 0.05$) in pigs fed cellulose and beef tallow-containing diet than in pigs that consumed the other diets. The ileal flows of C15:0, C16:1t, C17:0, C20:2 and C22:0 were greater ($P < 0.01$) in pigs fed pectin-containing diets than in pigs fed cellulose-containing diets. The ileal flow of 16:1n-7 was greatest ($P < 0.001$) in pigs fed the pectin and beef tallow-containing diet than in pigs offered the other diets, but pigs that consumed cellulose-containing diets had similar ileal flows of C16:1n-7. The ileal flow of 18:0 was lower ($P < 0.01$) in pigs fed diets supplemented with beef tallow compared with those fed diets supplemented with corn oil. The ileal flow of C18:1n-9 was greater ($P < 0.001$) in pigs fed the pectin and beef tallow-containing diet compared to pigs offered the other diets, however, those that consumed the cellulose and corn oil-containing diet had lower ileal flows of C18:1n-9 than the cellulose and beef tallow-containing diet-fed pigs.

The ileal flow of C18:1*n*-7 was greater ($P < 0.05$) in pigs fed beef tallow-containing diets than in pigs fed diets containing corn oil. The ileal flows of C18:2 were greater ($P < 0.01$) in pigs fed the cellulose and beef tallow- or pectin and corn oil-containing diets compared to pigs that consumed the pectin and beef tallow-containing diet or the cellulose and corn oil-containing diet. The ileal flow of C18:3*n*-6 was greater ($P < 0.05$) in pigs fed beef tallow-containing diets than in pigs fed the other diets, but among cellulose-containing diets the flows of C18:3n-6 was depressed more in corn oil-fed pigs. The ileal flow of C20:0 in pigs fed pectin and corn oil-containing diets was greater ($P < 0.05$) than in pigs fed the other diets. The ileal flow of C20:3*n*-3 in pigs fed cellulose and corn oil-containing diet was lower ($P < 0.05$) compared with pigs consuming the

other diets. The ileal flow of C20:5n-3 in pigs offered cellulose and corn oil-containing diet tended to be greater ($P = 0.095$) than in pigs fed the other diets. There were no differences ($P > 0.10$) in the ileal flows of C10:0, C12:0, C14:0, C14:1, C16:0, C20:1, C20:3n-6, C22:5n-6, C22:1n-9, C24:1n-9, C22:6n-3, C24:0 and C20:4n-6 among all diets.

In the cecum, an interaction ($P < 0.01$; **Table 6.10**) of dietary fiber and lipid type was observed on the flows of C8:0, C10:0, C14:0, C14:1, C15:0, C16:0, C16:1t, C16:1n-7, C17:0, C18:0, C18:1n-9, C18:1n-7, C18:2, C18:3n-6, C18:3n-3, C20:0, C20:1, C20:2, C20:3n-3, C20:4n-6, C22:0, C22:1n-9, C22:5n-3, C24:0 and C24:1n-9. In this case, the cecal flow of C8:0, C10:0, C14:0, C14:1, C15:0, C16:0, C18:1n-7, C18:3n-3, C22:0, C22:1n-9, and C22:5n-3 were greater ($P < 0.05$) in pigs fed pectin and beef tallow-containing diet than in pigs fed the other diets. The cecal flows of C16:1t, C16:1n-7, C17:0, C18:1n-9, C18:3n-6, C20:1 and C20:2 in pigs fed cellulose-containing diets were lower ($P < 0.05$) compared to those fed pectin-containing diets. The cecal flows of C18:0 in pigs fed pectin-containing diet were lower ($P < 0.01$) compared to those fed cellulose-containing diets.

The cecal flows of C18:2 and C20:0 in pigs fed diets containing cellulose were lowest ($P < 0.05$), followed by those fed the pectin and beef tallow-containing diet and greatest in pigs that consumed the diet containing pectin and corn oil. The cecal flows of C20:3n-3, C20:4n-6 and C24:1 in pigs fed pectin-containing diets were greater ($P < 0.05$) compared to those fed cellulose-containing diets. The cecal flows of C24:1n-9 in pigs fed corn and beef tallow- and pectin and corn oil-containing diets were lower ($P < 0.01$) compared to those fed cellulose and corn oil- and pectin and beef tallow-containing diets. However, there were no interactive effects of dietary fiber and lipid type that were observed on the cecal flows of C12:0, C20:3n-6, C20:5n-3, C22:4n-6 and C22:6n-3.

Table 6.9. Ileal fatty acid flow in pigs fed the experimental diets[1]

Item	Diet[2]				SEM	P[3]		
	CC	CB	PC	PB		Fiber	Lipid	F×L
Caprylic (C8:0)	0.11[c]	3.88[a]	0.53[b]	0.08[c]	0.146	0.001	0.015	0.023
Capric (C10:0)	0.00	0.02	0.01	0.00	0.007	0.846	0.429	0.111
Lauric (C12:0)	0.01	0.02	0.01	0.01	0.163	0.384	0.358	0.365
Myristic (C14:0)	0.11	0.26	0.17	0.17	0.159	0.399	0.456	0.155
Myristoleic (C14:1)	0.00	0.01	0.01	0.10	0.067	0.846	0.379	0.371
Pentadecyclic (C15:0)	0.01[b]	0.01[b]	0.02[a]	0.02[a]	0.004	0.071	0.878	0.001
Palmitic acid (C16:0)	9.13	12.84	13.92	12.17	3.010	0.186	0.596	0.372
Palmitelaidic (C16:1t)	1.60[b]	2.73[b]	7.42[a]	9.12[a]	2.254	0.043	0.633	0.001
Palmitoleic (C16:1, n-7)	0.05[c]	0.11[c]	0.20[b]	0.50[a]	0.165	0.001	0.258	<0.001
Margaric (C17:0)	0.46[b]	0.55[b]	1.55[a]	1.64[a]	0.146	0.025	0.849	<0.001
Stearic (C18:0)	1096.6[a]	547.8[b]	875.6[a]	407.5[b]	113.04	0.001	0.011	0.001
Oleic (C18:1, n-9)	284.8[d]	771.0[b]	648.0[c]	1137.0[a]	35.18	0.001	0.581	<0.001
Vaccenic (1C8:1, n-7)	14.53[c]	115.71[a]	81.38[b]	130.49[a]	12.478	0.011	0.034	0.043
Linoleic (C18:2)	8.13[c]	78.27[a]	79.18[a]	43.66[b]	6.545	0.031	0.777	0.001
Linolenic (C18:3, n-6)	0.08[d]	2.06[b]	1.31[c]	5.21[a]	0.123	0.024	0.042	0.048
α-Linolenic (C18:3, n-3)	0.01[b]	0.03[b]	0.04[b]	0.58[a]	0.234	0.024	0.682	0.079
Arachidic (C20:0)	0.44[b]	1.03[b]	2.02[a]	1.06[b]	0.354	0.083	0.094	0.024
Gadoleic (C20:1)	0.55[d]	3.39[a]	2.42[b]	0.84[c]	3.22	0.045	0.406	0.153
Eicosadienoic (C20:2)	0.04[b]	0.15[b]	0.19[a]	0.38[a]	13.04	0.001	0.878	0.001
Dihomo-γ-linolenic (C20:3, n-6)	0.01	0.01	0.01	0.03	0.015	0.248	0.189	0.348
Eicosatrienoic (C20:3, n-3)	0.02[b]	0.04[a]	0.04[a]	0.04[a]	0.008	0.051	0.347	0.031
Arachidonic (C20:4, n-6)	0.01[A]	0.03[B]	0.03[B]	0.04[B]	0.005	0.365	0.956	0.073
Eicosapentaenoic (C20:5, n-3)	0.01[B]	0.02[A]	0.01[B]	0.01[B]	0.004	0.489	0.564	0.095

	CC	CB	PC	PB				
Behenic (C22:0)	0.02[b]	0.02[b]	0.09[a]	0.09[a]	0.013	<0.001	0.932	<0.001
Erucic (C22:1, *n*-9)	0.10	0.05	0.12	0.02	0.072	0.745	0.792	0.244
Docosapentaenoic (C22:5, *n*-6)	0.08	0.11	0.08	0.05	0.064	0.024	0.682	0.129
Docosahexaenoic (C22:6, *n*-3)	0.01	0.06	0.01	0.01	0.004	0.738	0.439	0.735
Lignoceric (C24:0)	0.05	0.13	0.10	0.02	0.273	0.003	0.964	0.324
Nervonic (C24:1, *n*-9)	0.25	0.17	0.20	0.06	0.165	0.739	0.159	0.685

[1]Mean values with their pooled standard errors (n = 6 per treatment).

[2]CC, cellulose and corn oil-containing diet; CB, cellulose and beef tallow-containing diet; PC, pectin and corn oil-containing diet; PB, Pectin and beef tallow-containing diet.

[3]Contrast statements: Fiber = Cellulose vs. Pectin; Lipid = Corn oil vs. Beef tallow; F×L = Interaction of dietary fiber and lipid type.

[a,b,c]Mean values within a row with unlike superscripts differ ($P < 0.05$).

There was an interaction ($P < 0.05$; **Table 6.11**) between dietary fiber and lipid type on faecal flow of C8:0, C10:0, C12:0, C14:1, C16:1t, C16:1n-7, C18:2, C20:0, C20:3n-6, C20:5n-3, C22:1n-9, C22:5n-3 and C24:0. In this regard, the faecal flow of C8:0 in pigs fed pectin and corn oil-containing diet was greater ($P < 0.05$) than in pigs fed the other diets, although the flows of C8:0 in pigs fed cellulose-containing diets were similar. The faecal flows of C10:0, C16:1t, C16:1 and C20:3n-6 in pigs fed diets containing cellulose were lower ($P < 0.001$) than in pigs fed diets containing pectin. The faecal flow of C12:0 and C22:5n-3 was greater ($P < 0.05$) in pigs fed corn oil-containing diets compared to those fed diets containing with beef tallow. The faecal flow of C14:1 was greater ($P < 0.05$) in pigs fed the pectin and corn oil-containing diet than in pigs offered the diets supplemented with beef tallow and that in pigs which consumed cellulose and corn oil-containing diet was intermediate. The faecal flow of C18:2 and C20:0 in pigs fed the pectin and corn oil-containing diet was greater ($P < 0.05$) than in pigs fed the other diets. The faecal flow of C22:1n-9 in pigs fed the cellulose and corn oil-containing diet was lower ($P < 0.05$) than in pigs offered the other diets. Although there were no ($P > 0.05$) dietary effects on the faecal flow of C14:0, C15:0, C16:0, C17:0, C18:0, C18:0, C18:1, C18:1n-7, C18:3n-6, C18:3n-3, C20:1, C20:2, C20:4n-6, C22:4n-6, C20:5, C22:0 and C24:1n-9 ($P > 0.05$).

6.5. DISCUSSION

Given the importance of VFA and dietary lipids in pigs, it is crucial to understand the interactive effects of DF solubility and lipid types on production and absorption of VFA, FA flows, fermentability and digestibility of dietary components in different segments of the GIT. As expected, the diets containing pectin and those containing cellulose differed substantially in the content of SDF, IDF and TDF. Moreover, the slightly higher fat content in beef tallow-containing

Table 6.10. Cecal fatty acid flow in pigs fed the experimental diets[1]

Item	Diet[2]				SEM	P[3]		
	CC	CB	PC	PB		Fiber	Lipid	F×L
Caprylic (C8:0)	0.26[b]	0.22[b]	0.86[b]	11.52[a]	3.658	0.086	0.595	0.042
Capric (C10:0)	0.01[b]	0.00[b]	0.01[b]	0.03[a]	0.008	<0.001	0.296	<0.001
Lauric (C12:0)	0.00	0.01	0.01	0.02	0.011	0.254	0.745	0.648
Myristic (C14:0)	0.12[b]	0.09[b]	0.18[b]	0.29[a]	0.054	<0.001	0.878	0.001
Myristoleic (C14:1)	0.01[b]	0.01[b]	0.01[b]	0.63[a]	0.137	0.587	0.547	<0.001
Pentadecyclic (C15:0)	0.01[b]	0.01[b]	0.01[b]	0.32[a]	0.024	0.014	0.578	0.001
Palmitic acid (C16:0)	14.08[b]	13.37[b]	19.99[b]	33.34[a]	4.012	0.301	0.383	0.041
Palmitelaidic (C16:1t)	1.57[c]	2.04[c]	8.29[b]	19.89[a]	2.655	<0.001	0.228	0.011
Palmitoleic (C16:1n-7)	0.04[c]	0.03[c]	0.16[b]	0.38[a]	0.085	<0.001	0.972	0.033
Margaric (C17:0)	0.31[c]	0.29[c]	0.60[b]	1.15[a]	0.146	0.061	0.695	0.010
Stearic (C18:0)	1287[a]	1091[a]	750[b]	585[b]	130.42	<0.001	0.878	0.001
Oleic (C18:1n-9)	201[c]	346[c]	741[b]	1116[a]	139.42	<0.001	0.041	<0.001
Vaccenic (C18:1n-7)	14.1[b]	16.0[b]	92.8[b]	185.3[a]	28.38	<0.001	0.564	0.003
Linoleic (C18:2)	10.9[c]	7.08[c]	136.9[a]	54.5[b]	16.72	<0.001	0.932	<0.001
Linolenic (C18:3n-6)	0.03[c]	0.06[c]	1.39[b]	2.62[a]	0.634	0.024	0.635	0.029
α-Linolenic (C18:3n-3)	0.01[b]	0.01[b]	0.03[b]	0.19[a]	0.064	0.534	0.286	0.039
Arachidic (C20:0)	0.35[c]	0.36[c]	2.10[a]	0.89[b]	0.173	0.003	0.357	0.024
Gadoleic (C20:1)	0.94[c]	0.88[c]	1.66[b]	3.72[a]	0.222	0.045	0.406	0.043
Eicosadienoic (C20:2)	0.05[c]	0.07[c]	0.15[b]	0.27[a]	0.034	<0.001	0.878	0.001
Dihomo-γ-linolenic (C20:3n-6)	0.01	0.01	0.02	0.02	0.033	0.245	0.668	0.175
Eicosatrienoic (C20:3n-3)	0.01[b]	0.01[b]	0.05[a]	0.03[a]	0.018	0.012	0.511	<0.001
Arachidonic (C20:4n-6)	0.01[b]	0.01[b]	0.06[a]	0.04[a]	0.011	<0.031	0.581	0.001
Eicosapentaenoic (C20:5n-3)	0.01	0.01	0.01	0.01	0.012	0.121	0.478	0.313
Behenic (C22:0)	0.03[b]	0.01[b]	0.12[b]	0.30[a]	0.042	0.083	0.932	0.001
Erucic (C22:1n-9)	0.07[b]	0.07[b]	0.13[b]	0.41[a]	0.035	0.456	0.145	0.035
Docosatetraenoic (C22:4n-6)	0.00	0.00	0.01	0.01	0.002	0.035	0.541	0.468

Docosapentaenoic (C22:5n-3)	0.01[b]	0.02[b]	0.12[b]	0.28[a]	0.064	0.024	0.682	0.029
Docosahexaenoic (C22:6n-3)	0.01	0.01	0.02	0.02	0.003	0.365	0.245	0.258
Lignoceric (C24:0)	0.01[b]	0.01[b]	0.13[a]	0.15[a]	0.023	0.003	0.964	0.024
Nervonic (C24:1n-9)	0.33[a]	0.11[b]	0.25[b]	0.39[a]	0.073	0.045	0.011	0.042

[1]Mean values with their pooled standard errors (n = 6 per treatment).

[2]CC, cellulose and corn oil-containing diet; CB, cellulose and beef tallow-containing diet; PC, pectin and corn oil-containing diet; PB, Pectin and beef tallow-containing diet.

[3]Contrast statements: Fiber = Cellulose vs. Pectin; Lipid = Corn oil vs. Beef tallow; F×L = Interaction of dietary fiber and lipid type. [a,b,c]Mean values within a row with unlike superscripts differ ($P < 0.05$).

Table 6.11. Faecal fatty acid flow in pigs fed the experimental diets[1]

| Item | Diet[2] | | | | SEM | P[3] | | |
	CC	CB	PC	PB		Fiber	Lipid	F×L
Caprylic (C8:0)	0.46[b]	0.42[b]	0.61[a]	0.26[c]	0.129	0.001	0.595	0.041
Capric (C10:0)	0.23[b]	0.19[b]	0.35[a]	0.33[a]	0.031	<0.001	0.429	<0.001
Lauric (C12:0)	3.17[a]	1.61[b]	3.05[a]	1.69[b]	0.475	0.325	0.976	<0.001
Myristic (C14:0)	26.96	14.17	20.12	21.90	5.623	0.456	0.878	0.563
Myristoleic (C14:1)	8.88[ab]	3.19[b]	11.45[a]	4.57[b]	3.125	0.069	0.036	<0.001
Pentadecyclic (C15:0)	42.33	20.03	32.26	28.06	7.634	<0.001	0.014	0.751
Palmitic acid (C16:0)	537.1	410.9	443.6	464.4	72.36	<0.001	0.799	0.101
Palmitelaidic (C16:1t)	0.22[b]	0.25[b]	0.79[a]	0.55[a]	0.136	0.446	0.633	<0.001
Palmitoleic (C16:1n-7)	1.38[b]	1.08[b]	2.68[a]	2.85[a]	0.357	0.369	0.489	<0.001
Margaric (C17:0)	38.95	21.10	25.69	26.29	14.332	0.098	0.695	0.631
Stearic (C18:0)	671.6	740.9	588.8	654.9	75.04	<0.001	0.878	0.151
Oleic (C18:1n-9)	92.5	90.0	172.5	82.3	115.12	0.745	0.581	0.761
Vaccenic (C18:1n-7)	10.03	8.09	9.38	14.27	4.122	0.332	0.564	0.156
Linoleic (C18:2)	14.4[b]	18.6[b]	99.5[a]	13.8[b]	16.72	0.864	0.932	<0.001
Linolenic (C18:3n-6)	1.79	0.68	0.87	1.36	2.171	0.385	0.111	0.458
α-Linolenic (C18:3n-3)	3.05	2.18	1.09	3.87	4.243	0.824	0.333	0.564
Arachidic (C20:0)	25.06[b]	28.23[b]	36.08[a]	27.73[b]	2.735	0.687	0.964	0.032
Gadoleic (C20:1)	7.76	5.62	11.00	7.44	3.223	0.045	0.406	0.153
Eicosadienoic (C20:2)	0.33	0.24	0.96	0.59	0.354	0.354	0.878	0.381
Dihomo-γ-linolenic (C20:3n-6)	0.00[b]	0.02[b]	0.12[a]	0.06[a]	0.021	0.375	0.406	0.024
Eicosatrienoic (C20:3n-3)	0.55	0.71	0.92	0.84	0.258	<0.001	0.041	0.101
Arachidonic (C20:4n-6)	0.12	0.16	0.79	0.49	0.522	<0.001	0.581	0.521
Eicosapentaenoic (C20:5n-3)	0.08[b]	0.04[b]	0.33[a]	0.00[b]	0.022	<0.001	0.034	0.043
Behenic (C22:0)	8.41	10.28	16.58	11.92	6.679	<0.001	0.014	0.201
Erucic (C22:1n-9)	0.67[a]	0.07[b]	0.53[a]	0.73[a]	0.272	0.348	0.932	<0.001
Docosatetraenoic (C22:4n-3)	0.00[b]	0.07[b]	0.28[a]	0.00[b]	4.231	0.824	0.333	0.564

Docosapentaenoic (C22:5*n*-3)	43.72[a]	22.55[b]	37.89[a]	23.45[b]	6.112	0.024	0.682	0.011
Lignoceric (C24:0)	8.70[b]	0.07[c]	24.37[a]	10.49[b]	2.733	0.003	0.079	0.024
Nervonic (C24:1*n*-9)	3.84	2.56	4.36	4.39	2.734	0.633	0.484	0.578

[1]Mean values with their pooled standard errors (n = 6 per treatment).

[2]CC, cellulose and corn oil-containing diet; CB, cellulose and beef tallow-containing diet; PC, pectin and corn oil-containing diet; PB,

Pectin and beef tallow-containing diet.

[3]Contrast statements: Fiber = Cellulose vs. Pectin; Lipid = Corn oil vs. Beef tallow; F×L = Interaction of dietary fiber and lipid type.

[a,b,c]Mean values within a row with unlike superscripts differ ($P < 0.05$).

diets than in corn oil-containing diets is attributable to high inclusion in diet formulation to achieve an iso-caloric value across all diets as it is well-known that beef tallow has a lower energy value than corn oil. However, the total FA were similar among all diets but the profiles for the content of SFA, UFA, MUFA and PUFA greatly differed for diets supplemented with beef tallow compared with diets supplemented with corn oil. These differences were largely due to the result of adding beef tallow which is characterized by a high SFA and MUFA, in comparison with corn oil that is rich in PUFA. The FA profiles of the diets in this experiment were similar to those reported by Reese (2003). However, although the SFA composition of diets containing beef tallow in this study were slightly below the values reported by Świątkiewicz et al. (2016) but the UFA, MUFA, PUFA and iodine values were considerably higher compared with those reported in the same study. The differences in the calculated iodine values between diets supplemented with tallow and those supplemented with corn oil is attributed to the differences in the concentrations of FA between these two lipid-rich ingredients.

The difference in the solubility of dietary fiber sources or NSP compositions explains the higher cecal VFA concentrations after *in vitro* fermentation for pectin-containing diets than for cellulose-containing diets. Insoluble fiber is generally less fermentable than soluble fiber (Agyekum and Nyachoti, 2017). Pectin is a soluble and branched polymer of galacturonic acids that are linked by α-(1→4) glycosidic linkages. Conversely, cellulose is classified as insoluble fiber and is made up of glucose units that are linked by β-(1→4) bonds. More appealing evidence of the interaction of DF and lipid type was observed on a similar trend noticed for the predicted production of cecal and colonic VFA except for propionic acid. In this regard, the predicted production and absorption of propionic acid was lower for beef tallow-supplemented diet compared with corn oil-enriched diets, across all pectin-containing diets. A similar trend by which

animal fat depressed VFA production and absorption was also noticed for cellulose-containing diets wereby the predicted production and absoption of colonic butyric acid was lower for diets supplemented with beef tallow compared with corn oil-supplemented diets. It is possible that FA in beef tallow depressed some fibrolytic activities by forming a film of adsorbate on the surface of the fiber matrices, resulting in slow caption of amino acids and production of ATP needed to support bacterial fermentation (Galbraith and Miller, 1973). Moreover, the galacturonic acids of pectin are likely to be responsible for the high proportion of acetic acid in pigs fed pectin-containing diets (Drochner et al., 2004). Supporting our findings are reports by Englyst et al. (1987) that fermentation of pectin produced acetate and Wang et al. (2004) also reported corroborating findings that *in vitro* incubation of ileal digesta of pigs fed 120g/kg DM sugar beet pulp contains considerable great proportions of pectin (Drochner et al., 2004; Pecka-Kielb et al., 2016), yielded greater proportions of acetic acid.

The respective values for the predicted production of total VFA in the cecum of pigs fed pectin-containing diets supplemented with corn oil and beef tallow were 806 and 683 mmol/kg DMI, and higher than those for cellulose-containing diets which were 210 and 211 mmol/kg DMI, respectively. In the colon, the respective amounts of the predicted production of total VFA for pectin-containing diets supplemented with corn oil and beef tallow were 507 and 446 mmol/kg DMI, and higher than those for cellulose-containing diets that were 175 and 133 mmol/kg DMI, respectively. Our findings from Chapter 5, Christensen et al., 1999 and Montoya et al., 2016 also, using a combined in vivo (ileal-cannulated pigs) and *in vitro* technique (faecum inoculum), reported lower values of predicted hindgut production of total VFA than in this current experiement. In Chapter 5 we used flaxseed meal and oat hulls and the prediced VFA produced ranged between 540 and 1340 mmol/kg DMI and the other studies used wheat flour, wheat bran

and oat bran as fiber sources and VFA produced ranged from 369–850 mmol/kg DMI. The difference in the amounts of predicted hindgut production of VFA reported in Chapter 5, Christensen et al. (1999) and Montoya et al. (2016) and predicted cecal or colonic production of VFA in our studies may be explained partly by the difference in the inclusion level and type of fiber sources used as well as the differences in sources of inoculum and substrate used during the *in vitro* fermentation assay. Previous studies inoculated ileal digesta using feces (Giusi-Perier et al., 1989; Christensen et al., 1999; Montoya et al., 2016) but to the best of our knowledge, this experiment is the first in which fresh cecal digesta and feces were used to inoculate ileal and cecal digesta, respectively.

To the best of our knowledge, several studies incuding the experiment reported in Chapter 5 have predicted the production and absorption of VFA in the hindgut (Giusi-Perier et al., 1989; Christensen et al., 1999; Montoya et al., 2016) but this is the first study to estimate production and absorption of VFA in the cecum and colon of pigs. The VFA were produced and absorbed in greater proportions, irrispective of the diet, more in the cecum compared with the colon. Previous studies have demonstrated that bacterial population, fermentation and proliferation are greatest in proximal regions of the GIT where substrate is in high concentration (Topping and Clifton, 2001). Therefore, a decline in VFA concentrations in distal regions corroborates the notion that the substrate has been depleted and that bacterial population size or composition changed during transit in response to changes in substrate supply. These results are supported by a trend reported by Jaworski and Stein (2017) which indicated that soluble fiber is most fermented before it reaches the colon. By extrapolation of data for VFA production in cecum and colon, the normalized faecal concentration of VFA represented approximately 2.1–2.7% and 1.1–1.2% of the predicted hindgut (cecum + colon) VFA production when examined for cellulose- and pectin-containing diets,

respectively. The amounts of the normalized faecal concentrations of VFA in the present study are comparable with the values (0.5–1.6%) reported by Montoya et al. (2017) for ileal cannulated pigs fed 25–50 g/kg of kiwifruit fiber. These concentrations agree with the previous notion by Den Besten et al. (2013) that approximate 95% of the total VFA produced in the hindgut are absorbed in the GIT. Although interactions between DF and lipid type were observed on the concentration of acetic acid in the feces, ileal, and cecal contents, it is important to note that the concentration of acetic acid in cecal digesta contents was higher for cellulose and beef tallow-containing diet compared to cellulose and corn oil-containing diet. The higher concentration of acetate in the former could be attributed to the assumption that gastrointestinal walls were not adapted to increase absorption of VFA as evidenced by a decrease in the determined and predicted VFA production, in beef tallow-supplemented diets, especially in the colon. However, more work is needed to investigate interaction of dietary fiber and lipid type on gene expression of transporters or assess absorption kinetics for VFA using other *in vitro* methodology such as the everted sac technique or the Ussing chamber. Interestingly, relative to the total predicted production of total VFA, the values for normalized faecal VFA concentration are far too low. Owing to this and the within treatment differences observed between acetic and butyric acids in the ileum, cecum and feces, the use of intestinal or faecal concentrations of VFA to describe VFA production can be misleading. However, the values could be used as reliable estimates for the quantity of the unabsorbed VFA rather than VFA production. Our findings also supports the reports (McNeil et al., 1978; Cummings and Macfarlane, 1991; Topping and Clifton, 2001; Montoya et al., 2016), all of which have strongly emphasized that VFA flows and concentrations are not accurate discriptors for VFA production.

The predicted amounts of VFA absorbed in the cecum and acetic and valeric acids in the colon were higher for diets containing pectin than for cellulose-containing diets. It is noteworthy that values for predicted apparent VFA absorption in the cecum and colon for cellulose containing diets (75.1-95.4%) in this study were lower but those for pectin-containing diets were within the range of 95-99.8% reported in Chapter 5, Roy et al. (2006), Nakamura et al. (2010) and Montoya et al. (2016), for the entire hindgut. The difference in the extend of absorption can be explained by the difference in the amounts of butyric acids produced that were lower for cellulose-containing diets compared with pectin-containing diets. For cellulose-containing diets, production and absorption of butyric acid in the colon was depressed even more in diets supplemented with beef tallow compared to those fortified by corn oil. Butyric acid is an immediate progenator for energy needed to boost absorption by colonocytes (Bergman, 1990). Thus, it can be speculated that a decrease in intestinal butyric acid fluxes could have contributed to reduction in the extent of absorption of other VFA in cellulose diets.

Evidence exists on interactions between DF and lipid type on the determined apparent digestibility and fermentability of OM in GIT segments. In this regard, the predicted and determined digestibilities and fermentabilities of OM showed a similar trend in which pectin-containing diets were degraded at a greater extent compared to cellulose-containing diets. It is noteworth that for pectin-containing diets, the determined fermentability in the ileum, the predicted fermentability in the cecum and the total tract digestibility of OM were lower in diets supplemented with beef tallow compared to those fortified with corn oil. These differences support the same trend observed in this study for the predicted amounts of propionic acids in the cecum and colon, and the quantities of acetic and propionic acids in the colon. As mentioned above, reduction is fermentability could also be ascribed to the masking effects that saturated fatty acids in beef tallow

impose on the fiber matrices thereby inhibiting microbial degradation (Galbraith and Miller, 1973). In general, all the predicted and determined total tract digestibilities of OM were similar statistically, supporting the application of the combined *in vivo-in vitro* methodology (Coles et al., 2013; Montoya et al., 2016) to assess fermentability of dietary components. However, the differences between the determined and predicted total tract digestibilities for cellulose-containing diets and cecal fermentability of OM across all diets is difficult to explain.

The observation that AID of DM was greater and ACD of DM tended to be greater in pectin-containing diets compared with cellulose-containing diets is in agreement with findings by Drochner et al. (2004). Drochner et al. (2004) predicted that the extent of depression of organic matter digestibility when 5% pectin is included in a diet will not be as high as with cellulose. Our data show that AID, ACD and ATTD of IDF and TDF fractions in diets containing pectin were greater compared with cellulose-containing diets, which is in line with previous observations on the uniqueness of the constituent monomers between the two polysaccharides. Unlike cellulose which is made up of sugars joined together to give it a hydrophobic and crystalline structure that is not accessible to microbial degradation, pectins are polymers of galacturonic acids that are soluble and accessible to microbial fermentation (Bach Knudsen and Hansen, 1991; Wilfart et al., 2007). The greater AID, ACD and ATTD of insoluble and TDF fractions in the present study for pectin-containing diets than in cellulose containing diets may be related in part to the reduction of digesta passage, resulting in more complete degradation of the DF constituents (Mateos et al., 1982).

Interestingly, the interactive effects of DF and lipid type on AID of SDF in the diet containing pectin and corn oil was lowest, followed by cellulose diets, compared to the diet containing pectin and beef tallow that was greatest. In this case, the observation that addition of corn oil depressed

fermentabilty was unexpected and contradicts the observation that beef tallow supplementation reduced the amounts of predicted cecal propionic acid produced, and predicted propionic and acetic acids produced in the colon. Considering that we did not estimate VFA production in the ileum as illustrated in the companion paper (**'?' in Figure 6.2**), this paradoxical relationship is difficult to explain. Moreover, the contradiction is exacerbated more by the observation that ACD of soluble DF is greater in cellulose-containing diets compared with pectin-containing diets but when compared with corn oil beef tallow supplementation reduced fermentability among all diets regardless of the DF source. Fatty acids in beef tallow depressed some fibrolytic activities by forming films of adsorbate on the surfaces of the fiber matrices, resulting in slow caption of amino acids and production of ATP needed to support bacterial fermentation (Galbraith and Miller, 1973). These discrepancies are attributable to the variation in microbial population and diversity in the ileum, cecum and colon and further suggests that fermentability of different DF fractions differs depending on the segment of the gut as well as level of saturation of the added dietary lipid. Thus, one plausible explanation for the reduced fermentability of SDF fractions in pectin-containing diets that were fortified with corn oil compared with those supplemented with beef tallow is most likely that some of FA in corn oil are bound within the fiber matrices thus depressing microbial degradation (Adam and Jensen, 1984). Furthermore, it is well established that the concentration and source of DF enormously influence digestibility of different DF fractions (Just et al., 1980; Hansen et al., 2006; Jarwoski and Stein, 2017), however, data to support this phenomenon on the effects of dietary lipid saturation on degradability of soluble or insoluble DF fractions in different segments of the lower gastrointestinal tract is scantily documented. Thus, the lower AID of SDF fractions in pectin and corn oil diets and reduced ACD of soluble DF components in pectin-containing diets compared
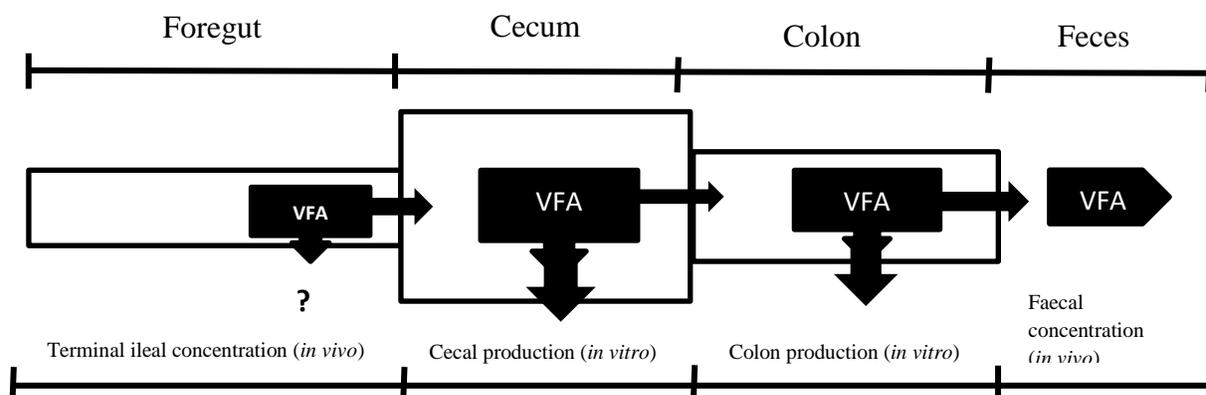
Figure 6. 2. Principle of the in vivo-in vitro methodology to determine production and absorption of VFA in the cecum and colon. The '?' represents the VFA absorbed in the foregut, which were not measured in the current study (modifications of schematic diagram reported by Montoya et al. (2016).

with cellulose-containing diets was unexpected, but could be ascribed to the lower concentration of analyzed soluble DF fractions in cellulose-containing diets compared with pectin diets that was competitively available for fermentation. Thus, gastrointestinal microbiota had a greater effect on apparent fermentability at smaller amounts of SDF than at greater amounts.

The apparent digestibility of TFA at the end of the ileum and over the entire gastrointestinal tract was lower for pectin-containing diets than for diets containing cellulose. This is likely a result of greater concentration of soluble DF fractions in digesta of pigs fed diets containing pectin compared to those fed diets containing cellulose. Our findings are in agreement with observations by Drochner (2004) who observed that pectin depressed fat digestibility by binding bile acids and reducing their capacity to emulsify fats. Moreover, the finding in the present study that digestibility of lipids was further depressed in pectin and beef tallow-containing diets compared with pectin and corn oil-containing diets can be ascribed to the presence of C16:0 and C18:0 that contribute approximately 35% SFA in beef tallow. These findings are consistent with previous reports that AID of SFA decrease with increasing chain lengths (Cera et al., 1988; 1989). Moreover, heating of lipids reduces apparent digestibility because of oxidation and polymeration of the FA in the fat (Kim et al., 2013; González-Muñoz et al., 2003). It is, therefore, highly likely that the heating (60°C) taking place to liquefy the beef tallow during packaging or feed mixing contributes to the reduced digestibility in diets that are fortified with bovine fat compared with those supplemented with corn oil. Dietary supplementation with beef tallow increased the ileal digestibility of SFA, but the increment was pronounced more in pectin-containing diets compared with cellulose-containing diets. Because a greater proportion of pectin disappears in the small intestines implying that there is less interaction between DF fractions from pectin and the SFA, yet in cellulose diets more IDF fractions from cellulose reaches the distal regions of the intestines.

Previous reports by Pomerenke et al. (2003) and Davis et al. (2015) reported that addition of 30% insoluble DF from DDGS reduced digestibility of C16:0 and total SFA in comparison with addition of 0 or 5% fiber from DDGS, which was likely due to an interaction between DF and FA. Kim et al. (2013) also proposed that fiber matrix surround the triglycerides and consequently impede the digestion process by preventing lipases from assessing the lipid. Therefore, observations in the current study and the previous studies strongly support the notion that digestibility and absorption of SFA are reduced when tallow is added to a diet that is rich in insoluble DF compared with one supplemented with soluble DF. The same phenomenon was also noticed with apparent ileal digestibility of PUFA whereby addition of tallow in pectin diets increased PUFA digestibility but supplementation with cellulose depressed digestibility. Furthermore, our findings are also in agreement with previous reports suggesting that digestibility of SFA and C16:0 in particular is greater in beef tallow-containing diets compared with other vegetable-based lipid sources such as canola, sunflower and flaxseed oils (Ozimek et al., 1984; Duran-Montgé et al., 2011; Mitchaothai et al., 2007). Another plausible explanation for the decrease in the digestibility of SFA in diets supplemented with corn oil could be due to an increase in the ileal flow of C18:0 as a result of its formation from biohydrogenation processes of PUFA, and specifically C18:2 which increased in the gastrointestinal tract (Martínez-Ramírez et al., 2013). The dietary level of PUFA were high in corn oil-supplemented diets in the present study, however, biohydrogenation rates of PUFA are pronounced more in the hindgut compared to the distal ileum (Jørgensen et al., 2000b; Duran-Montgé et al., 2011).

It is well-established that MUFA represent over 50% of the total lipids in pork fat deposits (Xu et al., 2010) and represented almost a third of total FA in corn oil-supplemented diets and approximately one-half of the FA in beef tallow-containing diets in the present study. Unlike the

trends observed with ileal digestibility of SFA and PUFA it is difficult to explain why cellulose and corn oil-containing diet and pectin and beef tallow-containing diet had greater ileal digestibility values when compared with the other diets. However, the AID of MUFA ranged from 86.5 to 91.6% among all diets in the present studies is in agreement with the reported range of 75 to 99% (Jørgensen et al., 1993; 2000). Moreover, the observation that SFA were numerically less digestible compared with MUFA and PUFA agrees with previous reports (Jørgensen et al., 1993; Overland et al., 1994; Powles et al., 2003). This is ascribed to the postulation that due to their lipid structure, SFA have a lower ability to form micelles and cross the water layer compared with MUFA or PUFA (Jørgensen et al., 1993; Overland et al., 1994; Powles et al., 2003).

Apart from other dietary factors, the digestibility of lipids in the hindgut is influenced by a number of factors including endogenous secretion from mucosal cells, sloughing of epithelial cells, or *de novo* synthesis by microbial communities in the large intestines (González-Muñoz et al., 2003). It is interesting to note that regardless of the lipid or fiber source, the ACD of lipids was lower than the AID of lipids in all diets. The lower digestibility of ACD of lipids can be attributed to the effects of DF that initiated inefficient digestibility and sub-optimal absorption of dietary fat due to deconjugation of bile acids by increased bacterial activity in the intestinal lumen (Chapter 4 and 5). These processes reduce the solubility and emulsifying capacity of bile acids, and consequently they are bound to bacterial cells and DF thereby increasing their excretion and that of FA (Chapter 4; Chapter 5). Supporting these notions is the observed intestinal flow of FA that were virtually absent in the diets but increased in different sections of the gastrointestinal tract. The flow of FA such as C8:0, C17:0, C18:3n-6, C20:3n-3, C18:1n-7, C20:4n-6 and C24:1n-9 increased at the end of the ileum. The increase in the flow of n-3 and n-6 FA observed in the current study is in agreement with findings reported by Martinez-Ramirez et al. (2013) and these

indicate a likelihood of activity of the Δ6- and Δ5-desaturases and chain-elongases in the upper sections of the GIT of the pig. More work is needed to explain the role played by gastrointestinal microbiota in elongating or desaturating FA such as C18:2n-6 and C18:3n-3 and the extent by which microbial fermentation contributes to an increase in the presence of UFA in the digesta of pigs. There was an increase in the cecal flow of C18:3n-6, C20:3n3, C20:4n-6 and C22:5n-6 more in pigs that were fed pectin containing diets compared with those fed cellulose-containing diets suggesting that microbiota played a central role in the production of these long-chain FA. Nevertheless, even though long-chain FA cannot be absorbed in the hindgut, their production is obviously irrelevant to the NEFA pool in the porcine blood but could indirectly affect lipid metabolism by influencing microbial activity that play a central role in VFA production. Thus, the increase in flow of these FA could also be attributed to the action of microbiota such as *Butyrivibrio fibrisolvens* through biosynthesis or other unknown bacteria that can facilitate the biodehydrogenation processes (Martinez-Ramirez et al., 2013).

The ATTD of lipids was lower than ACD of lipids, except for diets containing cellulose and corn oil which had a greater ATTD of lipids than ACD of lipids in other experimental diets. The observation that the flow of C18:1 decreased along the gut occurred at the same time when C18:2 increased supports a fact that is well-established in ruminants that feeding high-fiber diets stimulated the synthesis of the former through Δ9 desaturation from C18:1 precursor (Bauman and Griinari, 2003). However, the increase in digestibility of lipids in diets containing cellulose and corn oil can be ascribed to the decrease in the flow of FA in the colon. Moreover, the lower ATTD of lipids in pectin containing diets can be partly attributed to the content of mucilage in pectin or microbial production of FA following conversation of SCFA into elongated forms of FA or the contribution of endogenous losses of FA from the host to the intestinal flow of FA.

In general, the AID of DF was lower than the ATTD values, whereas values for ACD of DF were intermediate. The ileal fermentability of DF fractions in the current study were expected and are consistent with reports by Bach Knudsen et al. (2013) and Jarwoski and Stein (2017) who reported that fermentability of NSP in pigs fall below 40%. Although the marginal differences between AID and ACD of DF fractions were pronounced more for IDF fractions in pectin-containing diets compared with cellulose-containing diets, they are in agreement with the values reported by Jarwoski and Stein (2017) that a significant part of DF fermentation occurs in the cecum. These authors also mentioned that the fermentability of DF fractions varies along the GIT. However, our data indicates that the source of lipids supplemented in high-fiber diets also influence the extent of DF fermentation.

In conclusion, dietary fiber fermentability, and production and absorption and of VFA in the cecum and colon of pigs depends on the source of the lipid. The predicted amounts of VFA produced and absorbed and fermentability of OM in the cecum and colon is greater in pigs fed pectin-containing diets compared with those fed cellulose-containing diets. Moreover, considerable quantities of butyric and propionic acids are produced and absorbed at lower proportions in diets enriched with beef tallow compared with those supplemented with corn oil. The interaction between DF solubility and lipid saturation modulates digestibility of lipids, FA flows and fermentability of DF fractions but differs for soluble and insoluble fiber, saturated and unsaturated fatty acids and varies in different gastrointestinal segments. The digestibility of lipids is depressed and FA flows are increased in pectin-containing diets but more in diets supplemented with beef tallow compared with all cellulose containing diets. Soluble fiber fractions from both pectin-and cellulose-containing diets are mostly digested in the upper gut and cecum but insoluble fiber from cellulose diets is fermented the most in the cecum and colon.

LIMITATIONS AND IMPLICATIONS OF CHAPTER 6

Although this study had some limitations, its results also have implications that could be of interests not only in swine nutrition but also in human nutrition, especially in understanding the interactions between DF solubility and the degree of saturation of FA in dietary lipids. One limitation was that, to achieve isocaloric and iso-nutritious diets in feed formulation, 150 g/kg of either pectin or cellulose was included in each diet resulting in a higher content of TDF in the cellulose-containing diet compared to the pectin-containing diet. Another limitation was that there is a general increase in the flow of FA from one segment to the other, but it is still difficult to identify the actual source of lipids excreted in the terminal ileum, cecum or feces. This implies that the use of isotope-labelled FA would open way for future studies to pinpoint if FA in different gastrointestinal segments originates from the diet, endogenous secretions, or microbial activity following deconjugation of BA and/or microbial bioconversion of dietary FA or endogenous lipids into microbial FA.

CHAPTER SEVEN

MANUSCRIPT 4

Flaxseed meal and oat hulls modulates colonic volatile fatty acids transport and metabolism and expression of genes for lipid metabolism in colonic and hepatic tissues of growing pigs [1]

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[1]Materials presented in this chapter are under review as a manuscript in Livestock Science journal. The authors of the manuscript are S. P. Ndou, S. Liu, E. Kiarie, C. Yang and C. M. Nyachoti. (**Paper 7**)

S.P.N. and C.M.N. designed the experiment. S.P.N conducted the animal study, lab and statistical analyzes, analyzed the data and wrote the manuscript. S.P.N., S.L. and C.Y. performed the molecular lab analyses. E.K., S.L., C.Y., and C.M.N. critically reviewed the manuscripts. C.M.N. was the principal investigator who supervised all aspects of the study.

7.1 ABSTRACT

This study investigated the effects of soluble fiber from flaxseed meal and insoluble fiber from oat hulls on transepithelial absorption and intraepithelial metabolism of volatile fatty acids (VFA) in the colon and expression of colonic and hepatic genes involved in lipid metabolism in pigs. A total of 48 barrows were housed in pairs and assigned to three corn-soybean meal-based diets without (control), or with flaxseed meal (FM) or oat hulls (OH), in a completely randomized design for 28 d. On d 29 and 30, pigs were slaughtered to isolate colon tissues for everted sac technique, and both hepatic and colonic tissue for RNA isolation for assessing gen expression. The mucosal disappearance and catabolic loss of acetic and butyric acids and serosal release of colonic acetic acid, and colonic expressions of mucin 2, free fatty acid receptor 2 and liver-type fatty acid binding protein genes was higher ($P < 0.05$) for the FM and OH diets compared to the control diet. The mucosal disappearance and catabolic loss of propionic acid and expression of peptide YY in the colon, and cytochrome P450 2C49 and free fatty acid receptor 3 in the liver was higher ($P < 0.05$) for the FM diet compared to the OH diet. Expression of colonic glucagon-like peptide-2 receptor was suppressed ($P < 0.05$) for the FM diet relative to the control and OH diets. In conclusion, consumption of soluble fiber from flaxseed meal and insoluble fiber from oat hulls comparatively modulated the adaptive capacity of colon epithelium and hepatocytes to transport and metabolize VFA. Soluble fiber from flaxseed meal intake increased expression of genes related to energy and lipid metabolism compared to insoluble fiber from oat hulls.

Key words: bile acids, energy metabolism, growing pigs, soluble and insoluble fiber.

7.1. INTRODUCTION

In recent years, the use of alternative cost-effective feed ingredients such as agro-industrial co-products has significantly increased in pig diets resulting in increased dietary fibre (**DF**). It is, however, intriguing that nutrient utilization and performance are depressed if pigs consume nutritionally balanced high-fiber diets that are composed of co-products plus supplementary fat (Bakker, 1996; O'Doherty et al., 2002; Ball et al., 2010). Dietary supplementation with soluble fiber from flaxseed meal depressed growth performance more than insoluble fiber from oat hulls (Chapter 4). In contrast, in Chapters 4 and 5 we also observed that both flaxseed meal and oat hulls decreased fat digestibility and serum lipids, but induced variable effects on the concentrations of microbial metabolites, histomorphological characteristics, and digesta- and mucosa-associated microbiota in growing pigs' intestine. Previous studies including our experiment in Chapters 5 and 6 of this thesis have also demonstrated that dietary inclusion of increasing levels of fibrous ingredients such as co-products induce comparable effects on the utilization of FA and DF (Jarwoski and Stein, 2017). However, DF content increased the predicted contribution of energy produced from hindgut VFA (Iyayi and Adeola, 2015; Montoya et al., 2016). Moreover, soluble fiber increased VFA production in the hindgut (Chapter 5) and cecum and colon (Chapter 6) compared with insoluble fiber.

The energy produced from hindgut VFA can contribute up to approximately 30% of total available energy in growing pigs (Varel et al., 1997; Anguita et al., 2006). These calculations assume that once absorbed no other factors change the metabolism of VFA. There is, however, accumulating evidence in human nutrition studies that VFA and bile acids (**BA**) are multifunctional molecules supporting maintenance of gut health, regulation of lipid and energy metabolism (Inagaki et al., 2006; Nie et al., 2015; Wahlström et al., 2016). The uptake of VFA by

epithelial cells or BA reabsorption into the enterohepatic circulation largely depends on carrier mediated transport. Moreover, the physiological effects of VFA and BA are also mediated by receptors that are well expressed in the intestines and liver, depending on dietary FA composition. Interestingly, as observed in Chapters 4 and 5, DF solubility and gastrointestinal microbiota shapes the molarity proportion and fluxes of VFA and BA in the intestines and liver (Ferrebee and Dawson, 2015). In turn, BA can activate farnesoid X receptors (**FXR**) and modulate gastrointestinal microbiota responsible for producing VFA (Nie et al., 2015; Wahlström et al., 2016). It is well-established that receptors differ in their affinity for individual molecules such as VFA or BA, tissue distribution and physiological roles (Den Besten et al., 2013; Zhang et al., 2013; 2014; Ferrebee and Dawson, 2015). To what extent activation of receptors by specific VFA or BA influences lipid metabolism in growing pigs fed different DF types remains to be established.

Given the importance of VFA, it is important to reveal how DF modulate the mechanisms underlying their transepithelial transport and intraepithelial metabolism in relation to intestinal and hepatic expression of genes. However, the mechanisms behind VFA absorption in the cecum and colon are complex and have not been established in pigs fed practical sources of soluble and insoluble fiber. Because there are several factors involved during absorption and production of VFA and theses processes may occur at the same time, it is, therefore, difficult to quantify absorption kinetics of VFA *in vivo*. Differences in intestinal VFA uptake in various non-ruminant species have been reported *in vitro* using the Ussing chamber technique (Engelhardt et al., 1993; Busche et al., 2002ab). The everted sac technique (**EST**) as introduced by Wilson and Wiseman (1954) is well established for measuring transport of electrolytes in different regions of the GIT.

Therefore, the first objective of this experiment was to use the EST to investigate the effects of adding soluble and insoluble fiber in pig diets on mucosal disappearance, catabolic loss, tissue

concentration and serosal release of VFA in the colon. The second objective was to investigate the effects of dietary addition of soluble and insoluble fiber on the colonic and hepatic expression of host genes encoding proteins during lipid metabolism in growing pigs.

7.3. MATERIALS AND METHODS

7.3.1. Diets, Pigs, Experimental Design and Everted Sac Technique

The experimental procedures and use of animals were approved by the Animal Care Committee of the University of Manitoba (Protocol Number: F14-041/1/2) and pigs were cared for according to the guidelines of the Canadian Council on Animal Care (CCAC, 2009). The pigs were offered corn-soybean meal-based diets without (control diet) or with 12% flaxseed meal (**FM**) or 10% oat hulls (**OH**) (**Table 7.1**). The diets were formulated to contain similar standardized ileal digestible (**SID**) AA contents, energy and other nutrients and were similar to those reported in Chapters 5 and 6 of this thesis. Diets met energy and nutrient specifications for 25 to 50 kg pigs (NRC, 2012).

A total of 48 Genesus crossbred barrows [(Yorkshire-Landrace dam) $\times$ Duroc sire] with an initial BW of 26.8 ± 0.44 (mean $\pm$ s.d.) kg were penned in pairs. The pigs were randomly offered one of the three diets in a completely randomized design for 28 days, to give 8 replicates per treatment. Feed and water were provided at all times throughout the experiment. Room temperature was maintained at 22.0 ± 1.7 ºC (mean $\pm$ s.d.) with a 14 h light-10 h dark cycle throughout the study. After 28 days, pigs were sacrificed and immediately eviscerated within 15 mins *post mortem*. Two pieces of tissue from the colon, each about 4 to 6 cm were removed and prepared for everted sac technique (**EST**). The EST was performed following a method adapted from Wilson and Wiseman (1954) and elegantly described by Lloyd et al. (2010). In brief, the

Table 7.1. Compositions of the control, flaxseed meal and oat hulls diets[1]

Item	Diet[1]		
	Control	FM	OH
Ingredient composition (%)			
Corn	64.53	57.40	52.99
Oat Hulls	-	-	10.00
Flaxseed meal	-	12.00	-
Soybean meal, 44% CP	31.00	25.49	31.50
Vegetable oil	1.354	2.048	2.390
Limestone	0.683	0.667	0.640
Monocalcium phosphate	0.752	0.637	0.750
Salt	0.35	0.35	0.35
Vitamin-mineral premix[2]	1.00	1.00	1.00
L-Lysine HCl	-	0.086	0.010
DL-Methionine	0.031	0.010	0.060
Threonine	-	0.012	0.010
Titanium dioxide	0.30	0.30	0.30
Total	100	100	100
Analyzed compositions[3]			
Crude protein (N $\times$ 6.25), %	19.20	19.30	19.36
Gross energy, kcal/kg	3,900	3,995	3,980
Acid detergent fiber	3.7	5.0	7.6
Nitrogen detergent fiber	9.3	18.2	18.7
Soluble dietary fiber	5.65	6.9	4.5
Insoluble dietary fiber	15.2	17.6	21.1
Total dietary fiber	20.9	24.9	25.5

[1]FM = flaxseed meal-containing diet; OH = oat hulls-containing diet.

[2]Provided the following nutrients per kg of air-dry diet: 8,250 IU retinol (vitamin A); 200 IU cholecalciferol (vitamin D_3); 40 UI α-tocopherol (vitamin E); 4 mg vitamin K; 1.5 mg vitamin B_1; 7 mg vitamin B_2; 2.5 mg vitamin B_6; 25 µg vitamin B_{12}; 14 mg calcium pantothenate; 2 mg folic acid; 21 mg niacin (vitamin B_3); and 200 µg biotin (vitamin B_7). Minerals: 15 mg Cu (as copper sulphate); 0.4 mg iodine (as potassium iodine); 120 mg iron (as ferrous sulphate); 20 mg Mn (as manganese oxide); 0.3 mg Se (as sodium selenite); 110 mg Zn (as zinc oxide).

intestinal contents within the intestinal cylinder (lumen) were removed, rinsed with VFA-free physiological saline. One piece of tissue was immediately stored at -80 ^{o}C until analyzed for VFA concentrations (**VFA$_{TO}$**). The other piece was placed in a pre-warmed (37°C) buffer and everted using forceps. The tissues were inverted such that the serosal side faces inside and the mucosal side faces outside of the everted sac. The everted intestines were then tied off at one end using Ethicon Mersilk 3.5 metric to make a cylinder. The serosal side (inside) of the sac were filled with a pre-warmed (37°C) VFA-free buffer solution with VFA concentration (**VFA$_{SO}$**). The sac was then immersed in a pre-warmed (37°C) buffer in such a way that the mucosal side (outside) is exposed to a physiological mixture of VFA with a well-known VFA concentration (**VFA$_{MO}$**), for an incubation period of one hour. After incubation, an aliquot from the mucosal solute and another from serosal solute were obtained and stored at -80°C until analyzed for VFA concentration in the mucosal (**VFA$_{MF}$**) and serosal (**VFA$_{SF}$**) regions after incubation, respectively. The tissue samples were rinsed with ice-cold VFA-free physiological saline solution, dried by dapping off residual solution, weighed, and stored at -80 ^{o}C until analyzed for VFA concentrations after incubation (**VFA$_{TF}$**). The buffer solutions were prepared as described by Breves and Krumscheid (1997) and a physiological mixture of VFA were added to the solution that was exposed to the mucosal side (**Table 7.2**).

7.3.1. Ribonucleic Acid Extraction, cDNA Synthesis, and Quantitative Reserve Transcription-PCR

Total RNA was isolated from 50-75 mg of frozen colonic and hepatic tissue using RNAqueous Total RNA Isolation Kit (In vitrogen Canada Inc., Burlington, ON, Canada) according to the manufacturer's instructions. The concentration of RNA, OD280/OD260 and

Table 7.2. Buffer Formula Used in Everted Sac Technique Assay

	80mmol/L SCFA, pH 7.4 (serosal)	80mmol/L SCFA, pH 7.4 (mucosal)
NaCl	111	54.5
KCl	5	5
$NaHCO_3$	25	25
$Na_2HPO_4*2H_2O$	1	1
$NaH_2PO_4*H_2O$	2	1
$MgCl_2*6H_2O$	2	2
$CaCl_2*2H_2O$	1	1
Glucose	5	-
Mannitol	-	5
Na-Acetate	-	48
Na-Propionate	-	20
Na-Butyrate	-	12

OD260/OD230 were determined using Nanodrop 2000 (Thermo Scientific, Wilmington, DE, USA). The integrity of RNA was verified by visualization in an agarose gel. First strand cDNA was synthesized by reverse transcription using iScript^{TM} cDNA Synthesis Kit (Bio-Rad) following the suppliers'instructions.

Quantitative real time-PCR (**qRT-PCR**) was performed using SYBR Green Supermix (Bio-Rad) on a CFX Connect™ Real-Time PCR Detection System ((Life Science Research, Bio-Rad, Ontario, Canada). Glyceraldehyde 3-phosphate dehydrogenase (**GAPDH**) was used as an internal gene to normalize the expression of tested genes. The *GAPDH* was selected as the housekeeping gene because it is constitutively expressed at high levels in most tissues and it was assumed that glycolysis would not be different among fed the diets used in this experiment.

The Primers for qRT- PCR analysis were designed with Primer-Blast based on the published cDNA sequence in the DNA bank. All the primers spin at least two exons. Pairs of primers used for amplification of target genes and their sequences are presented in **Table 7.3**. The tested genes were mucin 2 (**MUC2**), monocarboxylate transporter 1 (**MCT1**), sodium-coupled monocarboxylate transporter 1 (**SMCT1**), basigin (**CD147**), phosphoenolpyruvate carboxykinase 1 (**PEPCK1**), free fatty acid receptor 2 and 3 (**FFAR2** and **FFAR3**), peptide YY (**PYY**), glucagon-like peptide-2 receptor (**GLP-2R**), liver-type fatty acid binding protein (**FABP1**), apolipoprotein A IV (**APOA4**), Acyl-CoA synthetase long-chain Family Member 5 (**ACSL5**), cholesterol 7α-hydroxylase (**CYP7A1**), and cytochrome P450 2C49 (*CYP2C49*). Incremental levels of dietary fiber and protein increase synthesis and secretion of mucin and increase *MUC2* expressions along the intestines (de Lange et al., 1989; Montagne et al., 2004; Gardiner et al., 2008). Both sodium-coupled monocarboxylate transporter and *MCT1*, a proton-coupled transporter, facilitates uptake of VFA, whereas *CD147* is responsible for *MCT1* function and its translocation to the plasma

Table 7.4. Porcine-specific primers utilized for quantitative reverse transcription-PCR.

Gene	Accession no.	Direction[1]	Primers	Size, BP[2]
MUC2[3]	XM_021082584.1	F	CCAGGTCGAGTACATCCTGC	90
		R	GTGCTGACCATGGCCCC	
MCT1[4]	NM_001128445	F	GGTGGAGGTCCTATCAGCAG	74
		R	AAGCAGCCGCCAATAATCAT	
SMCT1[5]	XM_003122908.1	F	AGGTCTACCGCTTTGGAGCAT	77
		R	GAGCTCTGATGTGAAGATGATGACA	
PEPCK1[6]	NM_001123158.1	F	CACAGCCCACGTTTTCTGATAA	76
		R	ATCTCACTTCCTGACCTCATTGTTACT	
CD147[7]	NM_001123086.1	F	CCGGATGAGGTCCTGGATGAT	80
		R	GCGAACGTTCTTGCCTTTGT	
PYY[9]	NM_001256528	F	TATGGGAAACGTGACAGCCC	101
		R	CATAGGTAGGCGCCTTCTGG	
FFAR2[10]	NM_001278758	F	TACTCGCGCTTTGTGTGGAT	123
		R	GTTATAGGGCCCGAAGCACA	
FFAR3[11]	NM_001315601	F	CACTGCAGCGTGGTCTACAT	142
		R	AAAGGACCACAGCCATCTCC	
FABP1[12]	NM_001004046	F	AGGGGACATCGGAAATCGTG	103
		R	TCACACTCCTCTCCCAAGGT	
GLP-2R[13]	NM_001246266	F	GACCCTCTCTTGTGTCTTCGTA	76
		R	AGAGGCAAACAGGTTCATGTG	
CYP7A1[14]	NM_001005352	F	AGAAAGCCAAGTCGCTCCTC	101
		R	TGCTTCAGGGCTCCTAATCG	
CYP2C49[15]	NM_214420	F	TGTTAAGGACCCTCGGGACT	77
		R	ATGCCACCTGGTGATTGTGT	
APOA4[16]	NM_214388	F	CTGAAGGCTGTGGTCCTGAG	108
		R	CAGCTGGCTGAAGTAGTCCC	
ACSL5[17]	NM_001195321	F	GGTAGCCGTCCCCTTGTATG	110
		R	GGATCAATGCCTTTTCGGGC	
GAPDH[18]	NM_001206359.1	F	GTGAACGGATTTGGCCGC	89
		R	AAGGGGTCATTGATGGCGAC	

[1]Direction of primer (F = forward; R = Reverse).

[2]Product length in base pair (BP).

[3]*MUC2* = mucin 2.

[4]*MCT1* = monocarboxylate transporter 1.

[5]*SMCT1* = sodium-coupled monocarboxylate transporter 1.

[7]*PEPCK1* = phosphoenolpyruvate carboxykinase 1.

[8]*CD147* = basigin.

[9]*PYY* = peptide YY.

[10] *FFAR2* = free fatty acid receptor 2.

[11]*FFAR3* = free fatty acid receptor 3.

[12] *FABP1* = liver-type fatty acid binding protein.

[13]*GLP-2R* = glucagon-like peptide-2 receptor.

[14] *CYP7A1* = cholesterol 7α-hydroxylase.

[15]*CYP2C49* = cytochrome P450 2C49.

[16]*APOA4* = apolipoprotein-A -IV.

[17]*ACSL* = Acyl-CoA synthetase long-chain Family Member 5.

[18]*GAPDH* = glyceraldehyde 3-phosphate dehydrogenase.

membrane (Kirk et al., 2000; Wilson et al., 2005; König et al., 2010). These genes were selected to elucidate information on intestinal concentrations and absorption of VFA predicted in Chapters 4 and 5 and determined using EST in this experiment. G protein-coupled receptors namely, *FFAR2* and *FFAR3* are stimulated by VFA and modulate energy metabolism (Gao et al., 2009; Den Besten et al., 2013; Zhang et al., 2014) and triggers the production and release of *PYY* (Cherbut et al., 1998; Samuel et al., 2008; Miyauchi et al., 2010). Peptide YY is a satiety stimulating hormone that are released in response to nutrient intake (Haenen et al., 2013) and was investigated due to the fact that bulkiness properties of fibrous ingredients are descriptors of gut fill. Glucagon-like peptide-2 control gastrointestinal growth by increasing intestinal cell proliferation and reduced apoptosis (Burrin et al., 2005; 2007) and modulate gastrointestinal function by decreasing gastric emptying, gastric acid secretion and gut motility (Wøjdemann et al., 1999; Guan et al., 2012). Phosphoenolpyruvate carboxykinase 1 is a gluconeogenic gene that control the enzyme involved in glucose metabolism and was selected based on the postulation that DF would reduce absorption of dietary glucose and, therefore, have increased gluconeogenesis in the liver (Yoon et al., 2001; Ferrebe and Dawson, 2015).

The expression of *APOA4* and *ACSL5* is dependent on lipid intake and on the saturation degree of FA and these were selected to test the effects of supplemental fat (Bjorntorp and Sjostrom, 1978; Hanniman et al., 2006; Navarro et al., 2004a). Intestinal-type fatty acid binding protein participate in the uptake, intracellular metabolism and/or transport of long-chain fatty acids and is responsible for modulating cell growth and proliferation. Two cytochrome P450 family members namely *CYP7A1* and *CYP2C49* were selected to assess BA metabolism as they modulate reactions regulating BA production and clearance in the liver and intestines (Moe et al., 2009). Colon tissue was tested for mRNA expression of *MUC2*, *MCT1*, *SMCT1*, *CD147*, *PYY*, *PEPCK1*,

FFAR1/2, *GLP-2R*, and *CYP7A1*, whereas, liver tissue was tested for *APOA4*, *ACL5*, *PYY*, *PEPCK1*, *FFAR1/2*, *FABP1*, *GLP-2R*, *CYP7A1* and *CYP2C49*.

7.3.2. Laboratory analyzes

Diets were ground through a 1-mm screen in a laboratory mill (Thomas Wiley Mill Model 4, Thomas Scientific, Swedesboro, NJ). The GE, DM, CP, NDF and ADF contents of the diets were determined as described by Ndou et al. (2017). The FA profiles of the diets were analyzed following the methodology described by Folch et al. (1957) using a 2:1 chloroform:methanol mixture (**Table 7.4**). The extracted FA were methylated to FA methyl esters using modifications based on methods described by Metcafe and Schmitz (1961). The soluble, insoluble and total DF contents were analyzed in all diets according to method 991.43 of AOAC (2012) using the Ankom^{TDF} Dietary Fibre Analyzer (Ankom Technology). The VFA concentrations were analyzed using the modification of procedures described in Chapter 4.

7.3.3. Calculations and statistical analysis

The mucosal disappearance (**VFA$_M$**) and serosal release (**VFA$_S$**) were determined using the values of VFA contents in the tissue and buffer at the beginning and end of incubation.

$$\textbf{VFA}_M = (\textbf{VFA}_{M0} - \textbf{VFA}_{MF})*\textbf{Buffer}_{Vol}$$

where, VFA$_{M0}$ = VFA concentration in the mucosal buffer before incubation (mmol/L); VFA$_{MF}$ = VFA concentration in mucosal buffer after incubation (mmol/L); Buffer$_{Vol}$ = Volume of buffer during incubation.

$$\textbf{VFA}_S = \textbf{(VFA}_{SF} - \textbf{VFA}_{S0})\textbf{*Buffer}_{Vol}$$

where, VFA_{S0} = VFA concentration in serosal buffer before incubation (mmol/L);

VFA_{SF} = VFA concentration in serosal buffer after incubation (mmol/L);

$Buffer_{Vol}$ = Volume of buffer during incubation (mL).

The concentration of VFA in tissue were determined by measuring their contents in tissues samples after incubation and compared to the values in untreated samples taken from the same part of the intestine. The tissue content ($\textbf{VFA}_T$) were calculated as follows:

$$\textbf{VFA}_T = \textbf{((VFA}_{TF} - \textbf{VFA}_{T0}))\textbf{*Eluant}_{Vol})\textbf{/2}$$

where, VFA_{TO} = VFA concentration in untreated tissue (mmol/L);

VFA_{TF} = VFA concentration in incubated tissue (mmol/L);

$Buffer_{Vol}$ = Volume of eluant (mL).

The catabolic loss of VFA ($\textbf{VFA}_{loss}$) were determined from the difference between mucosal disappearance and the sum of tissue content and serosal release.

$$\textbf{VFA}_{loss} = \textbf{VFA}_M - \textbf{(VFA}_S - \textbf{VFA}_T)$$

Statistical analyzes of data were performed using a generalized linear model procedure of SAS (SAS, Institute, Inc., Cary, NC). The model accounted for dietary effects on concentrations of gastrointestinal VFA and BA, VFA absorption and relative gene expressions in pigs fed the three

Table 7.4. Fatty acids (FA) profile of ingredients and experimental diets

Item	Ingredients				Diet[1]		
	Corn	Soybean	Flaxseed meal	Oat hulls	CON	FM	OH
Saturated fatty acids (SFA)							
Lauric (C12:0)	0.01	0.00	0.01	0.00	0.01	0.01	0.01
Myristic (C14:0)	0.04	0.07	0.07	1.20	0.07	0.06	0.08
Pentadecyclic (C15:0)	0.07	0.18	0.06	0.39	0.07	0.06	0.07
Palmitic acid (C16:0)	12.23	15.72	7.15	31.84	12.68	10.71	12.01
Margaric (C17:0)	0.09	0.20	0.06	0.26	0.11	0.10	0.11
Stearic (C18:0)	1.75	4.10	2.09	3.45	2.85	2.94	3.15
Arachidic (C20:0)	0.00	0.24	0.19	0.97	0.36	0.31	0.35
Behenic (C22:0)	0.18	0.36	0.15	0.81	0.24	0.23	0.27
Lignoceric (C24:0)	0.24	0.26	0.00	0.00	0.17	0.00	0.00
$\sum$SFA	15.06	21.14	9.95	39.48	16.57	14.59	16.22
Monounsaturated fatty acids (MUFA)							
Palmitoleic (C16:1n-7)	0.12	0.09	0.09	0.41	0.11	0.10	0.11
Palmitelaidic (C16:1t)	0.05	0.01	0.03	0.02	0.03	0.03	0.02
Oleic (C18:1n-9)	25.50	9.04	11.86	0.99	22.54	21.89	22.42
Vaccenic (C18:1n-7)	0.48	1.39	0.68	33.59	0.74	0.78	0.89
Gadoleic (C20:1)	0.35	0.15	0.14	1.58	0.27	0.23	0.25
$\sum$MUFA	26.51	10.61	12.81	37.17	23.69	23.04	23.70
Polyunsaturated fatty acids (PUFA)							
Linoleic (C18:2)	56.77	57.32	14.10	21.21	54.99	45.84	54.07
Linolenic (C18:3n-6)	0.00	0.00	0.00	0.00	0.00	0.01	0.01
α-Linolenic (C18:3n-3)	1.61	10.90	62.97	2.14	4.73	16.46	5.95
Eicosadienoic (C20:2)	0.02	0.03	0.08	0.00	0.03	0.04	0.03
Eicosatrienoic (C20:3n-3)	0.00	0.00	0.08	0.00	0.00	0.01	0.00
Eicosapentaenoic(20:5n-3)	0.03	0.00	0.00	0.00	0.00	0.01	0.02
Erucic (C22:1n-9)	0.00	0.00	0.01	0.57	0.00	0.01	0.01
Nervonic (C24:1n-9)	0.00	0.00	0.17	0.32	0.00	0.17	0.16
$\sum$PUFA	58.43	68.25	77.24	23.35	59.74	62.37	60.07
$\sum$FA	-	-	-	-	4.56	6.02	6.05
Calculated IV[2], g/100g	125	136	200	45	127	142	129

[1]FM = flaxseed meal-containing diet; OH = oat hulls-containing diet.

[2]IV = iodine value.

diets. The pen was the experimental unit. Comparisons of means were performed using the Tukey-Kramer honestly significance difference test. Significant differences among means were declared at $P < 0.05$, and trends declared for P values between 0.05 and 0.10 were discussed.

7.4. RESULTS

7.4.1. Volatile fatty acids fluxes rates under Everted Sac conditions in the colon

All pigs were healthy and consumed their daily feed allowance throughout the experiment. The mucosal disappearance and catabolic loss of acetic and butyric acids was higher ($P < 0.001$) in the colon of pigs fed the FM and OH diets compared to pigs fed the control diet (**Table 7.5**). The mucosal disappearance and catabolic loss of propionic acid in FM diet-fed pigs was higher ($P < 0.001$) than that in control and OH diets-fed pigs. The tissue contents of acetic and butyric acids were similar ($P > 0.10$) among all diets, but tissue content of propionic acid was lower ($P < 0.05$) in the colon of pigs fed the OH diet than those that consumed the control diet. The serosal release of acetic acid in the colon of pigs fed the FM and OH diets was higher ($P = 0.056$), compared to control diet-fed pigs. The serosal release of propionic and butyric acids in the colon of pigs fed the FM diet was higher ($P = 0.001$) than in pigs fed the control and OH diets.

7.4.2. Gene expressions in the colon and liver

Expressions for *MCT1*, *SMCT1* and *FFAR3* were similar among diets ($P > 0.05$) but the expressions of *MUC2* ($P < 0.01$) and *FFAR2* ($P < 0.05$) were greater in pigs fed FM and OH diets compared to those fed the control diet (**Table 7.6**). Pigs fed the control and OH diets tended to have higher ($P = 0.070$) expressions of the *CD147* gene in the colon compared to those that consumed the control diet. Expression of *PYY* in the colon was higher ($P < 0.01$) in pigs fed the

Table 7.5. Volatile fatty acids fluxes (µmol/hr/g) under everted sac conditions in colon of growing pigs fed the control, flaxseed meal and oat hulls diets[1] (n = 8)

Item	Control	FM	OH	SEM	P
		Diet[1]			
Acetic					
Mucosal disappearance	88.7[b]	206.1[a]	181.6[a]	19.23	<0.001
Catabolic loss	69.1[b]	152.3[a]	133.3[a]	14.29	0.005
Tissue content	1.9	2.2	1.1	0.47	0.217
Serosal release	17.7[b]	51.5[a]	47.7[a]	4.98	<0.001
Propionic					
Mucosal disappearance	55.0[c]	89.4[a]	68.7[b]	2.83	<0.001
Catabolic loss	33.5[b]	46.1[a]	35.4[b]	1.50	0.001
Tissue content	0.6[a]	0.4[ab]	0.3[b]	0.08	0.040
Serosal release	20.9[c]	42.9[a]	33.0[b]	1.32	<0.001
Butyric					
Mucosal disappearance	78.3[b]	152.2[a]	141.5[a]	8.09	<0.001
Catabolic loss	77.6[b]	148.4[a]	140.6[a]	8.02	<0.001
Tissue content	0.6	0.8	0.4	0.16	0.151
Serosal release	0.0[b]	3.0[a]	0.2[b]	0.07	<0.001

[1]FM = flaxseed meal-containing diet; OH = oat hulls-containing diet.

[a,b,c]Mean values within a row with unlike superscripts differ ($P < 0.05$).

control and FM diets compared to OH diet-fed pigs. A tendency was observed in which the expression of *PEPCK1* gene in the colon was greater ($P = 0.062$) in the control and OH diets-fed pigs compared to FM diet-fed pigs. The expression of *FABP1* gene in the colon was higher ($P < 0.01$) in pigs fed the control diet than in those fed the FM and OH diets. Expression of *GLP-2R* in the colon was higher ($P < 0.01$) in pigs that consumed the control and OH diets compared to pigs fed the FM diets. The expression of *CYP7A1* in the colon of pigs offered diets containing flaxseed meal and oat hulls were greater ($P < 0.01$) compared to pigs fed the control diet.

A significant increase ($P < 0.05$) in the expression of *CYP2C49* and *FFAR3* in the liver was noticed in pigs fed the FM diet compared to those that consumed the control and OH diets. The expression of *PYY*, *PEPCK1*, *CYP7A1*, *APOA4*, *FFAR2* and *GLP-2R* was not different ($P > 0.10$) among pigs fed the control, FM and OH diets. Dietary addition of flaxseed meal and oat hulls resulted in a higher ($P < 0.01$) expression of *FABP1* in the colon compared to the control diet. The expression of *ACLS5* in the liver tended ($P = 0.077$) to be higher in pigs fed the FM diet compared pigs fed the control and OH diets.

7.5. DISCUSSION

Dietary addition of flaxseed meal and oat hulls increased the mucosal disappearance and catabolic loss of acetic and butyric acids probably due to presence of DF which modulates adaptation of intestinal tissue to selectively absorb and metabolize VFA (Den Besten et al., 2013; Zhang et al., 2014; Agyekum et al., 2015). This finding concurs with observations from the *in vivo-in vitro* fermentation assay in Chapter 5 that predicted that hindgut absorption of acetic and butyric acids was greater in pigs fed high-fiber diets, regardless of the differences in DF solubility. Moreover, the observation that an increase in mucosal disappearance and catabolic loss in pigs fed the FM and OH diets are also in agreement with the observation in the current experiment that

Table 7.6. Relative mRNA gene expressions in the colon and liver of pigs fed control, flaxseed meal and oat hulls diets[1] (n = 8)

Item	Diet[1]			SEM	*P*
	Control	FM	OH		
Colon					
MUC2[2]	0.799[b]	1.717[a]	1.549[a]	0.1775	0.003
MCT1[3]	0.948	1.549	1.241	0.212	0.158
SMCT1[4]	6.528	7.428	4.174	2.069	0.527
CD147[5]	1.456[B]	2.424[A]	1.554[B]	0.307	0.070
PYY[6]	0.902[a]	0.511[b]	1.037[a]	0.090	0.001
PEPCK1[7]	0.647[A]	0.465[B]	0.862[A]	0.111	0.062
FFAR2[8]	1.614[b]	7.721[a]	6.267[a]	1.639	0.039
FFAR3[9]	14.389	18.466	6.721	5.223	0.293
FABP1[10]	1.612[a]	0.792[b]	1.059[b]	0.155	0.004
CYP7A1[12]	0.427[a]	0.169[b]	0.200[b]	0.058	0.001
Liver					
PYY[6]	1.396	2.323	1.599	0.626	0.555
PEPCK1[7]	1.004	1.478	0.863	0.342	0.428
CYP7A1[12]	0.29	0.37	0.27	0.07	0.605
CYP2C4P[13]	0.900[b]	1.496[a]	0.930[b]	0.117	0.045
APOA4[14]	0.474	0.923	1.614	0.621	0.440
ACLS5[15]	1.254[B]	2.332[A]	1.423[B]	0.340	0.077
FFAR2[8]	2.284	4.628	2.666	1.712	0.591
FFAR3[9]	1.331[b]	5.322[a]	2.044[b]	1.137	0.042
FABP1[10]	1.612[a]	0.792[b]	1.059[b]	0.155	0.004
GLP-2R[11]	0.674	0.652	0.997	0.168	0.287

[1]FM = Flaxseed meal-containing diet; OH = Oat hulls-containing diet.

[2]MUC2 = mucin 2

[3]MCT1 = monocarboxylate transporter 1

[4]SMCT1 = sodium-coupled monocarboxylate transporter 1

[5]CD147 = basigin

[6]PYY = peptide YY

[7]PEPCK1 = phosphoenolpyruvate carboxykinase 1

[8] FFAR2 = free fatty acid receptor 2

[9] FFAR3 = free fatty acid receptor 3

[10] FABP1 = liver-type fatty acid binding protein

[11] GLP-2R = glucagon-like peptide-2 receptor

[12] CYP7A1 = cholesterol 7α-hydroxylase

[13] CYP2C49 = cytochrome P450 2C49

[14] APOA4 = apolipoprotein-A -IV

[15] ACSL = Acyl-CoA synthetase long-chain Family Member 5.

consumption of flaxseed meal and oat hulls also exhibited similar effects on gene expression of *FFAR2* in the current experiment. Although *FFAR2* and *FFAR3* share the same chromosomal loci and 43% amino acid sequence identity (Den Besten et al., 2013; Zhang et al., 2014), there were no dietary effects on the former compared to the latter which increased in pigs fed high-fiber diets. This disparity can be explained by the differences in the affinity of specific SCFA between *FFAR2* and *FFAR3*. Thus, the increase in mucosal disappearance and catabolic loss of acetic and butyric acids in pigs fed high fiber diets in this experiment also supports the previous assertion that *FFAR2* has preference for binding to low molecular weight VFA (acetic and propionic acids) within micromolar affinity (Brown et al., 2003; Le Poul et al., 2003; Milligan et al., 2009). Conversely, *FFAR3* preferentially binds to propionic, valeric and butyric acids with relatively higher affinities and low affinities for acetic acid (Brown et al., 2003; Le Poul et al., 2003; Milligan et al., 2009).

The consumption of soluble fiber from flaxseed meal promoted the mucosal disappearance and catabolic loss of propionic acid compared to insoluble DF from OH diets which was similar to the control diet. This difference can be attributed to the increase in the luminal concentration (Chapter 4) and increased predicted and determined production fluxes of propionic acid in pigs fed soluble DF from flaxseed meal which selectively stimulated the absorption capacity and metabolism of propionic acids in the intestinal mucosal tissue. The finding from *in vitro* studies using Ussing chambers that the increase in intestinal lumen concentration of VFA stimulates their absorption strengthen this assumption (Herrmann et al., 2011). Furthermore, this suggestion is also supported by findings by Bach Knudsen et al. (2000), who assessed *in vivo* VFA concentration in portal vein of pigs fed fibrous diets. In agreement with results of the current study they reported that the molar proportion of butyrate in the blood stream increased after pigs consuming a diet rich in soluble DF from oat bran. Interestingly, no dietary effects were observed on the tissue contents

of acetic and butyric acids. The lower tissue content of propionic acid in the colon of pigs fed insoluble DF from OH than those that consumed the low-fiber in the control diet is difficult to explain.

The inclusion of flaxseed meal and oat hulls induce gastrointestinal fermentation thereby increasing intestinal VFA concentrations (Chapter 4) as well as VFA production and absorption in the entire hindgut (Chapter 5) and cecum and colon (Chapters 6). In the GIT, *MCT1* and *SMCT1* are widely distributed monocarboxylate transporters that are found in the colon of pigs and facilitates transportation of VFA. The expression of *MCT1* and *SMTC1* depends on substrate availability (Agyekum et al., 2015). In this context, the lack of dietary effects on the expression of *MCT1* and *SMCT1* was unexpected, and the reason for this is not clear. These findings also disagree with observations that the expression of *MCT1* increase with incremental level of DF in rats (Kirat et al., 2009) and pigs and indicates increased fiber fermentation and rapid absorption of VFA (Agyekum et al., 2015; Jarwoski et al., 2017). The tendency that serosal release of acetic acid in the colon of pigs fed the high-fiber diets also contradicts with the lack of expressions of *MCT1* and *SMCT1* but suggests that there could be other mechanisms that selectively facilitates the uptake of VFA. Apart from these monocarboxylate transporters, VFA can also diffuse across the epithelial cell membrane (Halestrap and Meredith, 2004). Metzler-Zebeli et al. (2012) demonstrated that expression of the *MCT1* gene in the colon of weaned pigs was positively corelated with butyric and propionic acids fluxes suggesting that increased concentration of VFA may upregulate expression of the *MCT1* gene. Thus, the differences observed in the serosal release of propionic and butyric acids in the colon of pigs fed soluble DF from flaxseed meal compared to insoluble DF from oat hulls may be due to differences in the form and fermentability of these fibrous ingredients which detects the molar proportions and fluxes of VFA produced (Chapters 4

and 5). These findings are in accordance with observations in our previous experiments that flaxseed meal supplementation increased production and predicted absorption of both butyric and propionic acids (Chapter 4 and 5). The predicted functional metagenomes indicating that butanoate metabolism in mucosa-associated microbiota of pigs fed flaxseed meal-containing diets also supports this assumption (Chapter 4).

The *CD147* is crucial for *MCT1* translocation to the plasma membrane as well as for *MCT1* transporter function (Kirk et al., 2000; Wilson et al., 2005). The paradox created by the similarities in gene expression of the monocarboxylate transporters and variabilities in serosal release of VFA, and propionic acid in particular in pigs fed high-fiber diets is further exacerbated by that the expression of *CD147* which tended to be higher in FM diet-fed pigs compared to the control and OH diets. The increase in the expressions of *MUC2* in pigs fed DF from flaxseed meal and oat hulls in this experiment concurs with previous studies that DF increase mucin gene mRNA expressions along the intestine of pigs and rats (Gardiner et al., 2008; Han et al., 2008; Montoya et al., 2010; Smith et al., 2011). Moreover, it is well-established that dietary factors such as incremental levels of dietary fiber and protein influence synthesis and secretion of mucin from goblet cells and its recovery in digesta (Montagne et al., 2004).

Although there were no dietary effects on hepatic expression of *GLP-2R*, the consumption of the control and OH diets increased the expression of colonic *GLP-2R* compared to that of FM diet. Glucagon-like peptide-2 increased intestinal cell proliferation and reduced apoptosis (Burrin et al., 2005; 2007) and decreased gastric emptying, gastric acid secretion and gut motility (Wøjdemann et al., 1999; Guan et al., 2012). In Chapter 4, we observed that metagenomic functional pathways related to apoptosis were enriched in ileal mucosa-associated microbiota and performance was depressed due to low feed intake on pigs fed flaxseed meal. It may, therefore, be

speculated that the greater *GLP-2R* expression in the control and OH diets-fed pigs observed in this experiment resulted in increased GLP-2 being synthesized, and eventually improved growth performance. Propionic acid was reported to inhibit food intake in humans (Arora et al., 2012), but the underlying molecular mechanisms and mediators are not known. Feed intake was reduced (Chapter 4), propionic absorption was increased (Chapter 5) and serosal release of propionic acids increased in the current experiment in pigs fed flaxseed meal and *FFAR3* has a high affinity for propionic acid (Brown et al., 2003; Le Poul et al., 2003; Milligan et al., 2009). It is possible that increase in propionic acid absorption increased the expression of *FFAR3* in the liver of pigs fed flaxseed meal in this experiment. Thus, the same mechanisms could have also contributed to the depression in feed intake in pigs fed flaxseed meal observed in Chapter 4. It has been suggested that the activation of *FFAR2* and *FFAR3* triggers the production and release of satiety-stimulating hormones GLP and PYY (Zhou et al., 2006; 2008; Samuel et al., 2008, Miyauchi et al., 2010). Thus, the higher expression of *PYY* in the colon of pigs fed the FM diet compared to the OH diet-fed diets could be due to upregulation of *FFAR2* in pigs consuming flaxseed meal. In addition to their roles as sources of energy to the host, together with BA, SCFA may bind with *FFAR2/3* thereby affecting the host's metabolic rate to regulate energy homeostasis (Kuwahara, 2014).

Bile acids are endogenous molecules synthesized from cholesterol in the liver and metabolized by the gut microbiota (de Aguiar Vallim et al., 2013). Microbial biotransformation of BA also modulates the signalling properties of BA via the nuclear FXR and the G protein-coupled membrane receptor 5, which regulate numerous metabolic pathways including BA synthesis by the host. The classical (or neutral) and alternative (or acidic) pathways have been proposed to account for BA production initiated by 7α-hydroxylation of cholesterol catalyzed by cholesterol 7α-hydroxylase (**CYP7A1**) and sterol-27-hydroxylase (**CYP27A1**), respectively (Russell, 2003;

Thomas et al., 2008; Wahlström et al., 2016). The *CYP7A1* and *CYP2C49* can be used as biomarkers for cholesterol metabolism (Zhang et al., 2013; Wahlström et al., 2016). Therefore, the increased expression of *CYP7A1* in the colonic tissue of pigs fed FM and OH diets is partly responsible for cholesterol lowering effects of DF from flaxseed meal and oat hulls (Zhang et al., 2013). Supporting this assumption are the hypocholesteremia (Chapter 4) and increase in excretion of secondary BA in pigs (Chapter 5) in pigs fed high fiber diets. Moreover, the predicted functional metagenomic analyzes in Chapter 4 demonstrated increase in biosynthesis of both primary and secondary BA in pigs fed flaxseed meal. It is also important to note that in the present experiment the increase in the expression of *CYP7A1* was observed in the colon but not in the liver most probably due to the fact that the mechanisms by which *CYP7A1* is inhibited by BA via the *FXR* vary between enterocytes and hepatocytes (Kim et al., 2007; Nie et al., 2015).

Porcine isoform CYP2C49 metabolize drugs, steroids, skatole and is involved in the synthesis of cholesterol from squalene and cholesterol conversion into steroid hormones and BA, which facilitates dietary fat digestion (Moe et al., 2009; Skaanild and Friis, 2005; Świątkiewicz et al., 2016). The increase in the expression of hepatic *CYP2C49* and *ACLS5* in FM diet-fed pigs compared to pigs consuming the control and OH diets was unexpected, since the SFA content were low in FM diet yet it is well-established that dietary incremental levels of SFA upregulate expressions of not only *CYP2C49* and *ACSL5* but also activated *APOA4* and *CYP7A1* in the present study (Świątkiewicz et al., 2016). It appears that the abovementioned differences in *CYP2C49* expressions between FM and OH diets reflect the variation in the effects of physicochemical properties of flaxseed meal and oat hulls that modulate BA reabsorption in the enterohepatic circulation of BA. Bile acid binding resin such as DF improves metabolic control through the induction of energy expenditure such that BA synthesis increase energy demand in muscles and

adipose tissue (Watanabe et al., 2012). Thus, it is highly likely that overexpression of *CYP2C49* in FM diet-fed pigs can also explain the poor growth performance observed in Chapter 4.

In addition to its role in lipid metabolism by regulating plasma lipid transport by decreasing expressions of *APO* and *FABP1*, *FXR* induction of *SHP* expression can decrease expression of *PEPCK*, a gluconeogenic gene that mediate glucose metabolism (Yoon et al., 2001; Ferrebe and Dawson, 2015). Although no dietary effects were observed on hepatic *APOA4* expression, *FABP1* suppression in pigs fed flaxseed meal and oat hulls-containing diets could be due to the down-regulation of this protein in an attempt to counter for the increase in dietary level of LCFA (Shi et al., 2012). Moreover, no dietary effects were observed on expression of *PEPCK* in the liver but consumption of flaxseed meal tended to reduce colonic expression of *PEPCK* in the enterocytes. Because it is well-established that DF would reduce absorption of dietary glucose and, therefore, increase gluconeogenesis (Yoon et al., 2001; Ferrebe and Dawson, 2015; Shulman and Petersen, 2012), the reduced colonic expressions of *PEPCK* expression indicates that the consumption DF did not compromise glucose absorption. This can be ascribed to that the quantity or proportion of major progenitors of glucose in the form of corn was lower in the FM and OH diets compared to the control. The *ACSL5* has a high preference for saturated and unsaturated FA, and it is suggested that it also a contributor during the modulation of triglycerides pool during endogenous synthesis of FA or absorption from GIT (Bjorntorp and Sjostrom, 1978). The tendency observed that hepatic *ACLS5* expression increased in FM diet-fed pigs that were low in SFA contents contradicts the assumption that overestimation of these gene is enhanced when pigs are fed diets with high SFA content (Świątkiewicz et al., 2016).

In conclusion, the consumption of high-fiber diets led to superior colonic capacity to absorb, transport and metabolize VFA compared to the control (low fiber) diet. The mRNA

expression of *MUC2* and *FFAR2* in the colon was greater in pigs fed soluble and insoluble fiber-enriched diets relative to the pigs fed the low-fiber diet. The consumption of soluble and insoluble fiber decreased the expression levels of *FABP1* and *CYP7A1* genes in the colon, and hepatic expression levels of *FABP1* gene. The consumption of soluble fiber from flaxseed meal increased the relative hepatic expression of *CYP249* and *FFAR3* but reduced the mRNA expressions of *PYY* in the colon when compared to consumption of oat hulls. Taken together, the data on gene expressions and the EST suggest that overexpression of *MUC2*, *FFAR2*, and *CYP249* are results of metabolic interventions or adaptive mechanisms to improve efficiency in use of high-fiber diets. However, these postulations and conclusion needs to be proven experimentally by subsequent studies.

CHAPTER 8

GENERAL DISCUSSION

The continued pressure in conventional feed ingredient market and increase in feed costs has resulted in trends for the livestock industry to seek alternative low-cost feedstuff options. One option is to use agro-industrial co-products, however, a characteristic feature of these alternative ingredients is the high content of dietary fiber (**DF**), which is indigestible and reduce energy density of the feed. Therefore, supplemental fats in the form of animal fat or vegetable oils are added in pig diets to improve the energy density of a balanced diet. It is, however, fascinating that growing pigs perform worse and have poor nutrient utilization when fed nutritionally-balanced high-fiber diets with a similar energy supply but composed of co-products plus and added fat.

As discussed in Chapters 1 and 2 of this thesis, this disparity in performance has been attributed to overestimation of fat energy value or interaction between DF solubility and dietary fatty acids (FA) types. Although incremental levels of DF reduce energy density of a pig diet, it is intiresting that DF is also a substrate for fermention by gastrointestinal microbiota that allows pigs to extract significant amounts of energy in the form of volatile fatty acids (**VFA**) (Anguita et al., 2006; Iyayi and Adeola, 2015; Montoya et al., 2016). This paradox is also exercerbated by that DF increase fermentation but also increased fecal fat excretion (Graham et al., 1986; Bach Knudsen and Hensen, 1991; Galassi et al., 2004). It is still not clear on whether fecal fat could be due to poor absorption of supplemental fats that is encapsulated within DF matrices or predisposed by microbial deconjugation of bile acids (**BA**) and microbial FA production by hindgut microbes. Furthermore, fecal fat may originate from endogenous losses from the gastrointestinal secretions but data to explain these paradoxes is scarce. Increased fat in feces as a result of increased

fermentability is obviously counter-productive to the use of fibrous feedstuffs as alternative feed ingredients in pig diets.

There is accumulating evidence that physiological effects of dietary FA, VFA and BA are mediated by receptors that are well expressed in the intestines and liver. The VFA and BA stimulate expression of genes involved in enteroprotection, energy and lipid metabolism (Inagaki *et al.,* 2006). Interestingly, BA can activate farnesoid X receptor (**FXR**) and modulate gastrointestinal microbiota responsible for producing VFA (Ding *et al.*, 1993; Lorenzo-Zúñiga *et al.*, 2003). In turn, gastrointestinal microbiota shapes the molarity proportion and fluxes of VFA and BA in the intestines and liver. In spite of the realization that the aforementined pig responses to dietary incremental levels of co-products are exclusively governed by DF content, conclusive studies on the influence of DF solubility have not been riched. Thus, more work is needed to improve nutrient utilization and increase production and absorption of VFA in pigs fed high-fiber diets. Therefore, the broad objective of the studies described in this thesis was to investigate the effects of DF source on growth performance, production, absorption, and metabolism of FA in growing pigs fed soluble and insoluble fiber-enriched diets.

The thesis research was conducted in 4 phases to achieve the overall objective. In the first phase, growing pigs were fed three corn-soybean meal-based diets without (control), or with flaxseed meal (**FM**) or oat hulls (**OH**) to evaluate the effects of soluble and insoluble DF on growth performance, fat digestibility, blood lipids, and intestinal VFA, BA and neutral sterols (**NS**) concentrations. The effects of soluble and insoluble fiber on histomorphological characteristics, structural and functional characteristics of digesta- and mucosa-associated microbiota were also investigated in Phase 1. In phase two, a combination of the *in vivo* (ileal cannulated pigs) and *in vitro* fermentation technique was used to investigate effects of DF solubility on digestibility of DF

and FA, ileal and fecal flows of FA and BA and hindgut VFA production and absorption in pigs fed the control, FM and OH diets. In phase 3, a combination of the *in vivo* (ileal and cecal cannulated pigs) and *in vitro* fermentation technique was used to investigate the interactive effects of dietary fiber and lipid type on digestibility of DF and FA, and flows of FA and BA in the ileum, cecum and feces, and production and absorption of VFA in the cecum and colon of growing pigs. Pigs in phase 3 were offered cellulose and pectin containing diets in a 2×2 factorial arrangement of treatment. In phase 4, the slaughter technique and an *in vitro* nutrient uptake model (Everted Sac Technique) were employed in this thesis to investigate the effects of soluble and insoluble DF on transport and metabolism of colonic VFA and expressions of genes involved in lipid metabolism in growing pigs fed the control, FM and OH diets.

In Chapter 1, flaxseed meal and oat hulls were selected based on previous studies and preliminary analysis in our lab that showed that they are high sources of soluble and insoluble fiber, respectively, and can be incorporated in pig diets (Eastwood et al., 2009; Jiménez-Moreno et al., 2009; Ndou et al., 2018b). The finding that insoluble DF depressed performance was unexpected, however, the observation that soluble DF depressed growth performance by reducing feed intake is in line with studies that DF compromises weight gain. As indicated in Chapter 4, the major reason for the differences in the responses between soluble DF- and insoluble DF-fed pigs could be due to the difference in the physicochemical properties of flaxseed meal and oat hulls. However, in some cases, different sources of DF may induce variable responses in pigs but using similar mechanisms. For example, as observed in Chapter 4, hypocholesteraemic effects of both soluble and insoluble DF can be ascribed to their ability to act as immediate substrates for intestinal fermentation which in turn decrease fat digestibility. Moreover, DF also act as BA sequestrant agents by binding these molecules within their fiber matrices thereby promoting cholesterol

ecretion by reducing BA reabsorption. On the other hand, insoluble DF from oat hulls reduced serum cholesterol more than soluble DF from flaxseed meal (Chapter 4). Furthermore, as mentioned also in Chapter 4, and in line with previous investigations, consumption of either soluble DF from flaxseed meal or insoluble DF from oat hulls modulates the structure of ileal and cecal microbiota and induced associations between gastrointestinal microbiota and concentrations of microbial activity-related metabolites (VFA, BA and NS) as well as their predicted metagenomic functions. The findings in Chapter 4 that functional pathways including primary and secondary BA biosynthesis were enriched in ileal mucosa-associated microbiota of FM diet-fed pigs supports this assumption. Thus, apart from the physicochemical properties of the fibrous ingredients, fecal fat excretion in pigs fed diets enriched with both soluble and insoluble DF could be due to microbial fat or inefficient digestion due to deconjugation of BA by increased bacterial activity in the GIT. Fat excretion could also have been due to encapsulation of dietary fat within the fiber matrices or endogenous losses of gastric secretions. Although the slaughter model used in Chapter 4 opened an avenue for us to investigate other variables (intestinal histomorphology and microbiota) that cannot be quantified *in vivo*, conclusions drawn from intestinal VFA and BA concentrations may not be accurate. Furthermore, without measurements of ileal fat digestibility in Chapter 4, it is difficult to estimate fat production in the hindgut. However, the slaughter method did not open way for sampling enough digesta needed to analyze fermentability of DF and flows of fat in the ileum and entire GIT. These are major limitations of this study that indicate that the conclusions drawn from this section of the thesis raised questions that need answers. Therefore, these created an opportunity for improving the models in the successive experiments of this thesis. The *in vivo-in vitro* fermentation assay was subsequently introduced using ileal cannulated pigs in the study in Chapter 5 to explain findings from Chapter 4 and elucidate the effects of DF solubility.

In the experiment described in Chapter 5, the observations that consumption of flaxseed meal depressed fat digestibility more than oat hulls supports the findings in Chapter 4 that the response of pigs to fibrous co-products largely depends on their physicochemical properties. However, it is interesting that in Chapter 5, apparent total tract digestibility of total FA was lower than apparent ileal digestibility of total FA, and there was an increase in the ileal and fecal flows of normalized secondary BA in pigs fed high-fiber diets. The reduced nutrient digestibility in pigs fed flaxseed and oat hulls observed in Chapter 5 can also be explained by the lower duodenal and ileal villi height values observed in pigs fed similar diets in Chapter 4. Supporting these observations are the findings also in Chapter 4 that metagenomic functional pathways associated with apoptosis were enriched in ileal mucosa-associated microbiota of FM diet-fed pigs. The increase in the apoptosis could have been a compensatory mechanism to open the way for replenishing mucosal cells following the damage which may be indirectly caused by the viscous characteristics of the non-starch polyssacharide mucilage in flaxseed meal (Stanogias and Pearce, 1985). We simultaneously observed an increase in production and absorption of hindgut VFA which was pronounced more in pigs fed flaxseed meal compared to those fed oat hulls. Although DF increased the predicted quantity of energy contribution from hindgut fermentation, a decrease in ileal fat digestibility indicates a loss in dietary energy and is counterproductive to utilization of fibrous co-products. It is well established that digestibility of intact fat is lower than that of added fat and differences in FA profiles induce variable effects of digestibility of lipids (Kil et al., 2010). Therefore, the presence of lipid residues within flaxseed meal and oat hulls and differences in DF fractions from corn and soybean meal among diets could have imposed confounding effects on responses to the interaction between added fat and DF source observed in Chapters 4 and 5. Another major confounding factor was that we used fecal inoculum to ferment ileal digesta to simulate hindgut

fermentation in Chapter 5 but this model ignores the difference between cecal and colonic microbiota composition. These limitations stimulated the need to improve the model so that the interactive effects of DF solubility and lipid type can be investigated separately in the cecum and colon and using well-characterized lipid-free sources of DF. Thus, interactive effects of DF solubility and lipid types on FA and DF utilization were investigated in Chapter 6 using ileal and cecal cannulated pigs. As mentioned in Chapter 6, the addition of the cecal cannula to the ileal-cannulated model reported in Chapter 5 opened an opportunity for sampling cecal digesta for assessing the flows of FA and DF flows. Fresh cecal digesta was also used for inoculating ileal digesta to model fermentation in the cecum, whereas, freeze-dried cecal digesta was inoculated with fresh feces to simulate colonic fermentation during the *in vivo-in vitro* fermentation assay. Our data in Chapter 4 demonstrated that DF sources induced diversity in structural and functional characteristics of microbiota between the ileum and cecum. Therefore, the *in vivo-in vitro* fermentation assay in Chapter 6 was designed based on the postulations that digesta quality and microbiota composition and activity differ between the cecum and colon. The interaction between DF solubility and lipid types modulated digestibility of lipids, FA flows and fermentability of DF fractions but differs for soluble and insoluble fiber, saturated and unsaturated fatty acids and varies in different gastrointestinal segments (Chapter 6). In this regard, the digestibility of fatty acids is depressed and FA flows are increased in soluble fiber-containing diets but more in diets supplemented with beef tallow compared with insoluble fiber-containing diets. The predicted amounts of VFA produced and absorbed and fermentability of OM in the cecum and colon is greater in pigs fed soluble fiber-enriched diets compared with those fed insoluble fiber-containing diets (Chapter 6). Moreover, considerable quantities of butyric and propionic acids are produced

and absorbed at lower proportions in diets enriched with beef tallow compared with those supplemented with corn oil.

In Chapter 4, we demonstrated that soluble and insoluble fiber increased the concentrations of VFA and BA in different segements of the GIT and then in Chapter 5 and 6, predicted hindgut VFA production and absorption were greater in pigs fed soluble fiber compared to insoluble fiber. These findings concurred with finding in Chapter 7 that uptake and metabolism of VFA and in particular butyrate and acetate were greater in pigs fed soluble and insoluble fiber compared to pigs fed the control diet. Taken together, findings from these three studies clearly indicated that the adaptation of the intestinal mucosa to absorb individual VFA is indirectly influenced by the fermentability of the fiber source and bioavailability of individual VFA. The mechanisms involved appear complex and raises many questions that warrant further investigations.

Understanding the mechanism by which DF solubility modulates metabolic processes may assist us to answer the question why growth performance is poor in pigs fed high-fiber diets. It is also important to note that our data from experiments in Chapters 4 and 7 revealed that different fiber sources induce metagenomic functions as well as stimulate expressions of genes involved in energy and lipid metabolism. These processes appear to be substrate-specific and are initiated more in growing pigs fed soluble fiber from flaxseed meal compared to those offered insoluble fiber from oat hulls. The consumption of flaxseed meal promoted the production and absorption of propionic acid (Chapter 5) and also increased metagenomic functions related to biosynthesis of bile acids (Chapter 4). Furthermore, pigs fed diets with flaxseed meal also had poor performance and excreted more lithocholic and ursodeoxycholic acids compared to those fed oat hulls (Chapter 4). The two metabolites represent the predominating forms of secondary bile acids that are excreted following bioconversion of primary bile acids. Primary bile acids are sythesized by hepatocyte

from cholesterol and their production occurs at the expense of metabolizable energy that might be needed to fuel growth performance. Therefore, strategies to reduce BA excretion must be put in place as possible interventions to promote fat absorption and reduce cholesterol excretion. The VFA produced from DF fermentation could have contributed to hypocholesterolemic effects mainly due to increased intestinal propionate production in pigs fed FM diets (Chapter 4 and 5), which has been reported to decrease endogenous synthesis of cholesterol in the liver (Vela´zquez et al., 2000; Yoon et al. 2006).

The hepatic expression of *CYP7A1* gene can be used as a biomarker for cholesterol metabolism (Zhang et al., 2013; Wahlström et al., 2016). Therefore, the overexpression of *CYP7A1* in the colon of pigs fed FM and OH diets is also associated the cholesterol lowering effects of DF from flaxseed meal and oat hulls (Zhang et al., 2013). This could be an adaptation mechanism to compensate for poor re-absorption of primary bile acids and excretion of secondary bile acids. Bile acids excretion could be due to bioconversion of primary BA into secondary forms that are poorly absorbed back into EHC system. Supporting these observations are previous studies which have also shown that approximately 95% of total BA are reabsorbed into the hepatic system (Graffner et al., 2016). More work is needed to investigate if this suggestion stands in pigs fed different fiber sources. Moreover, hindgut microbiota has been reported to facilitate biotransformation of primary BA into secondary forms through gut microbial 7α-dehydroxylation and/or 7α/β-epimerization (Stevens and Hume, 1998; Ahn et al., 2003; Wahlström et al., 2016). The observation that a decrease in weight gain in pigs fed FM diet coincided with an increase in concentration of secondary BA and reduction in fat digestibility concurs with findings by Mathlouthi et al. (2002). These studies indicate that increase in DF reduce bile acids capacity for fat solubilization and emulsification, thereby depressing growth performance. Moreover, it can be suggested that this

mechanism could be one way by which propionic acid contributed to the poor performances in pigs fed flaxseed meal in Chapter 4. Another possible way by which flaxseed meal could have depressed feed intake and consequently reducing performance is by increasing propionic acid absorption. This postulation is supported by previous report that propionic acid inhibit food intake in humans (Arora et al., 2012). However, the molecular mechanisms and mediators involved are yet to be investigated.

In Chapter 7, ingestion of soluble fiber from flaxseed meal promoted the mucosal disappearance and catabolic loss of propionic acid compared to insoluble fiber from oat hulls which was similar to the control diet (Chapter 7). Metzler-Zebeli et al. (2012) reported that expression of *MCT1* gene in the colon of weaned pigs was positively corelated with propionic acid fluxes suggesting that increased concentration of VFA may upregulate expression of the *MCT1* gene. Moreover, propionic acid may indirectly stimulate PYY, thereby inducing satiety and depressed feed intake as observed in pigs fed flaxseed meal. Production fluxes and absorption of propionic acid has been associated with hypocholestomic and inappetance, and both have direct negative effects on growth performance. The use of alternative feedstuffs that promote propionic acid production needs to be investigated further in relation to growth performance in growing pigs.

Overall, results obtained in the studies reported in this thesis indicates that growth performance in pigs fed diets containing fibrous ingredients depends on how physicochemical properties of DF sources modulates interrelationships between diet, gut microbiota, host's GI tract epithelium and GI metabolites. The mechanism involved are complex, and are mediated by receptors that regulate lipid metabolism and partly relies on amount and solubility of DF and microbial activity that are diversified from one GI segment to the other.

The research described in this thesis open ways for practical dietary interventions that could be implemented in swine nutrition and the feed manufacturing industry. Firstly, it revealed that the complexity by which dietary fiber and supplementary fat modulates growing pigs responses is not necessarily linked to fiber level but largely depends on interactions between fiber solubility and the degree of saturation of dietary fatty acids. This information enlightens the importance for the need to characterize and put into consideration the ratio of soluble: insoluble fiber for alternative feed ingredients during feed formulation. Several studies demonstrated that growth performance, fat digestibility is compromised when pigs are fed high-fiber diets that are enriched with supplementary fat, but very few have investigated the flow of fatty acids and bile acids. The work described in studies of this thesis highlighted fundamental principles regarding the interrelationships by which DF sources and intestinal microbiota comparatively influence fat digestibility by modulating bioconversion of bile acids. This also unveiled the importance of gastrointestinal microbiota in nutrient utilization and supports the notion that gut microbiota can be modified to impede deconjugation of bile acids that are important for emulsifying fat. Considering that we revealed that bile acids are associated with gut microbiota that produce VFA, fibrous ingredients that are less fermentable in the upper gut may reduce incidences of deconjugation of bile acids.

Another significant contribution of the work described in this thesis is that we characterized the effects of soluble and insoluble fiber sources on the extent to which they individually reduce digested energy and at the same time increase the energy supply from volatile fatty acids produced by gastrointestinal fermentation. In this regard, previous studies using the integrated *in vivo-in vitro* fermentation assays have reported the effects of fiber level on VFA production (Ayayi and Adeola, 2015; Montoya et al., 2016). Among these, only Montoya et al. (2016) predicted VFA

absorption, and none characterized neither production nor absorption of these VFA separately in the cecum and colon. Furthermore, to the best of our knowledge only one study has demonstrated the fermentability of DF components in pigs fed high-fiber diets, but very little attention has been focused on the interactive effects of DF solubility and degree of saturation of the dietary fatty acids. This research has therefore confirmed the differences in which DF components are degraded and also estimated the quantity of VFA produced in different sections of the gastrointestinal tract. Therefore, it can be postulated that as a mitigating strategy, there is need to combine fiber with different solubility and fermentability capacity to allow a constant supply of VFA during transit of digesta in different gastrointestinal segments.

CHAPTER NINE

CONCLUSIONS AND FUTURE STUDIES

9.1. CONCLUSIONS

The following conclusions can be drawn based on the studies described in this thesis:

1. Consumption of insoluble fiber reduced serum cholesterol more than soluble fiber and the latter depressed growth performance by reducing feed intake but the former did not affect growth performance.

2. The intake of soluble and insoluble fiber induced variable effects on histological attributes of small intestines, profiles of fermentation products, as well as ileal and cecal microbiota composition and their predicted metagenomic functions.

3. Dietary inclusion of soluble and insoluble fiber decreased fatty acids digestibility, increased flows of fatty acids in the terminal ileum, cecum and feces and promoted excretion of bile acids and microbial activity. Our data suggests that fatty acids excretion in pigs fed high-fiber diets could be due to inefficient digestion caused by deconjugation of bile acids predisposed by bacterial activity. Fecal fat could also be ascribed to fatty acids produced from microbial activity in hindgut.

4. The ileal and fecal digestibility of fatty acids is depressed and fatty acids flows are increased more in pectin-containing diets but more in diets supplemented with beef tallow compared with all cellulose containing diets.

5. Ingestion of soluble fiber reduced ileal digested energy to a lesser extent than did insoluble fiber but resulted in a corresponding increase in hindgut digested energy and energy contribution from hindgut VFA production.

6. The predicted VFA production and absorption, and fermentability of organic matter in the cecum and colon is greater in pigs fed soluble fiber compared to insoluble fiber.

7. Butyric and propionic acids are produced and absorbed at lower proportions in diets enriched with beef tallow compared with those supplemented with corn oil.

8. Soluble fiber fractions from are mostly digested in the upper gut and cecum but insoluble fiber is fermented mainly in the cecum and colon.

9. The interactive effects of DF and lipid types modulates digestibility of lipids, FA flows and fermentability of DF fractions but these effects differ for soluble and insoluble fiber, saturated and unsaturated fatty acids and varies in different gastrointestinal segments.

10. Consumption of soluble fiber and insoluble fiber altered the adaptive capacity of colon epithelium and hepatocytes to transport and metabolize VFA.

11. Soluble fiber activated expression of genes related to lipid metabolism compared to insoluble fiber.

9.2. FUTURE STUDIES

1. More work is needed to characterize the effects of the interactive effects of dietary fiber and fatty acids type on microbial species at genera level and the structural and functional microbiota composition in other gastrointestinal compartments such as jejunum, duodenum and colon.

2. The observation in Chapters 5 and 6 that the profiles of fatty acids flows dramatically changed in the ileum, cecum and feces stimulates the need for subsequent studies to quantify the contribution of fatty acids originating from the diet, endogenous secretions, or microbial activity following deconjugation of BA and/or microbial bioconversion of supplementary FA or endogenous lipids into microbial FA. This implies that the infusion of stable isotope-labelled FA

or water in future studies would facilitate the characterization of the sources of fatty acids detected in feces.

3.   Addition of either soluble or insoluble fiber increased the quantity of bile acids excreted and these bile acids interrelate with gut microbiota and are produced at the expense of cholesterol produced by the pig (Chapters 5 and 6). Thus, more research is also needed to estimate the quantities of bile acids excreted and how these are influenced by changes in physicochemical properties of digesta or microbiota composition from one gastrointestinal segment to the other.

4.   Because gastrointestinal fermentation and gut development is noticeable more as pigs age and gain more weight, and the capacity to consume and utilize fiber-rich diets increase with pig maturity and size, further studies are warranted to test all the hypotheses studied in this thesis using finishing pigs and sows.

5.   In Chapter 5 we used ileal cannulated pigs, whereas in Chapter 6 ileal- and cecal-cannulated pigs were used in the *in vivo-in vitro* fermentation assay. There is need for subsequent studies to investigate if differences exist between ileal cannulated pigs model and ileal- and cecal-cannulated pig model when used to predict VFA production using the integrated *in vivo-in vitro* fermentation techniques.

CHAPTER TEN

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CHAPTER 11

APPENDICES

APPENDIX 1
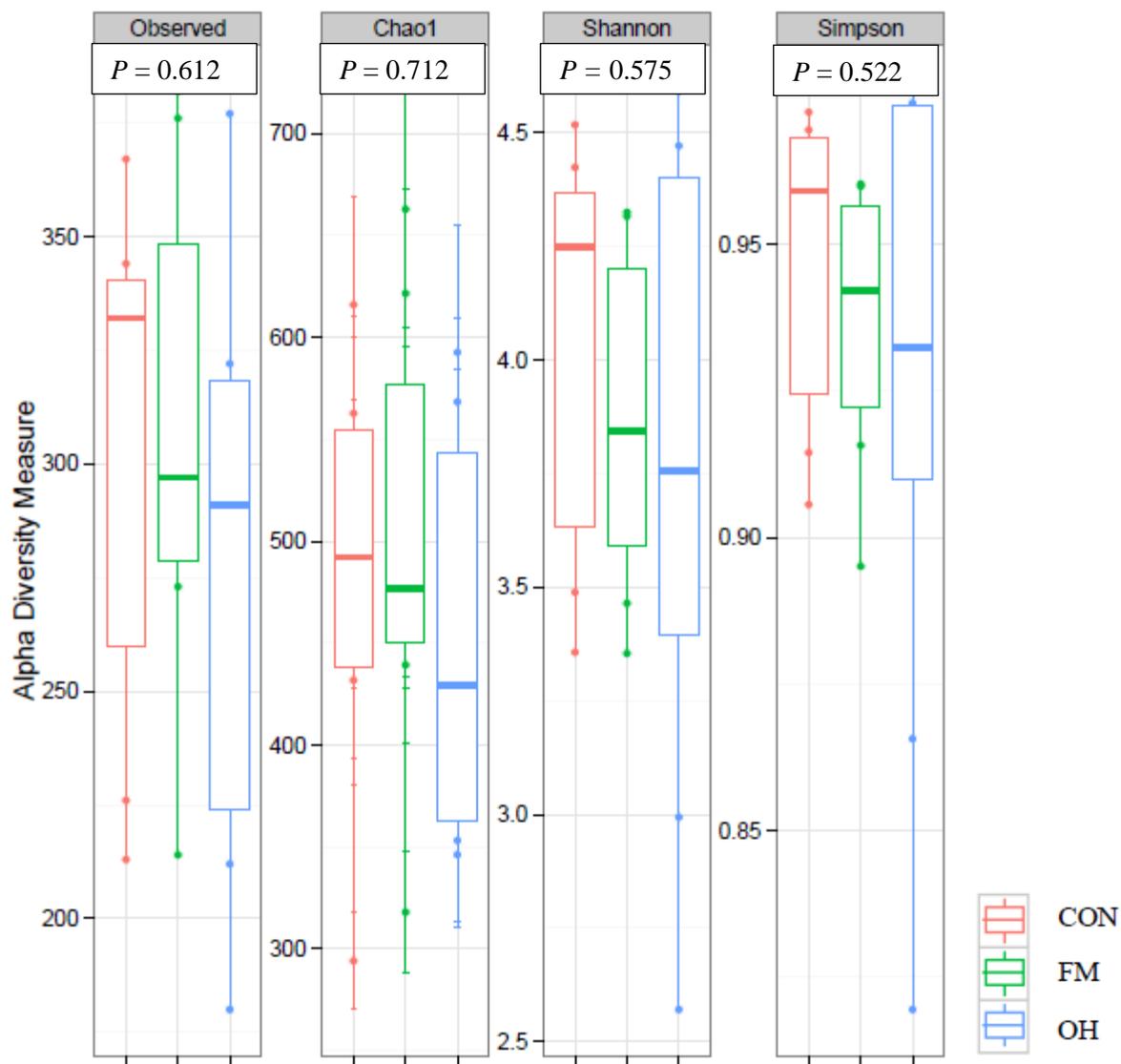
Supplementary Figure 4.1. Alpha-diversity indices of microbiota in ileal digesta of pigs fed with control (CON), flaxseed meal (FM) and oat hulls (OH) diets
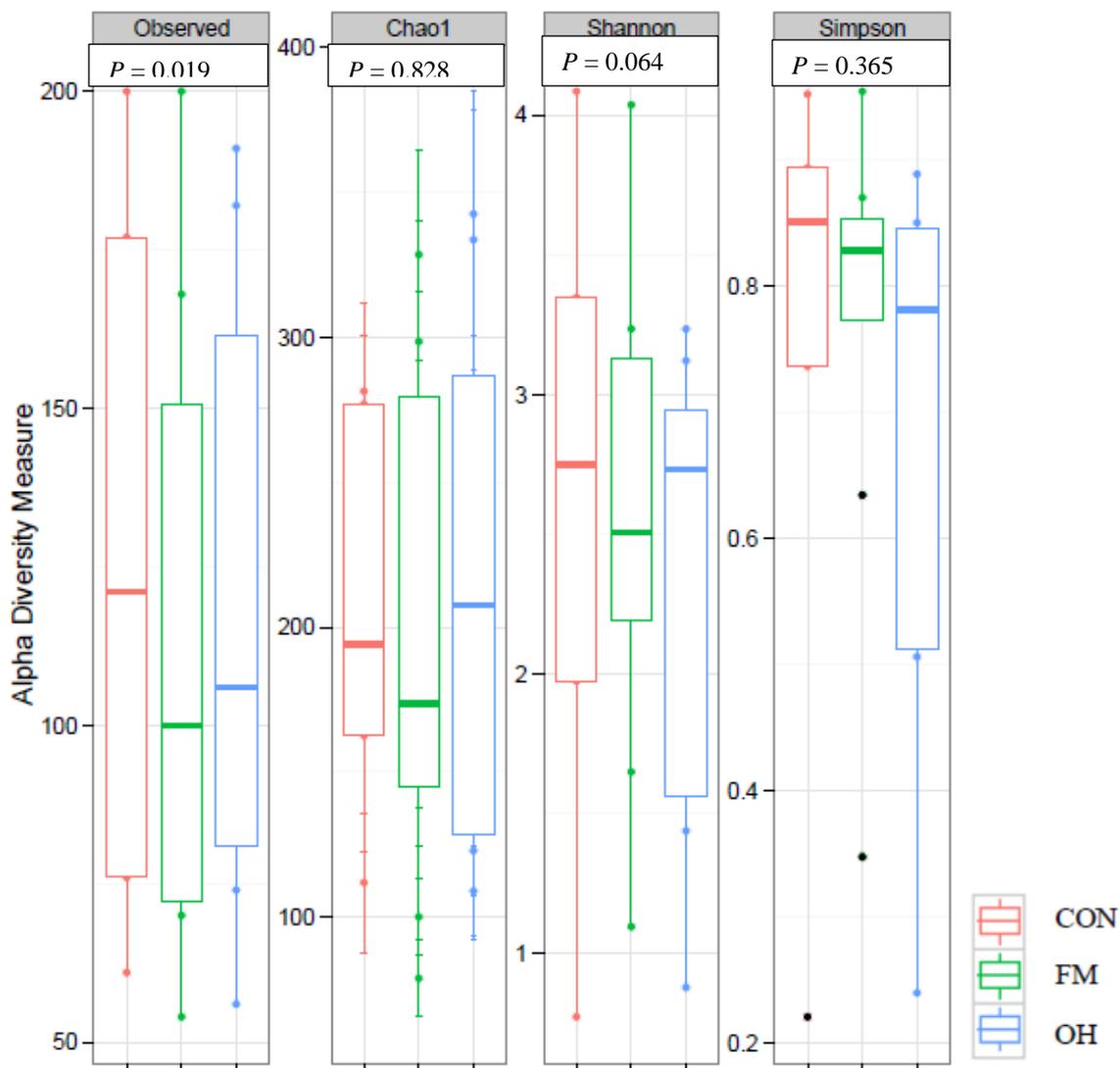
Supplementary Figure 4.2. Alpha-diversity indices of ileal mucosa-associated microbiota of pigs fed with control (CON), flaxseed meal (FM) and oat hulls (OH) diets
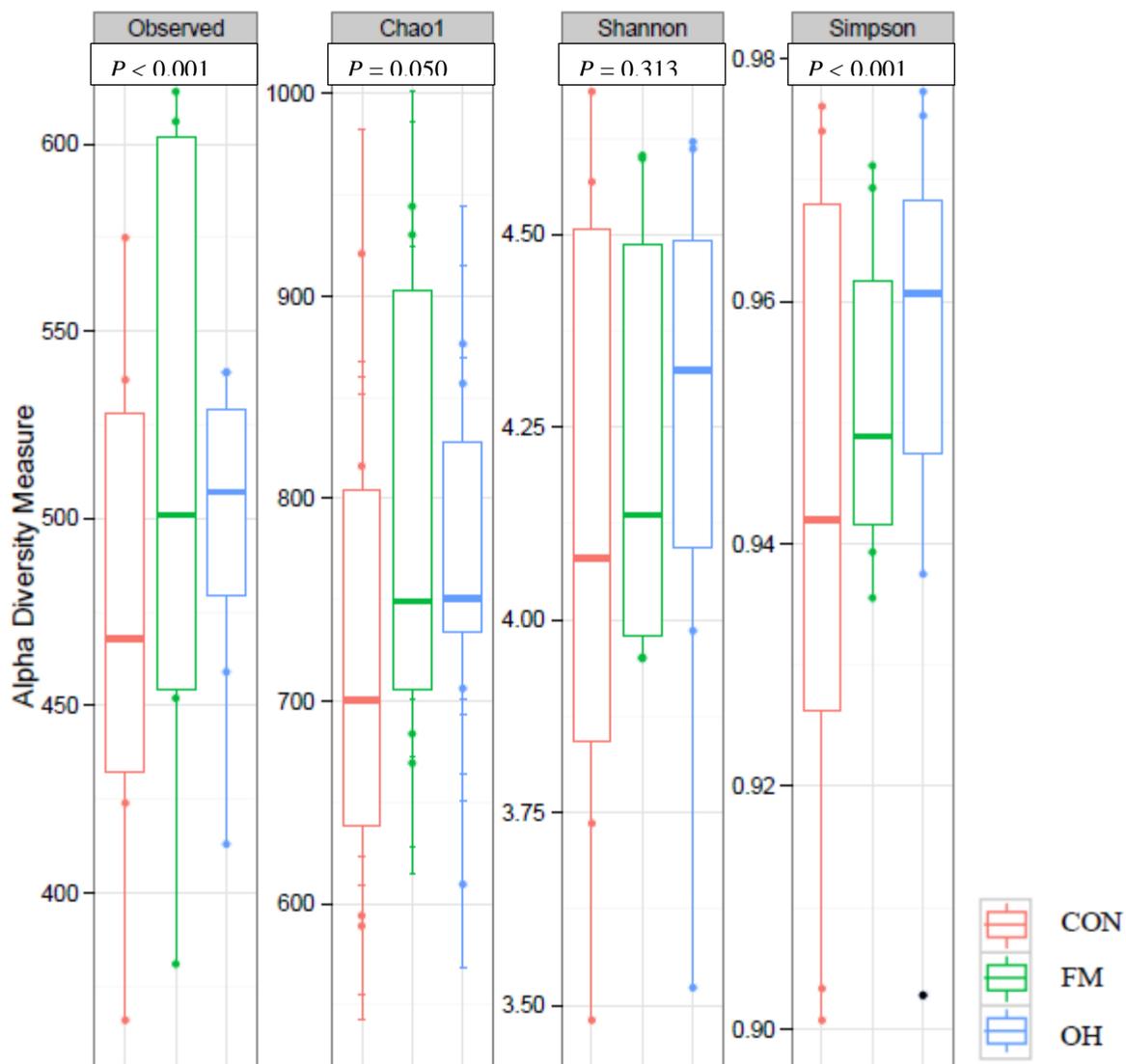
Supplementary Figure 4.3. Alpha-diversity indices of microbiota in cecal digesta of pigs fed with control (CON), flaxseed meal (FM) and oat hulls (OH) diets
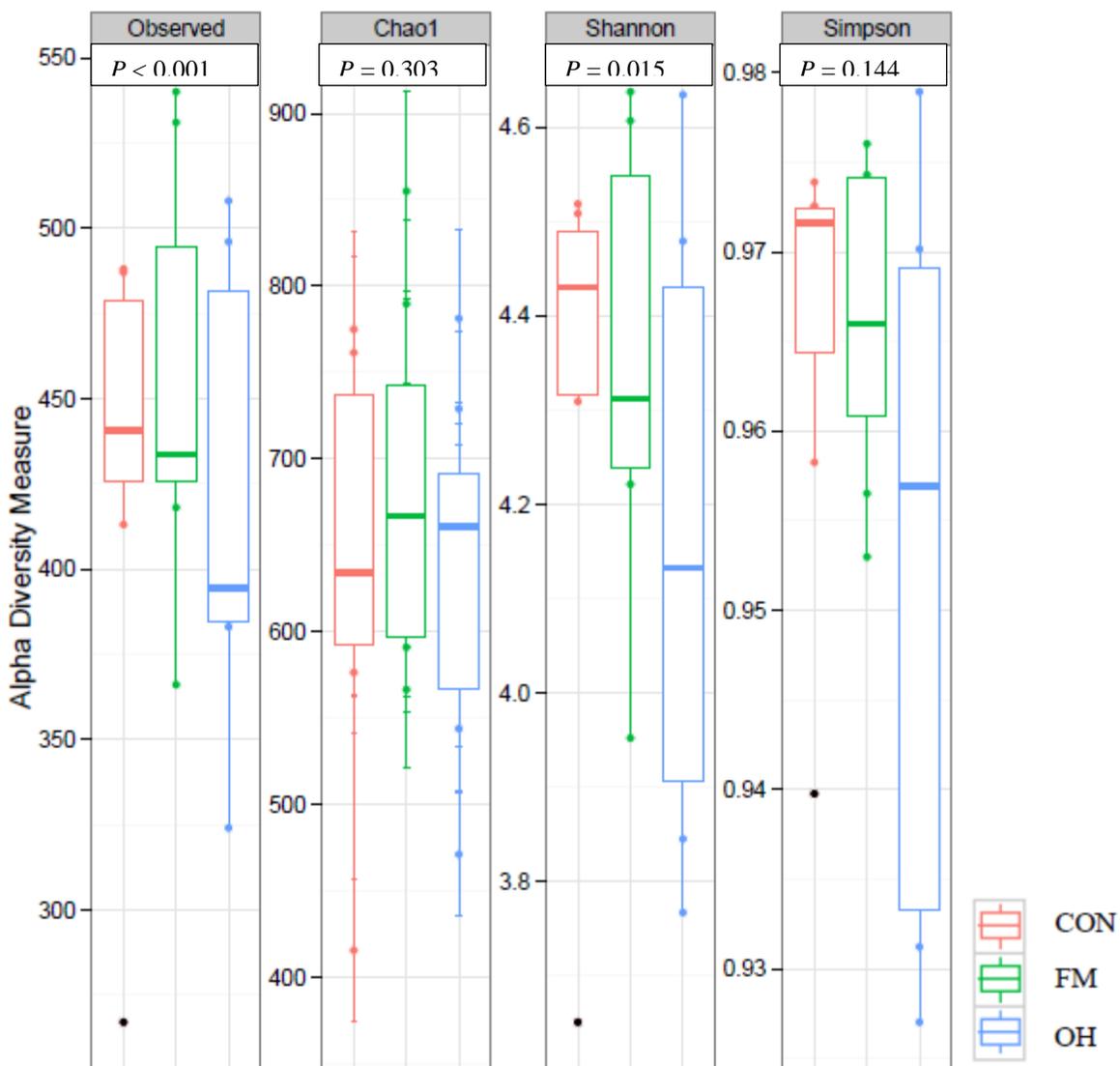
Supplementary Figure 4.4. Alpha-diversity indices of cecal mucosa-associated microbiota of pigs fed with control (CON), flaxseed meal (FM) and oat hulls (OH) diets.

Supplementary Table 4.1. Effect of diet on alpha-diversity indices[1] of cecal and ileal digesta microbiota of pigs fed control diet (CON), flaxseed meal (FM) and oat hulls (OH)

Item	Ileum					Cecum				
	[2]Diet			SEM[3]	*P*-value	[2]Diet			SEM[3]	*P*-value
	CON	FM	OH			CON	FM	OH		
Observed_species[4]	303	304	277	60.3	0.612	462[b]	595[a]	510[b]	23.85	<0.001
Coverage[5]	0.955	0.955	0.959	0.010	0.673	0.975	0.973	0.974	0.0016	0.799
Chao1[6]	485	494	453	104.7	0.712	722[C]	805[A]	769[B]	65.3	0.050
Shannon[7]	5.827	5.583	5.418	0.598	0.575	5.700	6.035	6.126	0.328	0.313
Simpson[8]	0.947	0.937	0.925	0.021	0.522	0.942[b]	0.951[a]	0.954[a]	0.0003	<0.001

[1]Least square mean values for alpha biodiversity indices.

[abc]Within a row, means with unlike superscripts differ ($P < 0.05$)

[ABC]Within a row, means with unlike superscripts differ ($P \leq 0.10$).

[2]CON, Control diet; FM, Flaxseed meal-containing diet; OH, Oat hulls-containing diet.

[3]Standard error of treatment mean (n = 8 replicates per treatment).

[4]Observed_species is the unique OTUs (97% distance) found in the samples.

[5]Coverage estimates the percentage of the total species sequenced in samples.

[6]Chao richness index estimates the number of species or OTU presenting in samples.

[7,8] Indices of microbiota diversity, which take into account species richness and abundance.

Supplementary Table 4.2. Effect of diet on alpha-diversity indices[1] of cecal and ileal mucosa-associated microbiota of pigs fed control diet (CON), flaxseed meal (FM) and oat hulls (OH)

Item	Ileum			SEM[3]	*P*-value	Cecum			SEM[3]	*P*-value
	[2]Diet					[2]Diet				
	CON	FM	OH			CON	FM	OH		
Observed_species[4]	91.6^b	151.3^a	115.8^{ab}	1487	0.019	393^b	504^a	445^{ab}	3044	0.002
Coverage[5]	0.973	0.963	0.962	0.021	0.246	0.978	0.967	0.978	0.0016	0.671
Chao1[6]	216.1	223.4	201.6	8.288	0.828	553^b	683^a	638^{ab}	9.103	0.039
Shannon[7]	2.323^B	3.945^A	3.611^B	0.601	0.064	6.249^a	6.279^a	5.761^b	0.130	0.015
Simpson[8]	0.770	0.840	0.732	0.022	0.365	0.966	0.966	0.953	0.0021	0.144

[1]Least square mean values for alpha biodiversity indices.

[abc]Within a row, means with unlike superscripts differ ($P < 0.05$)

[ABC]Within a row, means with unlike superscripts differ ($P \leq 0.10$).

[2]CON, Control diet; FM, Flaxseed meal-containing diet; OH, Oat hulls-containing diet.

[3]Standard error of treatment mean (n = 8 replicates per treatment).

[4]Observed_species is the unique OTUs (97% distance) found in the samples.

[5]Coverage estimates the percentage of the total species sequenced in samples.

[6]Chao richness index estimates the number of species or OTU presenting in samples.

[7,8] Indices of microbiota diversity, which take into account species richness and abundance.

Supplementary Table 4.3. Relative abundance of bacterial phyla in ileal digesta-associated microbiota of pigs fed control diet (CON), flaxseed meal (FM) and oat hulls (OH) [†]

Phyla present	[1]Diets			SEM[2]	*P-value*[3]
	CON	FM	OH		
-----------------------------------Abundant Phylum (>1% of population)-----------------------					
Firmicutes	60.21 ± 5.32^B	73.23 ± 3.84^A	72.92 ± 2.85^A	6.612	0.081^G
Bacteroidetes	25.72 ± 4.22	20.05 ± 8.59	22.98 ± 1.12	5.321	0.772^P
Proteobacteria	1.12 ± 0.24^b	5.01 ± 0.33^a	2.82 ± 0.68^a	0.097	$<0.01^G$
Unclassified	11.25 ± 4.23^a	1.05 ± 4.23^b	2.85 ± 3.53^b	2.361	0.023^G
---------------------------------Low-abundant Phylum (<1% of population)-------------------					
Spirochaetes	0.42 ± 0.22	0.35 ± 1.29	0.58 ± 0.42	0.831	0.362^P
Actinobacteria	0.21 ± 0.05	0.12 ± 0.08	0.33 ± 0.05	0.003	0.51^G
Cyanobacteria	0.02 ± 0.20	0.02 ± 0.02	0.03 ± 0.03	0.011	0.67^G
Tenericutes	0.01 ± 0.24	0.01 ± 0.33	0.02 ± 0.18	0.017	0.61^G
Elusimicrobia	0.01 ± 0.05	0.02 ± 0.18	0.01 ± 0.25	0.003	0.78^P
Fibrobacteres	0.01 ± 0.20	0.02 ± 0.12	0.03 ± 0.43	0.011	0.67^G
Synergistetes	0.01 ± 0.05	0.02 ± 0.08	0.01 ± 0.05	0.003	0.88^G

[1]CON, Control diet; FM, Flaxseed meal-containing diet; OH, Oat hulls-containing diet;
[2]SEM, standard error of means;
[†]Least square mean values (n = 8 replicates per treatment).
[abc]Mean values within a row with unlike superscripts are significantly different ($P < 0.05$).
[ABC]Mean values within a row with unlike superscripts are significantly different ($0.05<P < 0.10$).
[3]Methods of analysis denoted by [G](Gaussian), and [P](Poison).

Supplementary Table 4.4. Relative abundance of bacterial phyla in ileal mucosa-associated microbiota of pigs fed control diet (CON), flaxseed meal (FM) and oat hulls (OH) [†]

Phyla present	[1]Diets			[2]SEM	*P-value[3]*
	CON	FM	OH		
--Abundant Phylum (>1% of population)-------------------------					
Bacteroidetes	13.22 ± 1.54^{ab}	17.26 ± 2.56^{a}	9.01 ± 2.88^{b}	3.251	$<0.001^{G}$
Firmicutes	80.04 ± 5.97^{a}	66.25 ± 4.74^{b}	65.01 ± 4.81^{b}	6.872	0.001^{P}
Proteobacteria	5.08 ± 6.61^{b}	14.09 ± 2.36^{ab}	17.05 ± 3.13^{a}	3.924	0.100^{NB}
Unclassified	1.16 ± 1.69	2.11 ± 3.65	8.53 ± 6.59	6.542	0.361^{G}
---------------------------------------Low abundant Phylum (<1% of population)---------------------					
Actinobacteria	0.41 ± 0.62^{A}	0.03 ± 0.09^{C}	0.11 ± 0.25^{B}	0.002	0.050^{P}
Spirochaetes	0.11 ± 0.15	0.05 ± 0.08	0.07 ± 0.11	0.051	0.551^{G}
Cyanobacteria	0.09 ± 0.07	0.08 ± 0.08	0.10 ± 0.11	0.055	0.961^{G}
Deferribacteres	0.01 ± 0.02^{b}	0.02 ± 0.03^{b}	0.06 ± 0.03^{a}	0.015	0.011^{G}
Synergistetes	0.02 ± 0.03	0.01 ± 0.02	0.01 ± 0.02	0.013	0.771^{G}
WPS-2	0.01 ± 0.15	0.02 ± 0.02	0.02 ± 0.11	0.031	0.365^{G}
Elusimicrobia	0.01 ± 0.11	0.02 ± 0.08	0.03 ± 0.11	0.042	0.111^{G}

[1]CON, Control diet; FM, Flaxseed meal-containing diet; OH, Oat hulls-containing diet;
[2]SEM, standard error of means.
[†]Least square mean values (n = 8 replicates per treatment).
[abc]Mean values within a row with unlike superscripts are significantly different ($P < 0.05$).
[ABC]Mean values within a row with unlike superscripts are significantly different ($P < 0.10$).
[3]Methods of analysis denoted by [G] (Gaussian), [NB] (Negative Binomial) and [P] (Poison).

Supplementary Table 4.5. Phylogenetic composition of bacterial genus from illumina-sequencing of 16S rRNA sequences in ileal digesta of pigs fed experimental diets

Order (O), Family (F), genus (G)	Diets[1]			SEM[2]	*P-value[3]*
	CON	FM	OH		
....................................Actinobacteria (P).......................................					
Unclassified Coriobacteriaceae (F)	0.104^{AB}	0.154^{A}	0.079^{B}	0.031	0.068^{G}
....................................Bacteroidetes (P).......................................					
Unclassified Bacteroidales (O)	0.543	1.024	0.543	0.434	0.614^{P}
Bacteroides	0.117	0.370	0.100	0.137	0.114^{G}
Prevotella	22.44^{a}	14.47^{b}	16.85^{ab}	3.207	0.004^{P}
Parabacteroides	0.067	0.083	0.046	0.040	0.659^{G}
CF231	1.15	1.36	0.99	0.425	0.799^{P}
YRC22	0.083^{b}	0.342^{a}	0.163^{ab}	0.099	0.048^{G}
....................................Firmicutes (P).......................................					
Lactobacillus	17.96^{b}	28.70^{a}	23.36^{ab}	1.597	0.002^{P}
Streptococcus	5.56^{B}	9.08^{A}	7.09^{AB}	1.647	0.087^{NB}
Unclassified Clostridiales (O)	3.12^{b}	4.02^{a}	1.82^{c}	0.225	0.015^{G}
Unclassified Clostridiaceae (F)	3.94^{b}	8.54^{a}	4.40^{b}	1.681	0.002^{P}
Clostridium	0.395	0.355	0.267	0.142	0.658^{G}
Blautia	2.16	1.25	2.58	0.711	0.195^{P}
Coprococcus	0.531^{ab}	0.357^{b}	0.754^{a}	0.142	0.037^{G}
Dorea	0.305^{B}	0.395^{AB}	0.658^{A}	0.146	0.065^{G}
Roseburia	0.273	0.322	0.360	0.102	0.695^{G}
Unclassified Ruminococcaceae (F)	3.65	3.16	3.74	0.568	0.554^{G}
Ruminococcus	0.518	0.478	0.783	0.239	0.698^{P}
Faecalibacterium	0.512	0.475	0.433	0.126	0.823^{G}
Oscillospira	0.353	0.539	0.383	0.226	0.836^{P}
Peptococcus	0.0708	0.1084	0.1208	0.046	0.536^{G}
Unclassified Veillonellaceae (F)	2.48^{a}	1.62^{ab}	0.283^{b}	0.804	0.024^{NB}
Acidaminococcus	0.500	0.217	0.071	0.119	0.108^{P}
Anaerovibrio	0.842	0.938	0.967	0.265	0.880^{G}
Dialister	0.308	0.138	0.175	0.106	0.262^{G}
Mitsuokella	5.18^{ab}	12.50^{a}	0.133^{b}	4.945	0.031^{NB}
Megasphaera	16.75	15.50	17.41	4.527	0.913^{G}
Phascolarctobacterium	9.33^{a}	2.09^{ab}	1.11^{b}	5.212	<0.001^{NB}
Unclassified Erysipelotrichaceae (F)	0.0666^{AB}	0.0418^{B}	0.108^{A}	0.026	0.055^{G}
Bulleidia	1.32	1.92	0.900	0.924	0.557^{P}
....................................Proteobacteria.......................................					
Succinivibrio	0.154	0.154	0.146	0.058	0.986^{P}
Unclassified Enterobacteriaceae (F)	0.103^{b}	0.218^{b}	2.575^{a}	0.095	0.047^{NB}

[1]CON, Control diet; FM, Flaxseed meal-containing diet; OH, Oat hulls-containing diet;
[2]SEM, standard error of means (n = 8 replicates per treatment).
[abc]Mean values within a row with unlike superscripts are significantly different (P < 0.05).
[ABC]Mean values within a row with unlike superscripts are significantly different (P < 0.10).

[3]Methods of analysis denoted by G (Gaussian), [NB] (Negative Binomial) and [P] (Poison).

Supplementary Table 4.6. Phylogenetic composition of bacterial genus from illumina-sequencing of 16S rRNA sequences in ileal mucosa of pigs fed experimental diets

Order (O), Family (F), Genus	Diets[1]			SEM[2]	*P-value[3]*
	CON	FM	OH		
……………………………………Actinobacteria (P)………...………………………….					
Actinobacteria_unclassified	0.5694	0.0468	0.0103	0.332	0.169^G
………………………………..…...…….Bacteroidetes (P)……...…………...………………..					
Bacteroidales _unclassified (O)	4.70^b	24.48^a	20.42^a	6.788	<0.001^P
………………………….………Firmicutes (P)………...……………………………..					
Lactobacillus	9.95^b	58.83^a	59.48^a	11.223	<0.001^{NB}
Streptococcus	3.71^{AB}	2.91^B	5.57^A	1.059	0.083^P
Unclassified Clostridiales (O)	2.63^a	0.488^b	1.93^{ab}	1.101	0.048^{NB}
Unclassified Clostridiaceae (F)	31.38^a	0.283^b	1.02^b	7.591	<0.001^P
Unclassified Lachnospiraceae (F)	1.55	0.679	0.925	0.472	0.193^G
Unclassified Ruminococcaceae (F)	1.86	1.85	1.13	0.851	0.627^G
Unclassified Veillonellaceae (F)	7.53^a	2.46^b	5.09^{ab}	2.587	0.002^P
Veillonella	0.137^b	2.79^a	0.372^b	0.384	0.004^P
………………..…………….………..Proteobacteria (P)…………...…………………….					
Campylobacter	0.2088	0.2251	0.2327	0.109	0.973^P
Helicobacter	2.2358^a	2.4393^a	0.2583^b	0.939	0.024^P

[1]CON, Control diet; FM, Flaxseed meal-containing diet; OH, Oat hulls-containing diet;
[2]SEM, standard error of means;
†Least square mean values (n = 8 replicates per treatment).
[abc]Mean values within a row with unlike superscripts are significantly different ($P < 0.05$).
[ABC]Mean values within a row with unlike superscripts are significantly different ($P < 0.10$).
[3]Methods of analysis denoted by G (Gaussian), [NB] (Negative Binomial) and [P] (Poison).

Supplementary Table 4.7. Relative abundance of bacterial phyla in cecal digesta-associated microbiota of pigs fed control diet (CON), flaxseed meal (FM) and oat hulls (OH)

Phyla present	Diets[1]			SEM[2]	*P-value[3]*
	CON	FM	OH		
--------------------------------------Abundant Phylum (>1% of population)------------------------					
Bacteroidetes	34.71±11.72	29.04±7.91	33.63±3.07	4.762	0.421[P]
Firmicutes	60.14±1.97[b]	64.08±0.73[a]	59.70±0.67[b]	1.031	<0.001[G]
Proteobacteria	1.11±0.43[c]	5.65±0.91[a]	2.05±0.50[b]	0.633	0.049[P]
Unclassified	2.46±0.65	0.04±0.15	4.01±5.63	3.658	0.215[G]
------------------------------Low abundant Phylum (<1% of population)-----------------------					
Actinobacteria	0.236±0.06[A]	0.078±0.06[B]	0.139±0.051[AB]	0.007	0.060[G]
Chlamydiae	0.565±0.01	0.263±0.532	0.273±0.004	0.251	0.592[P]
Cyanobacteria	0.236±0.16[a]	0.078±0.031[b]	0.139±0.115[ab]	0.060	0.048[G]
Deferribacteres	0.053±0.01	0.005±0.013	0.027±0.06	0.018	0.351[G]
Elusimicrobia	0.031±0.05	0.025±0.041	0.052±0.032	0.011	0.123[P]
Fibrobacteres	0.023±0.02	0.022±0.018	0.044±0.032	0.016	0.344[G]
Synergistetes	0.028±0.02	0.029±0.017	0.022±0.019	0.010	0.736[G]
TM7	0.011±0.02	0.006±0.007	0.006±0.009	0.006	0.695[G]
Tenericates	0.028±0.02[a]	0.005±0.013[b]	0.021±0.011[ab]	0.010	0.039[G]
WPS-2	0.078±0.07	0.027±0.012	0.028±0.025	0.030	0.244[G]

[1]CON, Control diet; FM, Flaxseed meal-containing diet; OH, Oat hulls-containing diet;
[2]SEM, standard error of means;
[†]Least square mean values (n = 8 replicates per treatment);
[abc]Mean values within a row with unlike superscripts are significantly different ($P < 0.05$);
[ABC]Mean values within a row with unlike superscripts are significantly different ($P < 0.10$);
[3]Methods of analysis denoted by [G] (Gaussian), [n] (Negative Binomial) and [p] (Poison).

Supplementary Table 4.8. Relative abundance of bacterial phyla in cecal mucosa-associated microbiota of pigs fed control diet (CON), flaxseed meal (FM) and oat hulls (OH)

Phyla present	[1]Diets			[2]SEM	*P-value[3]*
	CON	FM	OH		
---------------------------------------Abundant Phylum (>1% of population)----------------------- --					
Bacteroidetes	59.16 ± 5.85^a	51.22 ± 5.06^{ab}	48.89 ± 14.42^b	4.104	0.008^P
Firmicutes	36.06 ± 5.26	38.22 ± 7.77	34.90 ± 13.66	5.272	0.257^P
Proteobacteria	3.05 ± 2.35^b	9.23 ± 6.26^{ab}	13.19 ± 9.37^a	3.341	$<0.001^P$
Unclassified	0.87 ± 1.326	0.514 ± 0.633	2.63 ± 1.11	1.363	0.635^G
--------------------------------Low abundant Phylum (<1% of population)----------------------- -					
Actinobacteria	0.028 ± 0.03	0.025 ± 0.01	0.021 ± 0.02	0.010	0.743^G
Cyanobacteria	0.296 ± 0.18	0.269 ± 0.24	0.252 ± 0.13	0.094	0.895^G
Deferribacteres	0.067 ± 0.14	0.011 ± 0.59	0.033 ± 0.76	0.048	0.882^P
Elusimicrobia	0.023 ± 0.03	0.016 ± 0.02	0.014 ± 0.02	0.011	0.682^G
Fibrobacteres	0.014 ± 0.02	0.028 ± 0.02	0.013 ± 0.02	0.010	0.241^G
Spirochaetes	0.292 ± 0.44^{ab}	0.389 ± 0.24^a	0.093 ± 0.11^b	0.099	0.024^G
Synergistetes	0.022 ± 0.01	0.035 ± 0.02	0.024 ± 0.02	0.010	0.286^P
TM7	0.027 ± 0.02	0.016 ± 0.01	0.013 ± 0.01	0.009	0.202^G

[1]CON, Control diet; FM, Flaxseed meal-containing diet; OH, Oat hulls-containing diet;
[2]SEM, standard error of means;
[†]Least square mean values (n = 8 replicates per treatment);
[abc]Mean values within a row with unlike superscripts are significantly different ($P < 0.05$);
[ABC]Mean values within a row with unlike superscripts are significantly different ($P < 0.10$);
[3]Methods of analysis denoted by [G] (Gaussian), [n] (Negative Binomial) and [p] (Poison).

Supplementary Table 4.9. Phylogenetic composition of bacterial genus from illumina-sequencing of 16S rRNA sequences in cecal digesta of pigs fed experimental diets

Order (O), Family (F), Genus	Diets[1] CON	FM	OH	SEM[2]	*P-value[3]*
………………………………Actinobacteria (P)…………...……………………………					
Bifidobaterium	2.45	0.809	0.497	0.811	0.115[NB]
Unclassified Coriobacteriaceae (F)	0.0511[b]	0.164[a]	0.0875[b]	0.021	<0.001[G]
……………………….…....………Bacteroidetes (P)………..…………………………………….					
Unclassified Bacteroidales (O)	4.03[AB]	2.77[B]	5.49[A]	0.873	0.066[P]
Bacteroides	0.0704[b]	0.736[a]	0.159[b]	0.196	0.006[G]
Parabacteroides	0.109	0.183	0.122	0.046	0.245[G]
Prevotella	30.43[b]	26.73[b]	38.37[a]	2.264	0.002[P]
CF231	1.53[A]	1.43[AB]	1.06[B]	0.192	0.070[G]
………………………….…..…Firmicutes (P)………...…………………………………..					
Lactobacillus	6.41[b]	16.13[a]	12.87[ab]	2.977	<0.001[P]
Streptococcus	8.29[a]	5.18[b]	7.03[ab]	0.816	0.004[G]
Unclassified Clostridiales (O)	3.51	3.10	3.14	0.858	0.875[P]
Unclassified Clostridiaceae (F)	0.210	0.0149	0.159	0.249	0.117[NB]
Clostridium	0.389[a]	0.139[b]	0.214[ab]	0.077	0.012[G]
Unclassified Lachnospiraceae (F)	0.572[a]	0.425[ab]	0.309[b]	0.109	0.078[G]
Blautia	1.263[AB]	1.061[B]	1.934[A]	0.397	0.094[G]
Coprococcus	0.453[b]	0.351[b]	0.824[a]	0.086	<0.001[G]
Dorea	0.347[b]	0.298[b]	0.491[a]	0.051	0.004[G]
Roseburia	0.950[a]	0.348[b]	0.458[b]	0.169	0.004[G]
Shuttleworthia	0.0364	0.0522	0.0094	0.022	0.152[P]
Unclassified Ruminococcaceae (F)	3.64	2.99	3.70	0.664	0.498[G]
Ruminococcus	1.01	0.685	1.03	0.035	0.078[NB]
Faecalibacterium	0.277	0.325	0.430	0.086	0.219[G]
Oscillospira	0.496	0.647	0.552	0.186	0.718[P]
Peptococcus	0.0948	0.0802	0.1078	0.024	0.527[G]

[1]CON, Control diet; FM, Flaxseed meal-containing diet; OH, Oat hulls-containing diet;
[2]SEM, standard error of means;
†Least square mean values (n = 8 replicates per treatment).
[abc]Mean values within a row with unlike superscripts are significantly different ($P < 0.05$).
[ABC]Mean values within a row with unlike superscripts are significantly different ($P < 0.10$).
[3]Methods of analysis denoted by G (Gaussian), [NB] (Negative Binomial) and [P] (Poison).

Supplementary Table 4.9. Continued

Order (O), Family (F), genus	Diets[1]			SEM[2]	*P-value[3]*
	CON	FM	OH		
………………………………..…Firmicutes (P)………...………….……………………….					
Unclassified Veillonellaceae	1.21[AB]	1.74[A]	0.658[B]	0.445	0.075[G]
Acidaminococcus	0.426[AB]	0.698[A]	0.148[B]	0.240	0.098[G]
Anaerovibrio	0.910	1.22	0.942	0.319	0.569[G]
Mitsuokella	0.0986	0.0655	0.102	0.032	0.477[G]
Megasphaera	18.85	19.64	16.04	4.035	0.650[P]
Phascolarctobacterium	1.33[b]	3.69[a]	2.70[ab]	0.628	0.011[P]
Unclassified Erysipelotrichaceae (F)	0.00765[b]	0.00953[b]	0.0203[a]	0.007	0.019[G]
Bulleidia	0.0421	0.0407	0.0328	0.016	0.815[G]
Catenibacterium	0.0282	0.0093	0.0250	0.010	0.134[G]
Eubacterium	0.0126[B]	0.0186[B]	0.0453[A]	0.013	0.053[G]
Sharpea	0.0405	0.0283	0.0313	0.024	0.872[G]
………………………….………………..Proteobacteria (P)………..…..……….…………………………..					
Unclassified Proteobacteria	0.777[b]	1.831[a]	1.109[ab]	0.364	0.026[NB]
Campylobacter	0.8139	1.6408	1.2250	0.651	0.460[P]
Succinivibrio	0.0951	0.1580	0.0797	0.056	0.352[G]
Sutterella	0.0155	0.0298	0.0266	0.008	0.186[G]

[1]CON, Control diet; FM, Flaxseed meal-containing diet; OH, Oat hulls-containing diet;

[2]SEM, standard error of means;

[†]Least square mean values (n = 8 replicates per treatment).

[abc]Mean values within a row with unlike superscripts are significantly different ($P < 0.05$).

[ABC]Mean values within a row with unlike superscripts are significantly different ($P < 0.10$).

[3]Methods of analysis denoted by G (Gaussian), [NB] (Negative Binomial) and [P] (Poison).

Supplementary Table 4.10. Phylogenetic composition of bacterial genus from illumina-sequencing of 16S rRNA sequences in cecal tissue of pigs fed experimental diets

Phylum, Order, Family, genus	Diets[1]			SEM[2]	*P-value*[3]
	CON	FM	OH		
............................Actinobacteria (P)............................					
Unclassified Coriobacteriaceae	0.0294	0.0248	0.0204	0.010	0.668^G
............................Bacteroidetes (P)............................					
Unclassified Bacteroidales (O)	1.83	1.92	1.40	0.426	0.437^G
Bacteroides	0.0865^B	0.938^A	0.187^B	0.376	0.069^P
Parabacteroides	0.171^b	0.372^a	0.213^b	0.060	0.007^G
Prevotella	7.79^{AB}	7.12^B	10.51^A	2.986	0.069^P
CF231	1.15	1.02	1.02	0.258	0.839^P
YRC22	0.445^A	1.111^A	0.215^C	0.049	0.095^P
............................Firmicutes (P)............................					
Lactobacillus	1.44^a	1.52^a	0.536^b	0.392	0.045^G
Streptococcus	1.03	0.618	0.757	0.193	0.118^G
Unclassified Clostridiales (O)	1.19	2.51	1.68	0.682	0.172^G
Unclassified Clostridiaceae (F)	0.414^A	0.239^B	0.234^B	0.083	0.071^G
Clostridium	0.165	0.141	0.121	0.034	0.453^G
Unclassified Lachnospiraceae (F)	0.414^a	0.239^b	0.234^b	0.083	0.071^P
Blautia	0.3131	0.3665	0.4364	0.253	0.888^G
Coprococcus	0.239^a	0.121^b	0.226^{ab}	0.045	0.029^G
Dorea	0.199^A	0.102^B	0.219^A	0.047	0.052^G
Lachnospira	0.193^a	0.0536^b	0.0875^b	0.044	0.008^G
Roseburia	0.588^a	0.219^c	0.392^b	0.063	<0.001^G
Unclassified Ruminococcaceae (F)	4.01	2.98	2.92	0.682	0.210^P
Ruminococcus	0.259a	0.135b	0.193b	0.032	0.008^G
Faecalibacterium	0.814^a	0.380^{ab}	0.334^b	0.187	0.032^G
Oscillospira	1.05^a	1.12^a	0.462^b	0.166	0.027^G
Unclassified Peptostreptococcaceae (F)	0.0147	0.0078	0.0124	0.001	0.342^P
Peptococcus	0.0246	0.0185	0.0146	0.007	0.413^{NB}

[1]CON, Control diet; FM, Flaxseed meal-containing diet; OH, Oat hulls-containing diet;
[2]SEM, standard error of means;
[†]Least square mean values (n = 8 replicates per treatment).
[abc]Mean values within a row with unlike superscripts are significantly different ($P < 0.05$).
[ABC]Mean values within a row with unlike superscripts are significantly different ($P < 0.10$).
[3]Methods of analysis denoted by G (Gaussian), [NB] (Negative Binomial) and [P] (Poison).

Supplementary Table 4.10. Continued

Phylum, Order, Family, genus	Diets[1]			SEM[2]	*P-value[3]*
	CON	FM	OH		
....Firmicutes (P)..					
Unclassified Veillonellaceae (F)	3.37a	1.17ab	1.08c	1.051	0.010[P]
Acidaminococcus	0.733b	3.14a	0.466b	1.190	0.001[P]
Anaerovibrio	1.98[b]	2.53[ab]	5.21[a]	1.069	0.005[NB]
Mitsuokella	0.2705	0.1454	0.2411	0.063	0.126[NB]
Megasphaera	13.40[a]	10.90ab	6.01b	1.956	0.003[NB]
Phascolarctobacterium	4.2594	4.7350	4.6488	0.649	0.741[G]
Veillonella	0.0142[b]	0.0765[a]	0.0034[b]	0.022	0.008[G]
Unclassified Mogibacteriaceae (F)	3.82[a]	3.58[ab]	1.29[c]	2.137	0.025[P]
Unclassified Erysipelotrichaceae (F)	0.0761	0.0727	0.1020	0.033	0.634[G]
....Proteobacteria (P)..					
Unclassified Proteobacteria	0.0755	0.302	0.330	0.156	0.341[G]
Campylobacter	1.02[b]	4.35[ab]	5.28[a]	2.401	0.029[NB]
Unclassified Succinivibrionaceae (F)	0.0647	0.318	0.221	0.148	0.216[G]
Succinivibrio	0.070[b]	0.101[ab]	0.164[a]	0.033	0.033[G]
Sutterella	1.22[b]	1.75[ab]	5.36[a]	3.084	<0.001[P]
Unclassified Desulfovibrionaceae (F)	0.034[B]	0.035[B]	0.083[A]	0.053	0.577[G]
Desulfovibrio	0.131	0.133	0.122	0.050	0.972[G]
Unclassified Enterobacteraceae (F)	0.110	0.375	0.520	0.268	0.336[G]
Unclassified Helicobacteraceae (F)	0.016[B]	1.59[A]	0.415[B]	0.897	0.097[P]
Flexispira	0.069[B]	0.213[A]	0.112[B]	0.080	0.092[G]
Helicobacter	0.099	0.0639	0.4830	0.326	0.379[G]

[1]CON, Control diet; FM, Flaxseed meal-containing diet; OH, Oat hulls-containing diet;

[2]SEM, standard error of means;

[†]Least square mean values (n = 8 replicates per treatment).

[abc]Mean values within a row with unlike superscripts are significantly different ($P < 0.05$).

[ABC]Mean values within a row with unlike superscripts are significantly different ($P < 0.10$).

[3]Methods of analysis denoted by G (Gaussian), [NB] (Negative Binomial) and [P] (Poison).

Supplementary Table 4.11. Correlations coefficients between selected bacterial taxa[a] and short chain fatty acids (SCFA), bile acids (BA) and neutral sterols (NS) in ileal digesta

Taxa	Variable[1]	Rho	*P*-value
YRC22	Cholesterol	0.3599	0.036
Lactobacillus	CA-CDCA	0.4273	0.023
	IDCA	-0.6441	<0.001
Streptococcus	Fat digestibility	0.4170	0.017
Clostridiales (O)	Propionate	0.4656	0.046
	LCA	0.4752	0.042
	DCA	0.5105	0.035
	IDCA	0.3875	0.007
	UDCA	0.6003	0.004
Clostridiaceae (F)	LCA	0.3806	0.042
Erysipelotrichaceae (F)	Cholesterol	0.3431	0.046
	Triglycerides	0.6132	0.003
Mitsuokella	Triglycerides	0.5700	0.008
Coriobacteriaceae (F)	Propionate	0.5607	0.003
Veillonellaceae (F)	Acetate	0.5341	0.002
	LCA	-0.3456	0.041
	IDCA	-0.4135	0.011
Acidaminococcus	CA-CDCA	-0.4640	0.033
	Triglycerides	0.5066	0.028
Phascolarctobacterium	Propionate	0.4327	0.015

[a]Bacterial taxa with relative abundance ≥0.05% of the community were used for the correlation analysis. Only significant correlations are presented in the Table. [1]CA = Cholic acid; CDCA = Chenodeoxycholic acid; DCA = Deoxycholic acid; IDCA = Isodeoxycholic acid; LCA = Lithocholic acid; UDCA = Ursodeoxycholic acid..

Supplementary Table 4.12. Correlations coefficients between selected bacterial taxa[a] and short chain fatty acids (SCFA), and bile acids (BA) in ileal mucosa.

Taxa	Variable[1]	r	*P*-value
Lactobacillus	Propionate	0.7307	<0.001
	LCA	0.5252	0.043
	DCA	0.6176	0.008
	IDCA	0.6402	0.005
Streptococcus	Acetate	0.7306	<0.001
	DCA	0.4729	0.004
Veillonella	Propionate	0.8992	<0.001
	Butyrate	0.4581	0.044
	Valerate	0.5252	0.027

[a]Bacterial taxa with relative abundance ≥0.05% of the community were used for the correlation analysis. Only significant correlations are presented in the Table. [1]DCA = Deoxycholic acid; IDCA = Isodeoxycholic acid; LCA = Lithocholic acid.

Supplementary Table 4.13. Correlations coefficient between selected bacterial taxa and short chain fatty acids (SCFA), bile acids (BA) and neutral sterols (NS) in cecal digesta.

Taxa	Variable[1]	r	*P*-value
Bacteroidales (O)	Butyrate	-0.3998	0.025
	Valerate	-0.4167	0.015
Bacteroides	UDCA	0.4356	0.022
Prevotella	Propionate	-0.3714	0.042
	Butyrate	-0.3908	0.036
	Valerate	-0.4616	0.024
	CA-CDCA	0.5745	0.011
	UDCA	0.5745	0.002
Lactobacillus	Coprostanol	0.5378	0.007
	DCA	0.6970	0.041
	UDCA	0.5666	0.039
Streptococcus	Acetate	-0.4622	0.045
	Valerate	-0.3419	0.022
	Fat digestibility	0.6188	0.004
	DCA	-0.5795	0.008
	UDCA	-0.5340	0.037
	Triglycerides	-0.5667	0.009
Lachnospiraceae (F)	CA-CDCA	-0.4578	0.041
	Coprostanol	-0.5132	0.022
Clostridium	Acetate	-0.4714	0.035
	Butyrate	-0.3530	0.043
	DCA	-0.5007	0.038
	UDCA	-0.4942	0.026
	Triglycerides	-0.4219	0.019
Coprococcus	Propionate	-0.5650	0.007
	Butyrate	-0.5007	0.013
	Valerate	-0.4789	0.043
	CA-CDCA	0.5822	0.004
	IDCA	0.6012	<0.001

Supplementary Table 4.13. Continued

Taxa	Variable	r	*P*-value
Dorea	Butyrate	-0.4504	0.034
	Valerate	-0.4861	0.046
	Fat digestibility	0.3802	0.025
	CA-CDCA	0.4904	0.016
	Coprostanol	0.4037	0.024
	IDCA	0.4904	0.044
	Triglycerides	-0.3708	0.039
Roseburia	Acetate	-0.5218	0.004
	CA-CDCA	-0.4552	0.028
	Coprostanol	-0.5245	0.032
	DCA	-0.5645	0.003
	UDCA	-0.5833	0.008
	IDCA	-0.4552	0.043
	Triglycerides	-0.4846	0.019
Coriobacteriaceae (F)	Acetate	0.6095	0.004
	Butyrate	0.3965	0.048
	Valerate	-0.4255	0.029
	DCA	0.4422	0.043
	IDCA	0.5541	0.002
	Triglycerides	0.7751	<0.001
	Coprostanol	0.4950	0.035
Acidaminococcus	Butyrate	0.5068	0.047
	Valerate	0.6991	<0.001
	Fat digestibility	-0.6062	0.004
Phascolarctobacterium	Coprostanol	0.3608	0.047
	DCA	0.4086	0.042
	UDCA	0.3899	0.015
Eubacterium	Coprostanol	0.4207	0.049

[a]Bacterial taxa with relative abundance ≥0.05% of the community were used for the correlation analysis. Only significant correlations are presented in the Table. CA = Cholic acid; CDCA = Chenodeoxycholic acid; DCA = Deoxycholic acid; IDCA = Isodeoxycholic acid; LCA = Lithocholic acid; UDCA = Ursodeoxycholic acid.

Supplementary Table 4.14. Correlations coefficient between selected taxa[a] and volatile fatty

acids, bile acids and neutral sterols (NS) in cecal tissue

Taxa	Variable	r	*P*-value
Bacteroides	DCA	0.3666	0.069
	UDCA	0.4050	0.042
Provotella	Cholesterol	-0.6174	0.008
	Triglycerides	-0.5827	0.005
	Acetate	0.4456	0.018
	CA-CDCA	-0.3948	0.037
	Coprostanol	-0.6315	0.006
Parabacteroides	Acetate	0.3706	0.045
	Butyrate	0.4061	0.011
	Coprostanol	0.4124	0.044
	DCA	0.4562	0.016
	UDCA	0.5190	0.007
YRC22	Propionate	0.3656	0.065
	LCA	0.3431	0.061
Clostridiaceae (F)	CA-CDCA	-0.4104	0.038
	Coprostanol	-0.6296	0.004
	DCA	-0.3893	0.057
Lachnospira	Butyrate	0.5034	0.006
	Fat digestibility	0.5512	0.003
	Coprostanol	-0.5478	0.002
	LCA	-0.3703	0.068
	DCA	-0.5556	0.005
	UDCA	-0.5801	0.011
Coprococcus	Propionate	-0.3701	0.055
	Butyrate	-0.3557	0.079
	Fat digestibility	0.6607	<0.001

Supplementary Table 4.14. Continued

Taxa	Variable	r	P-value
Dorea	Triglycerides	0.3889	0.044
Megasphaera	LCA	0.4200	0.015
Roseburia	Valerate	-0.4192	0.046
Veillonella	Fat digestibility	-0.3491	0.056
	CA-CDCA	-0.4002	0.088
	Coprostanol	-0.3649	0.072
	LCA	0.5264	0.005
	DCA	0.4311	0.014
	UDCA	0.5726	0.002
Mitsuokella	Acetate	-0.3522	0.091
	UDCA	-0.3762	0.055
Succinivibrionaceae	Acetate	0.3968	0.041
	Coprostanol	0.3541	0.073
	LCA	0.4244	0.014
	DCA	0.5464	0.005
	UDCA	0.4657	0.029
Veillonellaceae	Acetate	-0.5216	0.008
	DCA	-0.4513	0.047
	UDCA	-0.3881	0.062
Acidaminococcus	Butyrate	0.4308	0.037
	Valerate	0.6503	<0.001
	Cholesterol	0.3833	0.053
	Fat digestibility	-0.6984	<0.001
Anaerovibrio	Acetate	0.4888	0.046
	CA-CDCA	0.4791	0.023
	Coprostanol	0.6271	0.004
Shutella	Acetate	0.4422	0.039
	CA-CDCA	0.4553	0.016
	LCA	0.3933	0.031
	DCA	0.3862	0.025
Campylobacter	Acetate	0.5093	0.030
	CA-CDCA	0.4209	0.044
	Coprostanol	0.6016	0.007
Succinivibrio	Acetate	0.4423	0.014
	Coprostanol	0.4318	0.034
Flexispira	Valerate	0.4448	0.010
	Fat digestibility	-0.4661	0.057
Helicobacteraceae	Valerate	0.5951	0.008
	Cholesterol	0.3774	0.063
	Fat digestibility	-0.7192	<0.001

[a]Bacterial taxa with relative abundance ≥0.05% of the community were used for the correlation analysis. Only significant correlations are presented in the Table. CA = Cholic acid; CDCA =

Chenodeoxycholic acid; DCA = Deoxycholic acid; IDCA = Isodeoxycholic acid; LCA = Lithocholic acid; UDCA = Ursodeoxycholic acid.