DIETARY ENERGY UTILIZATION IN SWINE

By

Jong Woong Kim

A Thesis submitted to the Faculty of Graduate Studies of

The University of Manitoba

In partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

Department of Animal Science

University of Manitoba

Winnipeg, Manitoba

Canada. R3T 2N2

Copyright © 2019 by Jong Woong Kim

ABSTRACT

Of the available dietary energy systems, the net energy (NE) system provides a more accurate estimate of dietary energy compared to digestible (DE) and metabolizable energy (ME) systems although NE values of ingredients and diets are affected by several factors. A series of experiments was conducted to investigate the effects of these factors on NE values of feed ingredients and diets fed to growing pigs. Net energy contents of selected coproducts fed to growing pigs were also determined. The first experiment was conducted to determine the energy contents of hemp hulls (HH) and processed HH products and to compare the determined NE by the difference method using indirect calorimetry (IC) and predicted NE using prediction equations. The NE of HH, extruded HH, and a HH-pea blended product determined using IC were 2,204, 2,199, 2,190 kcal/kg (as-fed basis), respectively, and these values were 2.9, 7.1, and 5.2% higher, respectively, compared with those estimated using prediction equations. However, no significant differences were observed between determined and predicted values. In the second experiment, energy contents of camelina cake (CC) and the effects of dietary glucosinolates originating from CC on the additivity of energy values in mixed diets containing multiple ingredients were determined. The DE, ME, and NE contents of CC fed to growing pigs were 3,755, 3,465, and 2,383 kcal/kg (as-fed basis), respectively. In addition, additivity of DE, ME, and NE was observed in the mixed diets containing different inclusion levels of corn, soybean meal (SBM), and CC, which indicates that dietary glucosinolates originating from up to 30% of CC inclusion did not affect DE, ME, and NE of diets. The third experiment was conducted to determine the energy contents of canola meal (CM) and to investigate the effects of nutrient composition of basal diet and experimental methodology (difference method vs. regression method) on energy values of CM. Determined NE of CM fed to growing pigs was 2,099 kcal/kg,

as-fed basis. The difference and regression methods did not give different NE values of CM. However, when the goodness of fit for the linear model was compared, the r^2 of the regression analysis from the corn-SBM basal diet [adequate amino acids (AA)] was relatively greater than that from the corn basal diet (inadequate AA). The fourth experiment investigated the effect of increasing dietary NE concentrations adjusted by either dietary fat or fiber content on growth performance and energy intake of weaned pigs and the effect of physiological status (i.e., age) on NE value of diets. During a 3-week trial, no changes in the ADG, ADFI, and G:F of weaned pigs fed diets containing increasing dietary NE concentrations were observed, regardless of diet compositions. Also, daily DE and NE intake were not maintained but linearly increased as dietary energy concentration increased. Moreover, the NE contents of experimental diets increased as age of weaned pigs increased. The fifth experiment was conducted to determine the energy contents of high protein sunflower meal (HP-SFM) and to compare energy values of HP-SFM determined by using a phosphorus (P)-deficient diet or a P-adequate basal diet. The DE, ME, and NE of HP-SFM determined using the P-deficient basal diet were 3,328, 3,057, and 2,062 kcal/kg, as-fed basis, respectively, whereas, values determined using the P-adequate basal diet were 3,366, 3,037, and 2,151 kcal/kg, respectively. Although no differences were observed between the energy values determined using the two basal diets, measurements observed in the current study (e.g., urinary N excretion, RE as protein and lipid) may suggest that a diet containing adequate amount of P is a more suitable basal diet when the difference method is used for the calculation of NE value in a feed ingredient. Taken together, results from the current study indicate that NE value of a feed ingredient determined by the difference method using IC would be similar to the value predicted using prediction equations. Moreover, the difference and regression methods did not give different NE values for the ingredient, therefore both methods

can be used for determining NE contents of feed ingredients. However, essential nutrient contents (i.e., AA and P) in a basal diet may have significant effect on energy value of an ingredient, therefore nutrient contents in a basal diet should be carefully considered to avoid bias in the calculation of NE value. Antinutritional factor present in CC (i.e., glucosinolates) did not affect the energy value of diet and additivity of dietary energy. However, dietary NE value was affected by age of weaned pigs and increased with increasing age of pigs.

DEDICATION

This thesis is dedicated to my dear wife (Juhee) and our lovely daughter (Seyeon), and to my parents Chunil Kim and Sunsoon Kim

ACKNOWLEDGEMENTS

Several institutions and individuals have contributed to the success of my entire doctoral program. First and foremost, I would like to express sincere gratitude and appreciation to my advisor and mentor, Dr. Martin Nyachoti for believing in me and for supporting me with his thoughtful guidance throughout my doctoral program. His constructive criticism and passion on swine nutrition fields inspired me to expand the scope of my expertise in the area of swine nutrition. He has shown what the true educator is, I always want to be and will be one day. I would also like to thank other members of my advisory committee, Dr. Bogdan Slominski, Dr. Chengbo Yang, and Dr. Qiang Zhang for their contributions to this project. Their valuable comments and suggestions on this project are highly appreciated and incredibly improved the quality of thesis. Moreover, I truly thank my MSc. Supervisor, Dr. Dongyong Kil (Department of Animal Science and Biotechnology at Chung-Ang University) for his invaluable guidance and training in many ways.

I wish to appreciate the Swine Innovation Porc through the Canadian Swine Research and Development Cluster for funding this project. I would also like to acknowledge the financial support I received from the University of Manitoba which made it possible for me to complete this project.

I would like to extend sincere and heartfelt appreciation to graduate students, technical and research staffs at the Department of Animal Science for their willingness to help me whenever I needed help from them. My studies would not have been completed without their contribution. Special mention to Robert Stuski and Gemmar Maramot for animal care, Atanas Karamanov for technical assistance during my studies. Special thanks to the present and past colleagues in Dr. Nyachoti's lab: Dr. Manik Hossain, Balachandar Jayaraman, Deepak

Ettungalpadi Velayudhan, Gustavo Mejicanos, Bonjin Koo, Jinyoung Lee, and Jichen Song for taking their valuable time to assist me with the animal trials and even with lab analyses.

Finally, I am deeply indebted to my wife, Juhee Back and our lovely daughter, Seyeon Kim for their unconditional love and all sacrifices they have had to make over the years to support me. Many thanks to parents and relatives in Korea for their support and care which enabled me to survive when I had a hard time. I wish to convey my appreciation to my friends in Korea and Winnipeg for their sincere concern, contributions, and encouragement throughout my study.

FOREWORD

The thesis was written in a manuscript format according to the Journal of Animal Science format and it comprises five manuscripts corresponding to five chapters. Manuscripts I, II, and III has been published as follows:

Manuscript I: Kim J. W., and C. M. Nyachoti. 2017. Net energy of hemp hulls and processed hemp hull products fed to growing pigs and the comparison of net energy determined via indirect calorimetry and calculated from prediction equations J. Anim. Sci. 95:2649-2657.

Manuscript II: Kim, J. W., B. Koo, and C. M. Nyachoti. 2017. Digestible, metabolizable, and net energy of camelina cake fed to growing pigs and additivity of energy in mixed diets. J. Anim. Sci. 95:4037-4044.

Manuscript III: Kim, J. W., B. Koo, and C. M. Nyachoti. 2018. Net energy content of canola meal fed to growing pigs and effect of experimental methodology on energy values. J. Anim. Sci. 96:1441-1452.

Manuscript IV has been submitted to the Animal Feed Science and Technology. Manuscript V is under preparation.

TABLE OF CONTENTS

ABSTRACT ii

DEDICATION v

ACKNOWLEDGEMENTS vi

FOREWORD viii

TABLE OF CONTENTS ix

LIST OF TABLES xv

LIST OF FIGURES xviii

LIST OF ABBREVIATIONS xix

CHAPTER 1 1

GENERAL INTRODUCTION 1

CHAPTER 2 5

LITERATURE REVIEW 5

2.1 INTRODUCTION 5

2.2 ENERGY UTILIZATION 6

2.2.1 Energy Utilization for Maintenance 8

2.2.2 Estimation of Energy Requirement for Maintenance 10

2.2.3 Energy Utilization for Growth 13

2.3 ENERGY METABOLISM 14

2.4 ENERGY VALUES OF FEED INGREDIENTS AND DIETS FOR SWINE 15

2.4.1 Gross Energy 16

2.4.2 Digestible Energy 17

2.4.3 Metabolizable Energy 18

2.4.4 Net Energy 20

2.5 METHODS FOR DETERMINATION OF NET ENERGY VALUES 21

2.5.1 Comparative Slaughter Method 21

2.5.2 Indirect Calorimetry Method 22

2.5.3 Comparison of Net Energy Value Determined with the Comparative Slaughter Method and Indirect Calorimetry Method 25

2.6 FACTORS AFFECTING NET ENERGY VALUES 29

2.6.1 Experimental Methodology for Determination of Fasting Heat Production 29

2.6.2 Physical Activity 31

2.6.3 Temperature 31

2.6.4 Physiological Status 32

2.6.5 Genotype and Gender 32

2.7 ENERGY EVALUATION SYSTEM 34

2.7.1 Digestible and Metabolizable Energy Systems 35

2.7.2 Net Energy System 36

2.7.3 The French System 37

2.7.4 The Dutch System 38

2.7.5 The Danish System 39

2.7.6 Comparison of Energy Systems 40

2.8 CONCLUSION 42

CHAPTER 3 44

HYPOTHESES AND OBJECTIVES 44

CHAPTER 4 46

MANUSCRIPT I: Net energy of hemp hulls and processed hemp hull products fed to growing pigs and the comparison of net energy determined via indirect calorimetry and calculated from prediction equations 46

4.1 ABSTRACT 47

4.2 INTRODUCTION 48

4.3 MATERIALS AND METHODS 49

4.3.1 Experimental Animals and Diets 49

4.3.2 Experimental Design and Procedure 52

4.3.3 Sample Preparation and Chemical Analyses 53

4.3.4 Calculations 54

4.3.5 Statistical Analysis 56

4.4 RESULTS 56

4.4.1 Chemical Composition of Hemp Hulls and Processed Hemp Hull Products 56

4.4.2 ATTD of DM, GE, and CP and N balance 57

4.4.3 Net Energy of Hemp Hulls and Processed Hemp Hull Products 57

4.5 DISCUSSION 64

CHAPTER 5 69

MANUSCRIPT II: Digestible, metabolizable, and net energy of camelina cake fed to growing pigs and additivity of energy in mixed diets 69

5.1 ABSTRACT 70

5.2 INTRODUCTION 71

5.3 MATERIALS AND METHODS 72

5.3.1 Experimental Animals and Diets 72

5.3.2 Experimental Design and Procedure 75

5.3.3 Sample Preparation and Chemical Analyses 75

5.3.4 Calculations 77

5.3.5 Statistical Analysis 78

5.4 RESULTS AND DISCUSSION 78

5.4.1 Chemical Composition 78

5.4.2 Digestible Energy, ME, and NE of Camelina Cake and Additivity of Energy in Mixed Diets 81

CHAPTER 6 89

MANUSCRIPT III: Net energy content of canola meal fed to growing pigs and effect of experimental methodology on energy values 89

6.1 ABSTRACT 90

6.2 INTRODUCTION 91

6.3 MATERIALS AND METHODS 92

6.3.1 Animals, Housing, and Experimental Diets 92

6.3.2 Experimental Design and Procedure 96

6.3.3 Sample Preparation and Chemical Analyses 97

6.3.4 Calculations 99

6.3.5 Statistical Analysis 100

6.4 RESULTS 101

6.4.1 Energy and Nutrient Digestibility and Nitrogen Balance 101

6.4.2 Energy Balance and Energy Values of Experimental Diets 102

6.4.3 Energy Values of Corn and Canola Meal 103

6.5 DISCUSSION 111

6.5.1 Chemical Composition of Ingredients and Diets 111

6.5.2 Energy and Nutrient Digestibility and Nitrogen Balance 112

6.5.3 Energy Balance of Pigs and Energy Values of Experimental Diets 113

6.5.4 Energy Values of Corn and Canola Meal 114

CHAPTER 7 118

MANUSCRIPT IV: Pigs weighing less than 20 kg are unable to adjust feed intake in response to dietary net energy concentration regardless of diet composition 118

7.1 ABSTRACT 119

7.2 INTRODUCTION 120

7.3 MATERIALS AND METHODS 121

7.3.1 Animals, Housing, and Experimental Design 122

7.3.2 Experimental Diets and Procedure 122

7.3.3 Sample Preparation and Chemical Analyses 125

7.3.4 Calculations 126

7.3.5 Statistical Analysis 126

7.4 RESULTS 127

7.4.1 Chemical Composition of Ingredients and Experimental Diets 127

7.4.2 Growth Performance 127

7.4.3 Nutrient and Energy Digestibilities 128

7.4.4 Digestible and Net Energy Concentrations of Diets and Intake by Weaned Pigs 128

7.5 DISCUSSION 136

CHAPTER 8 140

MANUSCRIPT V: Net energy content of high-protein sunflower meal fed to growing pigs and effect of dietary phosphorus content on energy values 140

8.1 ABSTRACT 141

8.2 INTRODUCTION 142

8.3 MATERIALS AND METHODS 144

8.3.1 Animals, Housing, and Experimental Diets 144

8.3.2 Experimental Design and Procedure 149

8.3.3 Sample Preparation and Chemical Analyses 150

8.3.4 Calculations 152

8.3.5 Statistical Analysis 153

8.4 RESULTS 153

8.4.1 Apparent Total Tract Digestibility of Dry Matter, Gross Energy, and Crude Protein and Nitrogen Utilization of Diets 153

8.4.2 Energy Utilization and Content of Experimental Diets 154

8.4.3 Energy Values of High Protein Sunflower Meal 155

8.5 DISCUSSION 159

8.5.1 Chemical Composition of Ingredients and Diets 159

8.5.2 Apparent Total Tract Digestibility of Dry Matter, Gross Energy, and Crude Protein and Nitrogen Utilization of Diets 160

8.5.3 Energy Utilization and Content of Experimental Diets 161

8.5.4 Energy Values of High Protein Sunflower Meal Determined Using a Phosphorus Deficient Basal diet and a Phosphorus Adequate Basal Diet 162

CHAPTER 9 165

GENERAL DISCUSSION 165

CHAPTER 10 172

CONCLUSIONS AND FUTURE STUDIES 172

10.1. Conclusions 172

10.2. Future Studies 174

CHAPTER 11 175

LITERATURES CITED 175

LIST OF TABLES

Table 2.1. Net energy contents of feed ingredients determined by either the comparative slaughter (CS) or indirect calorimetry (IC) method 27

Table 2.2. Estimates of fasting heat production (FHP) measured in modern lean-type pigs using the indirect calorimetry method..... 30

Table 2.3. Relative DE, ME, and NE values of ingredients for growing pigs 41

Table 4.1. Composition and nutrient content of experimental diets (as-fed basis) 51

Table 4.2. Analyzed nutrient composition in ingredients (as-fed basis) 59

Table 4.3. Apparent total tract digestibility (ATTD) of DM, GE and CP of experimental diets fed to growing pigs..... 60

Table 4.4. Nitrogen (N) balance of pigs fed experimental diets..... 61

Table 4.5. Energy balance in growing pigs and energy values of experimental diets, hemp hulls, and processed hemp hull products determined by the indirect calorimetry method..... 62

Table 4.6. Comparison of determined and predicted NE of hemp hulls, and processed hemp hull products fed to growing pigs..... 63

Table 5.1. Composition and nutrient content of experimental diets, as-fed basis..... 74

Table 5.2. Analyzed nutrient composition of ingredients, as-fed basis..... 80

Table 5.3. Apparent total tract digestibility (ATTD) of DM and GE, energy retention, and DE, ME, and NE of experimental diets (as-fed basis) 82

Table 5.4. Digestible, metabolizable, and net energy of corn, soybean meal, and camelina cake fed to growing pigs (as-fed basis)..... 85

Table 5.5. Determined and predicted DE, ME, and NE contents of mixed diets containing different inclusion level of corn, soybean meal, and camelina cake, as-fed basis..... 88

Table 6.1. Analyzed nutrient composition of ingredients (as-fed basis) 94

Table 6.2. Ingredient composition and calculated and analyzed nutrient composition of experimental diets (as-fed basis) 95

Table 6.3. Apparent total tract digestibility (ATTD) of DM, GE, and CP and N balance of experimental diets fed to growing pigs 104

Table 6.4. Energy balance in growing pigs and energy values of experimental diets 105

Table 6.5. Energy content of corn fed to growing pigs determined using a corn-basal diet 107

Table 6.6. Regression coefficient used for estimating DE, ME, and NE (kcal/kg) of canola meal (CM) from two different basal diets (as-fed basis) 108

Table 6.7. Energy content of canola meal determined by the difference and regression methods 109

Table 6.8. Energy content of canola meal determined by corn-basal and corn-soybean meal basal diets 110

Table 7.1. Ingredient composition of experimental diets (as-fed basis)..... 124

Table 7.2. Analyzed nutrient composition of corn, soybean meal, flaxseed meal, corn distillers' dried grains with solubles (DDGS), canola meal, and oat hulls (as-fed basis)..... 129

Table 7.3. Energy and nutrient composition of experimental diets (as-fed basis) 130

Table 7.4. Growth performance of weaned pigs fed experimental diets..... 132

Table 7.5. Apparent total tract digestibility (ATTD) of dry matter, gross energy, and nutrients in diets fed to weaned pigs 133

Table 7.6. Digestible energy (DE) and net energy (NE) of diets and DE and NE intake of weaned pigs fed experimental diets (as-fed basis) 135

Table 8.1. Analyzed nutrient composition of corn, soybean meal, and high protein sunflower meal (HP-SFM), as-fed basis 146

Table 8.2. Ingredient and nutrient composition of experimental diets, as-fed basis 147

Table 8.3. Apparent total tract digestibility (ATTD) of dry matter (DM), gross energy (GE), and crude protein (CP) and nitrogen (N) balance of experimental diets fed to growing pigs 156

Table 8.4. Energy utilization of experimental diets fed to growing pigs and energy values of experimental diets 157

Table 8.5. Digestible (DE), metabolizable (ME), and net energy (NE) of high protein sunflower meal (HP-SFM) fed to growing pigs determined by the difference method using P-deficient basal and P-adequate basal diets 158

LIST OF FIGURES

Figure 2.1. Schematic representation of the effect of feeding level (FL) on energy retention and fasting heat production (FHP) in pigs.. 12

Figure 2.2. Relationship between protein and lipid deposition and energy intake (linear-plateau model). 14

Figure 2.3. Classical partitioning of energy in feeds. 16

LIST OF ABBREVIATIONS

AA	Amino acids
ADF	Acid detergent fiber
ADFI	Average daily feed intake
ADG	Average daily gain
AOAC	Association of official analytical chemists
ATP	Adenosine triphosphate
ATTD	Apparent total tract digestibility
BW	Body weight
Ca	Calcium
CC	Camelina cake
CH_4	Methane
CM	Canola meal
CO_2	Carbon dioxide
CP	Crude protein
CS	Comparative slaughter
DE	Digestible energy
DM	Dry matter
DP	Degree of polymerization
EE	Ether extract
EHH	Extruded hemp hulls
FHP	Fasting heat production

FI	Feed intake
FU	Feed unit
G:F	Gain to feed
GE	Gross energy
GIT	Gastrointestinal tract
HH	Hemp hulls
HHP	Hemp hulls with pea
HI	Heat increment
HP	Heat production
HP-SFM	High protein sunflower meal
IC	Indirect calorimetry
ISS	Immune system stimulation
ME	Metabolizable energy
ME_m	Metabolizable energy requirement for maintenance
NDF	Neutral detergent fiber
NE	Net energy
NE:ME	Efficiency of metabolizable energy utilization
NE_m	Net energy requirement for maintenance
NE_p	Net energy for production
NSP	Non-starch polysaccharides
O_2	Oxygen
OM	Organic matter
P	Phosphorus

PPE	Potential physiological energy
RE	Retained energy
RE_L	Retained energy as lipid
RE_P	Retained energy as protein
RQ	Respiratory quotient
SBM	Soybean meal
SFM	Sunflower meal
SID	Standardized ileal digestible
STTD	Standardized total tract digestible
THP	Total heat production
TiO_2	Titanium dioxide

CHAPTER 1

GENERAL INTRODUCTION

The primary goal of the swine industry is to maximize profitability with the least production costs. The cost of feed accounts for the largest part of the total production costs in any phase of swine production (>60%), and within that cost ingredients supplying dietary energy contribute the greatest portion of the total feed cost (Noblet and van Milgen, 2013). Energy is not a nutrient, but is an essential dietary component for swine diets because they are not able to continue maintaining all physiological processes within body, and developing organs and body tissues without supply of energy. Indeed, negative effects on development, growth performance, meat quality, environment, and overall profitability have been observed when insufficient dietary energy is provided to the pigs (Chiba, 2000). Another importance of dietary energy provision is that the dietary energy content has a great impact on voluntary feed intake of pigs. Pigs tend to consume feed until their energy requirements are satisfied (Nyachoti et al., 2004). Therefore, dietary energy contents have been used to predict voluntary feed intake of pigs and subsequently to determine essential nutrient requirements of pigs.

Due to the recent increase in feed costs, swine nutritionists have been forced to use cost-effective feed ingredients for diet formulation to avoid the competition for feed ingredients among different livestock sectors (Woyengo et al., 2014). Feeding a diet formulated with cost-effective feed ingredients may be a good strategy to reduce total production costs and maximize profits if the nutritive and energy values of these ingredients are accurately measured. However, one of the biggest challenges of feeding these ingredients to pigs is their high dietary fiber contents. Energy contents of these ingredients tend to be overestimated with the digestible

energy (DE) and metabolizable energy (ME) systems, thereby supplying insufficient amount of available energy to pigs. With this situation, maximum overall profitability from pork production may not be guaranteed because additional feed and days to market weight are required due to the slow growth of pigs resulting from insufficient supply of dietary energy. Thus, it becomes crucial to use an accurate energy evaluation system to determine the amount of available energy in feed ingredients and to supply adequate dietary energy rather than excessive or insufficient.

Not all gross energy (GE) that is ingested by pigs will be retained in the body because there are inevitable energy losses via feces, urine, gas, and heat (Noblet and van Milgen, 2013). Based on these energy losses during the process of digestion, absorption, and metabolic utilization of energy, dietary energy contents for pigs can be expressed in several different ways; namely the GE, the DE, the ME, and the net energy (NE). Firstly, the GE is the most basic form in which energy can be expressed and represents the maximum quantity of energy in a given sample. It is usually determined by the complete combustion of organic compounds using a bomb calorimeter. However, GE values are not commonly used for diet formulation because it provides no information on the amount of available energy to pigs. The DE content is calculated by subtracting fecal energy losses from GE intake. Digestible energy values take into account the digestion process of pigs, therefore DE value is a better estimate than the GE value in terms of supplying dietary energy to pigs. Metabolizable energy is defined as the DE intake minus energy losses in urine and fermentative gases. Therefore, fermentative gases produced from the digestion process in gastro-intestinal tract and urinary metabolites excreted during metabolism are corrected for in the calculation (Noblet, 2007). However, the measurement of gaseous energy losses are often ignored in the calculation of ME values because the energy losses as fermentative gases (mostly methane) are relatively small in growing pigs and difficult to measure

on a routine basis (Le Goff and Noblet, 2001). Finally, NE is defined as ME minus heat increment associated with energy losses during feed utilization (i.e., the energy cost of feed ingestion, nutrient digestion and absorption, and metabolic utilization of ME). Therefore, NE value is superior to DE and ME values because it takes into consideration all forms of unavailable energy to pigs in the calculation. In addition, NE is the only system in which energy requirements of pigs and the supply of dietary energy values are expressed on a same basis, which should theoretically be independent of the feed (Noblet et al., 2004). Although the NE value has been recognized as a better estimate to express available energy in diets and feed ingredients for pigs than DE and ME values, there is a concern that more accurate descriptions of available energy in diets increases the dependency on individual pigs (Boisen and Verstegen, 2000). Including the factors that affect DE and ME values (e.g., diet composition, processing technique, quality of ingredients, and physiological status of pigs) other factors such as experimental methodology, physical activity, temperature, physiological status, genotype, gender, and health status also have a significant effect on the NE value of diets and feed ingredients (Kil et al., 2013; Velayudhan et al., 2015a). These factors still need to be further investigated for accelerating a wider adoption of the NE system around the world.

The NE contents of diets and feed ingredients fed to pigs can be directly determined by the comparative slaughter and indirectly calorimetry (IC) methods (Noblet and van Milgen, 2013). If experimental circumstance does not allow for animal trials, the NE value of feed ingredients can be easily obtained from feeding tables (Sauvant et al., 2004; NRC, 2012) or estimated using prediction equations based on chemical composition of ingredients (NRC, 2012). However, direct determination of NE contents in some feed ingredients, especially coproducts which have recently become available, are still required because prediction equations were

generated without using these ingredients and there is also a possibility that antinutritional factors present in coproducts may result in the differences between determined and predicted values.

Taken together, more information on the NE content of specific feed ingredients for pigs should be determined and proper research methods for evaluating the potential factors that may affect NE value of ingredients and diets are required. Therefore, the main objectives of this research were to determine the NE content of selected coproducts and to test the hypothesis that several factors including nutrient composition of a basal diet, antinutritional factor present in a feed ingredient, experimental methodology, and physiological status (i.e., age) of pigs affect the NE values of diets and ingredients fed to growing pigs.

CHAPTER 2

LITERATURE REVIEW

2.1 INTRODUCTION

The supply of nutritionally adequate feeds in the right amounts to pigs is essential for optimizing pig production. To achieve this, accurate estimation and a good understanding of both energy and nutrient requirements of pigs and the availability of energy and nutrients in each feed ingredient are required. Although energy is not a nutrient per se, dietary energy provision is necessary for pigs to maintain all physiological functions within the body, and to develop organs and body tissues. In addition, feed is the single most expensive cost factor for swine production, which generally account for more than 60% of total cost of production, and within that cost ingredients supplying energy in swine diets contribute the largest portion (Kil et al., 2013; Noblet and van Milgen, 2013; Velayudhan et al., 2015a). Dietary energy content is also important because pigs tend to consume feed until their energy requirements are fulfilled. In other words, the dietary energy content determines voluntary feed intake (FI) of pigs (Nyachoti et al., 2004). Because the nutrients requirement of pigs are predicted based on their voluntary FI, swine diets should contain proper ratio of available energy to essential nutrients such as amino acids (AA) and phosphorus (P) for pigs to express their genetic potential for lean tissue gain and to ensure maximum profits (Kil et al., 2013; Patience et al., 2015).

Due to these important economic and physiological implications, different systems to express the energy value of diets and methods to measure the energy requirements of pigs have been developed. Moreover, the competition for feed ingredients among different livestock industries due to an increase in feed costs, and the use of feed ingredients for bioethanol and

biodiesel production and human nutrition challenge swine nutritionists to use cost-effective feed ingredients and energy evaluation systems that maximize the utilization of energy in swine diets. However, this requires an accurate definition of energy values of diets and energy requirements of pigs. During the past decades, a great deal of new knowledge on energy utilization by pigs has been generated and efforts have been made to develop energy evaluation systems for diets and feed ingredients, and to accurately measure energy requirements of pigs. Therefore, this review is focused on (i) current knowledge on energy utilization and (ii) energy evaluation systems for pig diets.

2.2 ENERGY UTILIZATION

Laws of Thermodynamics:

Energy is defined as the potential capacity to perform work. There are several forms of energy: chemical, mechanical, kinetic, positional, electrical, and heat. The first law of thermodynamics states that energy can be neither created nor destroyed, but can be transformed from one form to another. The second law states that all forms of energy are convertible to heat, so the driving force in all systems is to release energy as heat”

As per the first and second laws of thermodynamics, all forms of energy are quantitatively convertible to heat (Baldwin and Bywater, 1984). This concept has been applied in the field of animal nutrition to determine and quantify energy supply and energy utilization in pigs (Baldwin, 1995). Energy is generated from the oxidation of organic compounds (i.e., carbohydrates, lipids, and proteins) in diets. Chemical energy captured in dietary organic compounds can be converted into biochemical energy called adenosine triphosphate (ATP), the fundamental “currency” in energy metabolism, by the cellular respiration mechanism which

involves a set of metabolic reactions taking place in the cells, and then utilized for various biological functions (Brafield and Llewellyn, 1982; Emmans, 1999; Burrin, 2001; van Milgen and Noblet, 2003). In order to convert the chemical energy in the dietary components into available forms of energy to pigs, digestion and absorption processes in the gastrointestinal tract (GIT) are necessary steps (Pond et al., 1995). During the digestion process, polymers of organic compounds (i.e., carbohydrates, lipids, and proteins) in the feed are broken down into monosaccharides (from carbohydrates), monoacylglyrerols (from lipids), free fatty acids (from lipids), amino acids (from proteins), and small peptides (from proteins) by enzymatic hydrolysis in the GIT (Noblet and van Milgen et al., 2013). These molecules can be absorbed by the intestinal epithelium, thereby providing potential of energy sources. Approximately 70 to 90% of dietary gross energy (GE) in typical swine diets is digested, and then utilized, depending upon the chemical composition of the diet (Noblet et al., 1994; Le Goff and Noblet, 2001; Noblet, 2007).

Most caloric needs of pigs are provided by carbohydrates, fats, and proteins present in the diet. Carbohydrates are naturally occurring organic compounds composed of carbon, hydrogen and oxygen in the ratio of $C_n:H_{2n}:O_n$. Carbohydrates are the most abundant dietary energy source in swine diets, and generally accounts for 60-70% of total energy intake (Knudsen Bach et al., 2013). Dietary carbohydrates can be classified, according to their degree of polymerization (DP), into sugars (DP 1-2), oligosaccharides (DP 3-9), and polysaccharides (DP ≥10); the latter consists of starches and non-starch polysaccharides (NSP; Cummings and Stephen, 2007; Englyst et al., 2007). Sugar and starch are hydrolyzed by the action of pancreatic and brush boarder enzymes to monosaccharides in the small intestine then absorbed in the body, whereas NSP and some types of oligosaccharides (i.e., a-galactosides, fructooligosaccharides)

are subject to fermentation by the microflora mainly in the large intestine (cecum and colon) due to the lack of enzymes that can hydrolyze these components in the small intestine (Bach Knudsen and Jorgensen, 2001). It is estimated that oxidation of simple sugars such as glucose generates approximately 4 kcal/g. Fat molecules, on the other hand, consist of carbon and hydrogen, with very little oxygen in the carboxyl group. It yields approximately 9 kcal/g, more than twice the energy generated from carbohydrates (Blaxter, 1989). Proteins are macromolecules that consist of one or more chains of amino acids and contain considerable amounts of nitrogen (**N**) in addition to carbon, hydrogen, and oxygen. They serves a variety of nutritional needs within the body such as maintenance functions and lean tissue gain, but also can be metabolized as source of energy when needed, yielding approximately 4 kcal/g.

However, not all energy digested and absorbed by pigs is ultimately utilized. The undigested or unabsorbed energy fraction (i.e., voided feces) corresponds to non-fermentable carbohydrates, undigested proteins and fats, and unabsorbed volatile fatty acids (Bastianelli et al., 1996) and microbial mass. Additionally, after absorption a significant amount of energy is inevitably lost as heat during metabolic utilization of energy (Kil et al., 2013; Velayudhan et al., 2015a).

2.2.1 Energy Utilization for Maintenance

Energy utilization for maintenance is associated with the continuous maintenance of vital physiological functions within the body such as thermoregulation, blood circulation, respiration, muscle tone, ion balance, immune responses, tissue turnover, and the maintenance of acid-base homeostasis as well as physical activity, and ingestion and digestion of feeds (Baldwin and Bywater, 1984; Milligan and Summers, 1986; Baldwin, 1995; Black and de Lange, 1995). In

growing pigs, energy required for maintenance is assumed to be the first priority and energy intake in excess of the energy requirement for maintenance is stored as protein or lipids in the body (Lizardo et al., 2002). It represents about 33% of total dietary energy utilization and the remaining portion is retained as proteins or lipids in growing pigs (Black and de Lange, 1995; NRC, 2012). However, the ratio of energy utilization for maintenance or for growth is also dependent on the physiological status of pigs [i.e., age or body weight (BW)], genotype, housing conditions, and chemical composition of the diet.

The energy requirement for maintenance in pigs has typically been estimated as the metabolizable energy (ME) requirement for maintenance (ME_m) or the net energy (NE) requirement for maintenance (NE_m). Generally, ME_m and NE_m are expressed proportionally on a metabolic basis (aBW^b; NRC, 2012). This expression has been widely adopted due to the fact that there is a proportional relationship between fasting heat production (FHP) and metabolic BW of animals (Kleiber, 1975). Theoretically, BW raised to the exponent and the ME_m or NE_m would be consistent if an appropriate exponent is chosen (Chwalibog, 1991). The exponent of 0.75 has been most widely used for most mammals due to a linear relationship between FHP and $BW^{0.75}$ in mature or fasting animals among various species (Kleiber, 1975). However, several studies have shown that this exponent function is less than 0.75 ranging from 0.54 to 0.75, and therefore would be inappropriate due to the inaccuracy when applied to FHP within a species or for rapidly growing pigs (Thonney et al., 1976; Tess, 1981; Baldwin and Bywater, 1984). Indeed, Chwalibog (1991) reported decreased ME_m with increasing BW of growing pigs when 0.75 was chosen as the exponent. This result might have been due to changes in body composition (i.e., deposition rate of protein and lipid) and the relative weight of visceral organs to total BW during growth stage of pigs (Tess et al., 1984; Noblet et al., 1991). Therefore, it has been suggested that

an appropriate exponent to predict the energy requirement for maintenance in growing pigs is closer to 0.60 rather than 0.75 (Brown, 1982; Noblet et al., 1994; van Milgen et al., 1998; Noblet et al., 1999). The exponent of 0.60 is currently adopted to express metabolic BW for growing pigs (Noblet et al., 1994; NRC, 2012). The exponent of 0.75, however, is still used for predicting the energy requirement for maintenance in gestating and lactating sows (NRC, 2012) because it has not been clearly demonstrated whether the different exponents other than 0.75 more accurately express energy requirement for maintenance in mature animals (Chwalibog, 1991; Noblet et al., 1999; Noblet and van Milgen, 2013).

2.2.2 Estimation of Energy Requirement for Maintenance

The energy requirement for maintenance has been determined using fasting pigs (Noblet and van Milgen, 2013). It can also be estimated by extrapolating HP measured at different feeding levels to zero ME intake (Figure 2.1; Extrapolated FHP; Kil et al., 2013). However, the latter method has been criticized due to the following important limitations although it had been widely used in the past (de Lange et al., 2006; Labussière et al., 2011). First, the extrapolated FHP is typically measured with FI levels ranging 60-100% of ad libitum FI, which leads to significant inaccuracies in the slope and intercept of regression analysis (slope, x-intercept, and y-intercept represent efficiency, $ME_{m,}$ and extrapolated FHP, respectively). Second and more important, as shown in Figure 2.2, previous feeding level affects the estimate of FHP, therefore FHP is markedly inconsistent with different feeding levels, especially in growing animals (Koong et al., 1982; de Lange et al., 2006; Labussière et al., 2011). This phenomenon is likely attributable to the ability of pigs to adapt their basal energy expenditure to the feed intake level and/or physiological status (i.e., growth intensity). The significantly less extrapolated FHP

compared to directly measured FHP has been observed along with subsequent lower dietary NE value and efficiency of ME utilization, and a higher HI (Koong et al., 1982; de Lange et al., 2006; Labussière et al., 2011). Taken together, these observations question the use of extrapolated FHP as an estimate for FHP to determine NE values. Direct measurement of FHP immediately after a fed period according to indirect calorimetry (IC) method has been recommended for the measurement of NE_m (Noblet et al., 1994; Noblet, 2007; Noblet and van Milgen, 2013). The ME_m has been estimated by assuming that the HP is equal to ME intake at maintenance. Therefore, based on the NE_m estimation, the ME_m is conventionally calculated by solving the linear regression equation (x-intercept; Figure 2.1). It can also be determined by subtracting total energy intake from the energy used for deposition of protein and lipid if the efficiencies of ME for protein or lipid deposition are known (Birkett and de Lange, 2001).

The estimates of ME_m for pigs varies from 92 to 160 kcal/$BW^{0.75}$ and the average estimate of 106 kcal/$BW^{0.75}$ had been used as ME_m (NRC, 1998). With the exponent of 0.60, regarded as more accurate exponent for metabolic BW in growing pigs, estimates for ME_m range from 186 to 250 kcal/$kg^{0.6}$ (Just et al., 1983; Noblet et al., 1991; Noblet et al., 1994; van Milgen et al., 1998; de Lange et al., 2006). Therefore, in NRC (2012), the estimate of 197 kcal/$BW^{0.60}$ has been recommended to use as ME_m for growing-finishing pigs. However, ME_m is still estimated as 100 and 110 kcal/$BW^{0.75}$ for gestating and lactating sows, respectively.

Estimation of NE_m is calculated as the sum of FHP and energy used for physical activity (van Milgen et al., 2008). However, NE_m is generally assumed to be equal to FHP (de Goey and Ewan, 1975; Noblet et al., 1994; Ewan, 2001; Noblet, 2007; Noblet and van Milgen, 2013). When 0.75 was used as the exponent for metabolic BW, estimates of FHP for growing pigs were reported to be between 71 and 81 kcal/$BW^{0.75}$ (Robles and Ewan, 1982; Just et al., 1983).

However, as discussed earlier, the exponent of 0.60 has also been recommended to estimate FHP in growing-finishing pigs (NRC, 2012). Reported values for FHP vary from 117 to 229 kcal/$BW^{0.60}$ (Schiemann et al., 1972; Just et al., 1983; Noblet et al., 1994; van Milgen et al., 1998; de Lange et al., 2006; Ayoade et al., 2012a; Heo et al., 2014; Velayudhan et al., 2015b; Kim and Nyachoti, 2017; Kim et al., 2018) and 179 kcal/$BW^{0.60}$ is the most widely used estimate for calculating NE values of diets and feed ingredients for growing-finishing pigs (NRC, 2012; Noblet, 2007; Noblet and van Milgen, 2013).

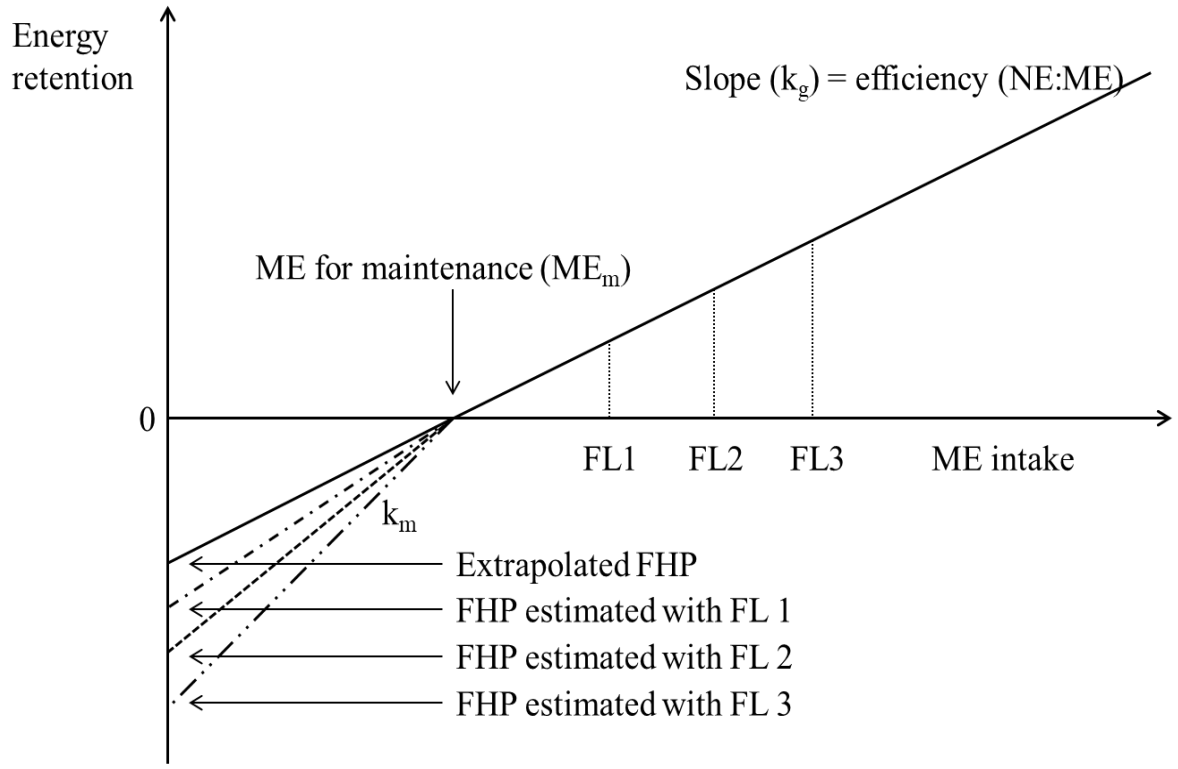


Figure 2.1. Schematic representation of the effect of feeding level (FL) on energy retention and fasting heat production (FHP) in pigs. Adapted from Koong et al., 1982; de Lange et al., 2006; and Labussière et al., 2011. Extrapolated FHP; estimated by extrapolating HP measured at different feeding levels (i.e., FL 1, FL 2, and FL 3) to zero ME intake. FHP estimated with FL 1, 2, and 3; determined FHP immediately after a fed period feeding at FL1, 2, and 3, respectively.

2.2.3 Energy Utilization for Growth

Additional energy intake in excess of the energy requirement for maintenance can be utilized for growth or deposition of body tissues (lipids and proteins) once the energy requirement for maintenance is fulfilled (Lizardo et al., 2002). It has been reported that the additional energy for pigs is first prioritized to deposit lean tissues (Close, 1996). However, the protein and lipid deposition simultaneously increases with a similar rate until the genetic potential for lean tissue gain is maximized or other factors start limiting protein deposition. (Figure 2.2; Close, 1996; van Milgen and Noblet, 2013). Among the efforts that have been made to represent the relationship between protein deposition and energy intake (e.g., ARC, 1981), the linear-plateau model has been most widely adopted. According to this model, protein deposition linearly increases up to the energy requirement for maximal protein gain then any additional energy intake after energy requirement for maximal protein deposition will lead to significant increase in lipid deposition with modest increase in protein (Figure 2.2; van Milgen and Noblet, 2013). Therefore, lipid deposition rate is much higher above the point where the protein deposition is at a maximum (Close, 1996; van Milgen and Noblet, 2013).

For a typical corn-soybean meal (SBM) based swine diet, it has been proposed that the efficiencies of ME utilization are 60% and 80% for proteins and lipids deposition, respectively (Noblet et al., 1999). The energy concentrations of body proteins and lipids are approximately 5.7 and 9.4 kcal/g, respectively. Therefore, the calculated ME requirements for protein and lipid are then 9.5 (5.7 / 0.6) and 12 (9.4 / 0.8) kcal ME/g, respectively. Energy utilized for growth can be calculated by the sum of retained energy (RE) as protein and RE as lipid. The RE as protein is typically calculated using N retention (N × 6.25 × 5.7 kcal/g) and RE as lipid is calculated as the difference between total RE and RE as protein (Kim et al., 2018).

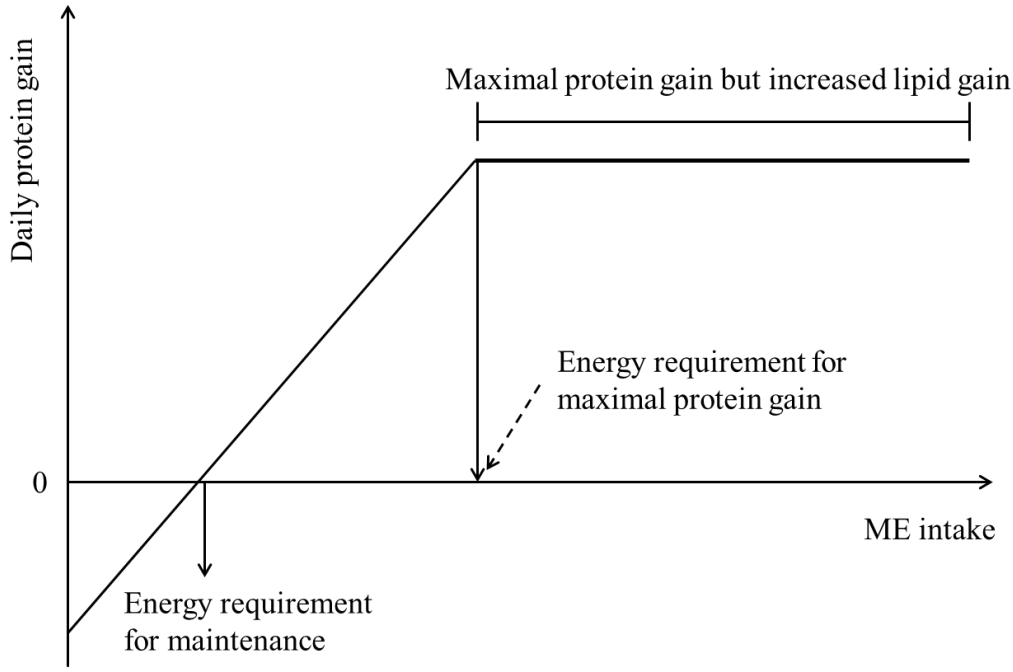


Figure 2.2. Relationship between protein and lipid deposition and energy intake (linear-plateau model). Adapted from Close (1996); van Milgen and Noblet, (2013).

2.3 ENERGY METABOLISM

The term metabolism is defined as all biochemical processes in a living organism by which nutritive component is built up into living matter, or by which complex molecules are broken down into small molecules that can be utilized (Mayes, 2000). Energy metabolism is divided into two categories; catabolism and anabolism. Catabolism releases energy by oxidation of organic compounds in exergonic reactions, and then released energy is utilized for other biochemical reactions or dissipated as heat, while anabolism synthesizes body components such as proteins and nucleic acids in endergonic reactions by capturing the energy released from the exergonic reaction (Mayes, 2000). These two reaction processes must be a coupled exergonic-endergonic system and subsequently, the overall net change in energy must be exergonic (Mayes,

2000). Oxidation of carbohydrates, lipids, and protein generates different amount of ATP. Oxidation of fatty acids in lipids is estimated to generate 112 to 146 moles of ATP depending upon the length of carbon chain, whereas glucose broken down from carbohydrate yields 38 moles of ATP by oxidation. Oxidation of AA generates 6 to 42 moles of ATP relying on the residual group of individual AA (Blaxter, 1989). However, not all energy generated by nutrient oxidation is captured in the form of ATP and the remaining portion is lost as heat. The energy in ATP is also partially utilized and is dissipated as heat when ATP is utilized for biological functions within the body (Blaxter, 1989; Kil et al., 2013).

2.4 ENERGY VALUES OF FEED INGREDIENTS AND DIETS FOR SWINE

Not all dietary energy consumed by pigs will be retained; there must be energy losses via feces, urine, gas, and heat during digestion, absorption, and metabolism of nutrients (Figure 2.3). Based on these losses, dietary energy values are expressed in different ways by subtracting sequential energy losses: digestible energy (DE) is the difference between GE intake and fecal energy losses; ME is the difference between DE intake and urinary and gaseous energy losses; and NE is the difference between ME intake and heat increment (HI).

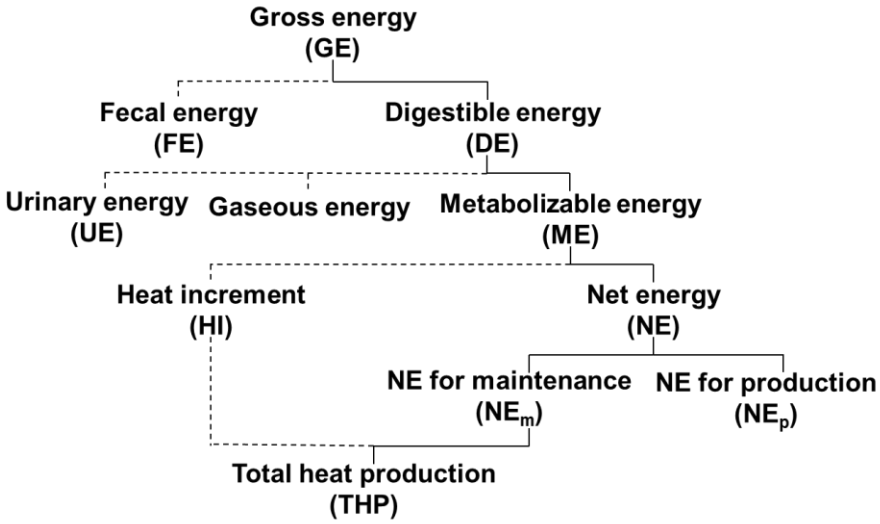


Figure 2.3. Classical partitioning of energy in feeds. Adapted from van Milgen and Noblet, (2013)

2.4.1 Gross Energy

Gross energy or heat of combustion is the most basic form in which energy can be expressed and represents the maximum quantity of energy in a given sample. It is usually determined by the complete combustion of organic compounds using a bomb calorimetry. The GE content of a feed or an ingredient is dependent on the extent of oxidation of organic compounds and the ratio of carbon and hydrogen to oxygen in the ingredient (McDonald et al., 2002). For example, average GE values of glucose, starch, protein, and fat are 3.7, 4.2, 5.6, and 9.4 kcal/g, respectively, whereas fermentative products have GE values of 3.5 kcal/g for acetate, 5.0 kcal/g for propionate, 5.9 kcal/g for butyrate, and 13.1 kcal/g for methane (McDonald et al., 2002). Therefore, dietary GE content can be predicted if the chemical composition of the diet or ingredient is analyzed. However, GE value is rarely used for a swine diet formulation because it provides no information on the amount of energy actually available to the pig.

2.4.2 Digestible Energy

The DE content of a diet is calculated by subtracting energy losses in feces from GE intake. Therefore, energy that is ingested by the animal and is not excreted in the feces is assumed to be digested and available to pigs (de Lange and Birkett, 2005). It is often referred to as apparent measurement because endogenous sources such as digestive enzymes, sloughed intestinal cell, and microbial mass are not corrected in the calculation (Just, 1982).

The DE content can be determined by either the total collection method or index method (Adeola, 2001) using following calculations.

Total collection method:

Digestible energy, kcal/kg = (total energy intake, kcal – total fecal energy excretion, kcal) / feed intake, kg

Index method:

Digestible energy, kcal/kg = {1 − [(index concentration in diet, g/kg / index concentration in feces, g/kg) × (energy concentration in feces, kcal/kg / energy concentration in diets, kcal/kg)]} × energy concentration in diets, kcal/kg

The ratio between DE and GE of most diets fed to pigs varies from 0.7 to 0.9, depending on chemical composition of the diet. According to previous studies conducted at the University of Manitoba, the average ratio of DE to GE in a typical corn-SBM diet fed to growing pigs was 0.88, whereas pigs fed the diet containing alternative feed ingredients which contains high level of dietary fiber had a much lower ratio (0.79) compared to that measured in pigs fed corn-SBM diets (Kim and Nyachoti, 2017; Kim et al., 2017, 2018). Many factors including level and source of dietary fiber, addition of lipids and minerals, FI level, and feed processing techniques have been reported to affect the utilization of GE (Just, 1982; Noblet, 1996; Black, 2000; Noblet and

van Milgen, 2013). Among these factors, dietary fiber is the primary reason for variations in DE values among diets (Noblet and van Milgen, 2013). The age or BW of animals also affects DE values of diets. Young pigs (or lower BW) may have less capacity for fiber fermentation compared to older pigs (or higher BW) because of their immature digestive system (Shi and Noblet, 1993; Noblet and van Milgen, 2004). In general, DE of diets and feed ingredients increase as BW of pigs increase (Noblet and van Milgen, 2013; Kil et al., 2013). When formulating swine diets, this factor should be carefully considered because DE values determined using older pigs may overestimate DE values for young pigs, especially in diets containing high level of dietary fiber (Shi and Noblet, 1993).

2.4.3 Metabolizable Energy

Metabolizable energy is equal to DE minus energy losses in urine and fermentative gases (NRC, 2012). However, the energy lost as fermentative gases (mostly methane) is relatively small in growing pigs (Le Goff and Noblet, 2001) and difficult to measure (Kil et al., 2013). Therefore, the energy losses in gases have often been ignored in the calculation of dietary ME value. Gaseous energy losses has been reported to range from 0.1 to 1.0 % of dietary DE in growing-finishing pigs (Noblet and Le Goff, 2001), whereas sows fed at maintenance level had higher proportion (Noblet and Shi, 1994). In general, energy losses in gases increase with BW (or age) of pigs and inclusion level of dietary fiber. Therefore, the estimated ME values are usually 0.1% to 3% higher than the actual ME depending on the level of dietary fiber and the BW (or age) of the animal. Recently, the following equation has been proposed to predict gaseous energy losses as CH_4 based on the fermentable fiber contents (Rijnen, 2003).

CH_4 loss (kcal/kg DM) = 2.94 × fermentable fiber (% DM) – 10.61,

where fermentable fiber equals to digestible organic matter (OM) – digestible crude protein (CP) – digestible ether extract (EE) – digestible starch – digestible sugar.

Urinary energy losses are dependent on concentration of N in urine (Le Bellego et al., 2001). Also, the urinary N excretion depends on the difference between digestible N and retained N or, in turn, the quantity of dietary CP and the capacity of the pig to retain lean tissue. As a result, the ME:DE ratio linearly decreases with increasing dietary CP contents and affected by physiological stage of the pig (Le Goff and Noblet, 2001). Therefore, excessive protein intake more than the amount required for lean tissue gain leads to greater urinary N excretion and contributes to underestimation of dietary ME value (Le Goff and Noblet, 2001). Practically, the use of a single ME value for a feed or a feed ingredient is suggested for formulating swine diets. With typical diets fed to growing pigs, on average, half of the absorbed N is utilized for maintenance functions and body protein synthesis, and the remaining half is excreted through the urine (Just, 1982; Sauvant et al., 2004). Therefore, the ME:DE ratio is relatively constant (approximately 0.96), however, this value is not applicable to individual feed ingredients which have totally different nutrient composition compared with typical diets (Noblet and van Milgen, 2004).

The procedure for measuring urinary energy losses may be tedious and laborious to be used on a routine basis. The following equations for predicting urinary energy is available to calculate ME value of feeds for growing pigs and adult sows, respectively (Le Goff and Noblet, 2001; Sauvant et al., 2004).

Urinary energy (kcal/kg DM) = $45.4 + 7.4 \times N_{urine}$

Urinary energy (kcal/kg DM) = $52.6 + 7.4 \times N_{urine}$

where N_{urine} is the N content in the urine, expressed as g of N per kg DM intake.

2.4.4 Net Energy

Net energy is defined as the ME minus HI associated with energy losses during feed utilization (i.e., the energy cost for feed ingestion, nutrient digestion and absorption, and metabolic utilization of ME). However, separation of HI from total heat production (THP; HI + NE_m) is difficult, and therefore, NE value is typically calculated as the sum of NE_m and NE for production (NE_p; retained energy) (Noblet and van Milgen, 2013). The NE value also can be expressed as a percentage of ME value, known as the efficiency of ME utilization for NE (NE:ME; Noblet et al., 1994). The NE:ME is, therefore, influenced by the amount of ME intake (e.g., maintenance purpose vs. production purpose) and physiological status of pigs (e.g., fat deposition vs. protein deposition vs. milk production). For example, the HI by pigs is lower when ME is supplied below the maintenance energy requirement than when it is supplied above the maintenance energy requirement (Noblet et al., 1994; Birkett and de Lange, 2001). The HI by pigs is also lower when ME is utilized to deposit fat tissues compared to lean tissues (Black, 2000). In addition, chemical composition of the diet fed to pigs also affect NE:ME because the energetic efficiencies of digestible nutrients are variable (Noblet et al., 1994; Noblet and van Milgen, 2013). The NE:ME of digestible components is 58, 58, 82, and 90% for protein, dietary fiber, starch, and lipids, respectively (Noblet et al., 1994). Variations in energetic efficiencies among digestible components are attributed to the fact that HI (per unit of energy) is higher when CP and dietary fiber are utilized for energy compared to starch or EE (Noblet et al., 1994). Therefore, it is evident that increases in dietary CP and fiber lead to an increase in HP by pigs. The NE:ME of typical swine diets is reported to be 74% (Noblet et al., 1994). Because differences in energetic efficiencies of digestible components are considered in the calculation of

NE, the NE values of diets and feed ingredients more closely represent the actually available energy to pigs than DE or ME values (Noblet et al., 1994; Moehn et al., 2005; Noblet, 2007). Also, NE value is the only value in which energy requirements of the animal and the supply of dietary energy are expressed on the same basis regardless of diet compositions (Noblet and Henry, 1993; Noblet and van Milgen, 2004), consequently provides a better prediction of pig performance (Velayudhan and Nyachoti, 2016).

2.5 METHODS FOR DETERMINATION OF NET ENERGY VALUES

Net energy content of a diet or a feed ingredient can be calculated by measuring either RE (directly measured when comparative slaughter method is used) or HP (directly measured when calorimetry method is used) by pigs (Noblet and van Milgen, 2013). Direct determination of RE can be achieved by the comparative slaughter (CS) method, whereas HP can be measured using either the direct or indirect calorimetry. Each method has advantages and disadvantages. The CS method requires simple techniques, but it is not only laborious but also time-consuming. Also it generates an estimate of the average RE over a longer period of time rather than specific point of time (Noblet and van Milgen, 2013). Measuring HP by using direct or indirect calorimetry is less laborious and time consuming compared to CS method, however it involves expensive and sophisticated experimental equipment.

2.5.1 Comparative Slaughter Method

Comparative slaughter method has been widely used as the gold standard for determining NE content of diets and feed ingredients (Kil et al., 2011; Ayoade et al., 2012a; Stewart et al., 2013). This method involves determination of initial body composition (or initial

energy concentration) of pigs at the beginning of an experiment and final body composition (or final energy concentration) of pigs at the termination of the experiment to determine energy retention of pigs during the experiment (Ayoade et al., 2012a; Noblet and van Milgen, 2013). Then, the RE is calculated by the difference between the total energy contents of the initial and final slaughter groups (Kil et al., 2011; Ayoade et al., 2012a; Stewart et al., 2013). The CS method can easily be used in small animals such as poultry, but has more difficulties in large animals due to the sampling procedures for carcass and organ, and relatively long experimental periods. Another weakness of this method is inherent methodological errors related to the measurements of body energy retention. In order to calculate the RE of pigs, initial body energy concentrations of pigs have to be predicted from a different group of pigs that are slaughtered at the beginning of the experiment (Quiniou et al., 1995). To minimize variations in body chemical compositions among experimental pigs, a large number of pigs in the initial slaughter group which have same genetic background as pigs fed the experimental diets, and relatively long experimental periods are required (Boisen and Verstegen, 2000).

2.5.2 Indirect Calorimetry Method

Metabolic reactions associated with maintenance, production, and other non-productive purposes such as physical activity, homeostasis of hormones and temperature, and immune response generate heat in animals. According to Hess's law, HP by animals due to metabolic reactions is independent of the biochemical pathway between the initial and final states (Blaxter, 1989). In other words, complete oxidation of energy-yielding components or partial oxidation of these components together with production of intermediate products such as volatile fatty acids

and ketone bodies, which are subsequently transformed and oxidized at a later stage, will not produce different amount of heat (Blaxter, 1989).

Heat production measurements can be determined using either direct or indirect calorimetry. Determination of HP using direct calorimetry requires more expensive experimental equipment because heat produced is determined using sealed-insulated chamber surrounded by known mass of water based on the definition of calorie (the amount of energy required to raise the temperature of 1g of water by 1℃); therefore, indirect calorimetry has been widely used for determination of HP (Christensen et al., 1988). Indirect calorimetry measures HP of animals based on their oxygen (O_2) consumption, carbon dioxide (CO_2) and methane (CH_4) production, and urinary N excretion because there is a close correlation between HP and O_2 consumption, CO_2 and CH_4 production, and urinary N excretion (Brouwer, 1965), by which the prediction equation for HP is generated. The equation published by Brouwer in 1958 and later adapted by the "Sub-committee of Constants and Factors", published in 1965 has been most widely used for estimation of HP. The approach is based on O_2 consumption, CO_2 production and heat released as a result of combustion of 1 g of carbohydrate, fat, and protein.

$$HP = 16.18 \times O_2 + 5.023 \times CO_2 – 2.17 \times CH_4 – 5.989 \times Urine_N$$

where HP is in kJ; O_2 is O_2 consumption in liters, CO_2 is CO_2 production in liters, and CH_4 is CH_4 production in liters; $Urine_N$ is urinary N production in g. The urinary N production corrects for the protein catabolism, whereas incomplete oxidation of carbohydrates and protein in the GIT are corrected for by measuring CH_4 production.

The respiratory quotient (RQ) is calculated using following equation;

RQ = liters of CO_2 production / liters of O_2 consumption

The RQ value, however, is generally calculated without correction for CH_4 and $Urine_N$ because the production of CH_4 and its contribution to energy lost is negligible (less than 1%) in growing pigs. The RQ value of a diet fed to pigs is dependent on the dietary composition. The RQ of carbohydrate is 1.00 because the number of CO_2 molecules produced is equal to the number of O_2 molecules consumed (Elia and Livesey, 1992). The average RQ values for proteins and lipids are 0.82 and 0.70, respectively. Generally, the RQ value of mixed diets fed to pigs is slightly greater than 1.0 during the fed-state and around 0.75 during the fasting-state (Agyekum et al., 2016). This value linearly increases with increasing carbohydrates metabolism and linearly decreases as metabolisms of lipids and proteins increase (Liu et al., 2014; Kim et al., 2018).

Most ICs keep the test pig inside a sealed chamber with a single air inlet and outlet. Fresh air from outside continually flows into chamber and this type of IC is called an open-circuit IC (Young et al., 1975). There are two types of open-circuit IC; pull ventilation system and push ventilation system. An open-circuit IC that uses a fan installed at the air outlet to pull fresh air into chamber is known as a pull ventilation system and results in negative pressure in chamber. Whereas, the push ventilation system uses an air-blowing pump to push air through the air inlet into chamber and results in positive pressure (Brown et al., 1984). The O_2 consumption and CO_2 and CH_4 production then are calculated by the difference between fresh incoming gas and gas exiting the chamber after respiration of pigs. Volume of chamber, velocity of air flow, temperature, pressure, and humidity also have to be measured for complete calculation of gas exchange (Young et al., 1975).

The IC method has advantages on determination RE and HP because these can be measured in the same pig with relatively short experimental periods. In addition, repeated energy balance can be measured in the same pig over successive periods of time. However, this method

also has disadvantages regarding experimental procedures. Pigs are kept in confined respiratory chambers to measure HP, which may lead to a reduction in physical activity and thermoregulation response. Therefore, the HP measured by the IC method is likely underestimated compared to that determined with the group-housed pigs which would have normal physical activity (Baldwin and Bywater, 1984; Reynolds, 2000). Moreover, the application of the IC method requires various considerations, including accurate measurement of air flow rate, gas analysis and calibration of analyzers, ability to control temperature inside the chambers where animals are kept, and deriving the FHP.

2.5.3 Comparison of Net Energy Value Determined with the Comparative Slaughter Method and Indirect Calorimetry Method

Several studies have been carried out to determine NE value of a diet or a feed ingredient fed to pigs using either the CS or IC method or to compare these two methods in the same trial (Table 2.1). The CS method was recently used in a series of studies conducted at the University of Illinois at Urbana-Champaign, whereas, the IC method has been used in European countries, University of Manitoba, and China Agricultural University (Noblet et al., 1994, Kil et al., 2011, 2013; Kim and Nyachoti, 2017; Kim et al., 2017, 2018)

According to the summary of recently published NE values of feed ingredients fed to growing pigs, the average % difference between the NE determined by the CS method and book value in NRC (2012) was much higher than that between the IC method and book value (-53.6% vs. -27.4%). The values reported in NRC (2012) are not directly determined values but predicted values using the prediction equations based on dietary DE contents and chemical composition of ingredients according to Noblet et al. (1994). These equations were generated from the

relationship between chemical composition of diets and dietary NE contents that were determined by the IC method, which may explain the lower % difference in the IC method than the CS method. Another reason for the lower % difference found in the IC method could be underestimation of HP due to less physical activity of pigs during HP measurement. As discussed earlier, not enough space to have normal physical activity is provided during the measurement of HP, which may lead to a decrease in physical activity and thermoregulation response (van Milgen et al., 1998; Ewan, 2001; de Lange et al., 2006). Considering that HP due to physical activity accounts for approximately 15% of total HP in growing pigs (Noblet and van Milgen, 2013), lower NE values determined by the CS method compared to the IC method can be reasonably explained. Despite of these reasons, similar NE values determined by the CS and IC methods were also reported (Ayoade et al., 2012a). The NE values of corn-SBM based diets containing 0%, 15%, and 30% corn-wheat DDGS fed to growing pigs were determined to be 2,134, 2,153, and 2,152 kcal/kg and 2,271, 2,229, and 2,233 kcal/kg, respectively, using the CS method or the IC method. Although these differences were not significantly different, NE values determined by the CS method were, on average, 4.3% less than those determined by the IC method.

Table 2.1. Net energy contents of feed ingredients determined by either the comparative slaughter (CS) or indirect calorimetry (IC) method

	Method				
Ingredient	CS	IC	NRC (2012)	% difference[1]	Reference
Corn and coproducts					
Corn	1,780		2,672	-50.1	Kil et al., 2013
Corn		2,893	2,672	7.6	Liu et al., 2014
Corn[2]		2,621	2,672	-1.9	Li et al., 2107
Corn		2,845	2,672	6.1	Kim et al., 2018
Corn		3,095	2,672	13.7	Li et al., 2018b
Corn-wheat DDGS	2,410		2,367	1.8	Ayoade, 2012
Corn-wheat DDGS		2,405	2,367	1.6	Ayoade, 2012
Corn gluten feed[2]		1,572	2,043	-30.0	Lyu et al., 2018
Corn germ meal[2]		1,873	1,888	-0.8	Lyu et al., 2018
Oilseed meal					
Soybean meal		2,222	2,148	3.3	Liu et al., 2014
Soybean meal[2]		2,438	2,148	11.9	Li et al., 2017
Soybean meal (dry-extruded)[3]		2,362	-	-	Velayudhan et al., 2015
Canola meal (*B. napus* yellow)		1,893	1,890	0.2	Heo et al., 2014
Canola meal (*B. juncea* yellow)		2,108	1,890	10.3	Heo et al., 2014
Canola meal (*B. napus* black)		2,033	1,890	7.0	Kim et al., 2018
Canola meal (experller pressed)[2]		2,606	2,351	9.8	Li et al., 2107
Canola meal (solvent extracted)[2]		1,926	1,890	1.9	Li et al., 2107
Oil and fat					
Choice white grease	5,345		7,149	-33.8	Kil et al., 2011
Corn oil		8,126	7,549	7.1	Li et al., 2018a
Fish oil		8,071	7,496	7.1	Li et al., 2018a
Flaxseed oil		8,155	7,553	7.4	Li et al., 2018a

Palm oil		7,749	6,265	19.2	Li et al., 2018a
Poultry fat		7,937	7,361	7.3	Li et al., 2018a
Soybean oil	4,671		7,545	-61.5	Kil et al., 2011
Soybean oil		8,066	7,545	6.5	Li et al., 2018a
Fibrous coproducts					
Defatted rice bran[2]		1,005	1,553	-54.5	Lyu et al., 2018
Full fat rice bran[2]		2,704	2,281	15.6	Lyu et al., 2018
Hemp hulls[3]		2,204	-	-	Kim and Nyachoti, 2017
Soybean hulls	603		989	-64.0	Stewart et al., 2013
Wheat bran		896	1,646	-83.7	Jaworski et al., 2016
Wheat middlings	987		2,113	-114.1	Stewart et al., 2013
Average % difference					
CS method				-53.6	
IC method				-27.4	

[1]Percentage difference between the values determined by the CS or IC method and value published in NRC (2012).
[2]Dry matter content was not provided in the original paper; therefore, dry matter content reported in NRC (2012) was used for calculation.
[3]Net energy content of an ingredient has not been reported in NRC (2012).

2.6 FACTORS AFFECTING NET ENERGY VALUES

Although NE values have been recognized as better estimates to express available energy in diets and feed ingredients for pigs than DE and ME values, there is a concern that more accurate descriptions of available energy in diets increases the dependency on individual pigs. This may lead to variations in absolute NE values of diets due to variations among individual pigs (Boisen and Verstegen, 2000). Including the factors that affect DE and ME values such as diet composition, processing technique, quality of ingredients, and age of pigs, other factors also affect the NE value of a diet and a feed ingredient significantly. These factors include the experimental methodology for determination of FHP, physical activity, temperature, physiological status, genotype and gender, and health status.

2.6.1 Experimental Methodology for Determination of Fasting Heat Production

During the fasting state, energy from body reserves is mobilized to produce ATP for maintaining various physiological functions in the body. Therefore, the energy expenditure in pigs during fasting stage is represented by FHP. In other words, FHP is the sum of basal energy requirement for physiological functions and metabolic cost for generating available energy from body reserves (de Lange et al., 2006). The FHP can be measured directly in fasting pigs or estimated by extrapolating HP measured at different feeding levels to zero ME intake. The FHP determined with IC method immediately after a fed period is strongly recommended for the calculation of NE value because extrapolated FHP tends to be inconsistent due to several limitations (see the *Estimation of energy requirement for maintenance* section). Recently, exponential regression analysis was used to estimate the FHP of pigs with a range that covers

values below and above maintenance and the estimate of FHP (181 kcal/$BW^{0.6}$; Zhang et al., 2014) was similar to the literature value (179 kcal/$BW^{0.6}$; Noblet et al., 1994).

Recently reported estimates of FHP determined in modern lean-type pigs during the last 7 years are summarized in Table 2.2. The average FHP of 11 experiments is 193 kcal/$BW^{0.6}$, which is very close to the value (179 kcal/$BW^{0.6}$) reported by Noblet et al. (1994). However, it should be noted that FHP of pigs is highly influenced by several factors such as previous feeding level, standardization of physical activities, and the duration of fasting (Baldwin, 1995; Rijnen et al., 2003; de Lange et al., 2006). Therefore, these factors should be carefully controlled to minimize the variation in FHP, and therefore absolute NE values. The use of standardized experimental conditions may be one of best solutions for reducing variation among experiments.

Table 2.2. Estimates of fasting heat production (FHP) measured in modern lean-type pigs using the indirect calorimetry method

BW (kg)	FHP (kcal/kg)	FHP (kcal/$BW^{0.6}$)	References
21	1,577	252	Ayoade et al., 2012a
36	1,436	181	Liu et al., 2014
60	1,103	181	Zhang et al., 2014
26	1,078	161	Heo et al., 2014
17	1,468	238	Velayudhan et al. 2015b
54	1,131	193	Jaworski et al., 2016
23	1,320	198	Kim and Nyachoti, 2017
21	1,100	143	Kim et al., 2018
37	1,226	192	Lyu et al., 2018
42	1,312	196	Li et al., 2018a
41	1,419	184	Li et al., 2018b
Average			
34	1288	193	

2.6.2 Physical Activity

Physical activities of pigs are greatly affected by group size, space allowance, feeding frequency, and mixing of animals (NRC, 2012). However, most FHP values have been measured in confined respiratory chambers with an individual pig or small groups of pigs, which may lead to restriction of the physical activity of pigs. Therefore, group-housed pigs may have a higher HP than individually-housed pigs (van Milgen and Noblet, 2000). In growing pigs, the HP due to physical activity has been reported to be about 15% of total HP (Noblet and van Milgen, 2013). Therefore, direct extrapolation of values obtained under these conditions to practical feeding systems may not always be appropriate. Also, van Milgen and Noblet (2000) observed a large variation in HP among individual pigs, which may result from different amount of physical activity. This variation directly influences the calculation of dietary NE values.

2.6.3 Temperature

At ambient temperatures above the lower critical temperature, pigs dissipate heat to maintain their core body temperature with adjustments of FI and respiration rate (Noblet and van Milgen, 2013). Whereas, below the lower critical temperature, pigs reduce the heat dissipation to the environment but need to elevate their HP to maintain body temperature consistently. For example, the FHP of growing pigs is 16% lower at 33℃ than at 23℃ (van Milgen and Noblet, 2000). Likewise, it has been reported that pigs have 4% greater maintenance energy requirement for each 1℃ reduction in the ambient temperature when they are housed below the lower critical temperature due to an increase in HP to maintain body temperature (Close, 1996). The energy requirement for maintenance may also be elevated if the temperature is above the evaporative critical temperature due to an increase in respiration rate. As temperatures elevate sensible heat

losses of pigs decrease; the pig should depend more on latent heat losses to dissipate produced heat. Because pigs increase latent heat losses by panting, respiration rate increases as temperature increases, and therefore maintenance energy requirement, to maintain homeothermy (Brown-Brandl et al., 2001). Therefore, it can be concluded that temperature has significant effects on maintenance energy requirement of pigs, and consequently absolute NE values of diets and feed ingredients.

2.6.4 Physiological Status

The utilization of ME is related to HI originating from the energy expenditure due to ingestion and digestion of feed, and physical activity as well as the energy losses associated with metabolic reactions (e.g., the synthesis of lipid from glucose). The efficiency of ME utilization is dependent on the final utilization of energy (i.e., protein deposition or lipid deposition). For example, the efficiency of using ME is much higher when ingested energy is retained as lipid (approximately 80%) than as protein (approximately 60%) (Noblet and van Milgen, 2013). Also, the efficiency of ME utilization of pigs fed below maintenance energy requirements differs from those fed above maintenance energy requirements (Noblet et al., 1993b; Noblet et al., 1994; Birkett and de Lange, 2001). Therefore, the physiological status and feed intake level may affect NE values.

2.6.5 Genotype and Gender

The sizes of the GIT and other organs such as liver, kidney, and heart significantly influence the energy requirement for maintenance of pigs (Koong et al., 1983; van Milgen et al., 1998; Knap, 2000). The HP due to the activity of these organs represents 25-30% of FHP

(Baldwin and Bywater, 1984), which has 3 to 4 times greater contribution to FHP than muscles (van Milgen et al., 1998). Different genotype of pigs may have various size and weight of metabolically active organs and muscle, leading to variations in the energy requirement for maintenance (Tess et al., 1986; Baldwin, 1995; Noblet et al., 1999). Indeed, it has been reported that two different genotypes (Yorkshire × Hampshire × Duroc vs. Large white × Landrace) had different THP, consequently, dietary NE contents determined with these two genotypes were also different (Kiarie et al., 2015). Male pigs grow faster than female pigs and they have different protein to lipid deposition ratio during growth stage (NRC, 2012), which may influence the efficiency of ME utilization. Therefore, genotype and gender will significantly affect the absolute NE value of diets.

2.6.6 Health status

Immune system stimulation (ISS) caused by inflammation and infection demands a large amount of energy and nutrients (Lochmiller and Deerenberg, 2000; Johnson, 2012), which reprioritizes the hierarchy of energy and nutrients partitioning away from productive purposes. For example, ISS generally leads to a series of responses in the body such as production of cytokine, acute phase proteins, fever, and increased skeletal protein catabolism as a body defense mechanism (Heegaard et al., 2011). Studies conducted over the past years have shown that more AA are required for maintenance of body function and optimal growth during ISS in pigs (Reeds and Jahoor, 2001; Johnson, 2012). An increase in catabolism of muscle protein and production of various immune related components during ISS results in a redirection of protein or AA from muscle tissue and organ development to immune related tissues development, thereby negatively affecting growth performance of pigs (Reeds and Jahoor, 2001; Johnson, 2012). Besides the costs

of AA as a substrate for various components related to immune response, associated costs such as stimulated protein turnover for tissue repair and impact on metabolism due to hormonal regulations as an effort to cope with ISS may be even greater. Apart from the impact of ISS on AA metabolism, energy metabolism is also reported to be affected during ISS (Benson et al., 1993; Humphrey and Klasing, 2004). Responses such as fever and production of immune related compounds consume substantial amount of energy (Lochmiller and Deerenberg, 2000), which should be used for development of body tissue and organs. Theoretically, energy metabolism should also be increased during ISS because there is an extra metabolic cost for immune responses in the body. However, the reported estimates of FHP by pigs during ISS were not consistent or even decreased (Campos et al., 2014; van der Meer, 2017; Huntley et al., 2018). These results may result from the amount of FI by pigs during ISS because pigs had lower FI during ISS compared to the healthy pigs due to anorexia caused by infection. In a methodological point of view and as discussed above (see the *Estimation of energy requirement for maintenance* section), the amount of previous FI level before measuring FHP significantly influences the FHP of pigs (Noblet et al., 1994; Campos et al., 2014). Further study is required to determine the effects of health status on HP by pigs.

2.7 ENERGY EVALUATION SYSTEM

Apart from direct determination in pigs, energy values of feed ingredients can be obtained from the literature or from feeding tables (Sauvant et al., 2004; NRC, 2012). However, the utilization of these values are limited if chemical compositions in the feeding tables and actual ingredients are not similar to each other. In this situation, variations in chemical compositions can be taken into account by using an energy evaluation system that estimates

energy values of feed ingredients using prediction equations based on chemical composition of ingredient. Energy values of feed ingredients predicted by the equations are assumed to be additive in mixed diets formulated with multiple feed ingredients and are used to calculate the energy contents of mixed diets (Emmans, 1999). The amounts of energy required for maintenance and production also can be accurately estimated with an appropriate energy system. Furthermore, the hierarchy of energy values among various feed ingredients can be correctly ranked (Noblet et al., 1994; Rijnen et al., 2004).

2.7.1 Digestible and Metabolizable Energy Systems

The DE and ME systems are the most widely used energy evaluation systems for feed ingredients and diets fed to pigs because DE and ME values are relatively easy to measure and assumed to be additive in mixed diets (NRC, 2012; Kim et al., 2017). As discussed previously, DE value is affected by BW of pigs (Shi and Noblet, 1993; Noblet and van Milgen, 2004). It is, therefore, appropriate to use different DE values according to BW of pigs. From a practical point of view, the use of two DE values is suggested; one for piglets and growing-finishing pigs, and one for adult pigs both pregnant and lactating sows. Generally, values reported in most feeding tables (e.g., NRC, 2012) are determined using 30 to 60 kg pigs, and therefore should not be applied to adult pigs (Noblet and van Milgen, 2013). The DE values in adult pigs can be reasonably estimated from the DE values in growing pigs based on the relationship that difference in DE between adult and growing pigs is proportionally related to the amount of dietary indigestible OM fed to growing pigs (Noblet and van Milgen, 2004). Several prediction equations for DE of feed ingredients and diets for growing and adult pigs have been proposed.

1) DE (kcal/kg DM) = 4,168 – (91 × % Ash) + (19 × % CP) + (39 × % EE) – (36 × % NDF); NRC (2012) adapted from Noblet and Perez (1993).

2) DE (kcal/kg DM) = 1,161 + (0.749 × GE) – (43 × % Ash) – (41 × % NDF); NRC (2012) adapted from Noblet and Perez (1993).

3) DE (kcal/kg DM) = (53.7 × % CP) + (75.8 × % EE) + (41.1 × % starch) + (7.6 × % NDF) + (39.0 × % Residue), Residue equals to OM minus CP minus EE minus starch minus NDF; Sauvant et al. (2004).

where energy and chemical components are expressed on a DM basis in all equations. Source of dietary fiber in all prediction equations has a significant impact on the accuracy because the extent of fermentation in large intestine is highly variable among ingredients. Equation 1 is only applicable for complete diets and should not be used in ingredients, whereas, equation 2 and 3 can be used in both feed ingredients and complete diets but with inaccuracies because of their inability to take into account the nature of dietary fiber.

The approaches to predict ME values of feed ingredients and diets are similar to those described in the DE system. The DE value can be easily converted to the ME value by applying average value of ME:DE ratio (0.96; NRC, 2012). However, the ME:DE value can vary with BW of pigs, the level and quality of protein, and the level and source of dietary fiber in the diets (Le Bellego et al., 2001; Le Goff and Noblet, 2001).

2.7.2 Net Energy System

Major NE systems for pigs were developed in France (Institut National de la Recherche Agronomique, INRA), the Netherland (Centraal Veevoeder Bureau, CVB) and Denmark to overcome the limitations of DE and ME systems (de Lange and Birkett, 2005). The utilization of

ME for maintenance and for growth (Just et al., 1983; Noblet et al., 1994) or for fattening (Schiemann et al., 1972) are combined in all NE equations based on the assumption that pigs have similar efficiencies for maintenance and energy retention. The French NE system (INRA) is the most commonly used system and has been described and demonstrated in several papers (Noblet et al., 1994; Noblet, 2007; Noblet and van Milgen, 2013). The system used in the Netherlands (CVB) was adapted from the equations proposed by Schiemann et al. (1972) and literature data. The Danish NE system is also known as potential physiological energy (PPE) system because this system is developed based on the combination of *in vitro* digestible nutrients and biochemical coefficients for ATP production potential from the complete oxidation of nutrients (Boisen and Verstegen, 2000). The prediction equations in the French and the Dutch systems are generated using the data obtained from the chemical compositions of diets and digestibility of nutrients, and determined dietary NE values, whereas the Danish system is established based on the PPE released from ATP bonds at the cellular level of pigs (Boisen, 2007).

2.7.3 The French System

The French NE system was proposed by Jean Noblet and his colleague who conducted a large set of experiments at the INRA in France. In short, as described by Noblet et al. (1994), the DE, ME, and NE values of 61 diets containing variety feed ingredients at a high ME intake (550 kcal of ME/kg $BW^{0.6}$) or at a low ME intake (330 kcal of ME/$BW^{0.6}$) were determined in lean-genotype growing pigs (45 kg of Large white boars) using the IC method. The digestibility of nutrients and energy at ileal and total tract level were measured for each diet, and then NE values of each diet were calculated as the sum of FHP and RE in pigs fed at high ME intake. Regression

equations for predicting dietary NE values in complete diets and feed ingredients were proposed using DE, ME, chemical composition, and digestible chemical composition. As a result, a total of 11 prediction equations were developed and the following 3 equations are applicable for both complete diets and feed ingredients for growing-finishing pigs (Sauvant et al., 2004; NRC, 2012; Noblet and van Milgen, 2013).

1) NE (kcal/kg DM) = (0.700 × DE) + (16.1 × % EE) + (4.8 × % starch) – (9.1 × % CP) – (8.7 ×% ADF).

2) NE (kcal/kg DM) = (0.726 × ME) + (13.3 × % EE) + (3.9 × % starch) – (6.2 × % CP) – (8.3 × % ADF).

3) NE (kcal/kg DM) = (27.3 × % digestible CP) + (83.7 × % digestible EE) + (34.4 × % starch) + (28.9 × % digestible residue), digestible residue equals to digestible OM – digestible CP – digestible EE – starch – digestible ADF.

In all equations, energy and chemical components were expressed on a DM basis.

2.7.4 The Dutch System

The Dutch NE system was developed by CVB in the Netherlands. The digestible nutrients contents in feed ingredients were used to predict the NE values of feeds and feed ingredients in this system, similar to the approach used in French NE system (Rijnen et al., 2004). However, in the Dutch NE system, total digestible carbohydrates (i.e., starch and sugar) are separated into enzymatically-digestible carbohydrates and fermentable carbohydrates. This is because energetic utilization of carbohydrates in small intestine (enzymatically-digestible carbohydrates) and in large intestine (fermentable carbohydrates) is different in pigs (Blok, 2006). Unlike French NE system, enzymatic procedure with amyloglucosidase is used for analyzing

starch content rather than Ewers polarimetric procedure, which results in differences in starch contents in the ingredients. Enzymatically degradable sugars are determined from the total sugars and the remaining part is considered fermentable sugars. In addition, digestible lipids are determined using the acid hydrolyzed ether extract method for both feeds and feces. Whereas, in the French NE system, lipids are determined using the acid hydrolyzed ether extract method only for feces. The NE of diets and feed ingredients can be predicted in the Dutch system using the following equation (Blok, 2006):

1) NE (kcal/kg DM) = (28.0 × % digestible CP) + (85.4 × % digestible EE) + (33.8 × % starch-e) + (30.5 × % sugar-e) + (23.3 × % fermentable carbohydrates)

where energy and chemical components are expressed on a DM basis; starch-e equals to enzymatically digestible starch; sugar-e equals to enzymatically digestible sugar; fermentable carbohydrates equals to fermentable starch + fermentable sugar + digestible NSP.

2.7.5 The Danish System

A concept called the PPE system was proposed by Boisen and Verstegen (1998) for estimating the NE value of swine diets. This system is based on the theoretical biochemical utilization of energy (i.e., ATP) at cellular level of pigs. Therefore, it is closer to a mechanistic model compared to other NE systems. The value used in the PPE system is the potential energy value for ATP production if digestible nutrients are completely oxidized by animals (Boisen, 2007). The PPE calculated from different feed ingredients or digestible nutrients are additive in diets containing multiple sources of feed ingredients and are independent of animal factors because it is assumed that the PPE of nutrients is independent of their metabolic utilization (e.g., oxidation or retention) (Kil et al., 2013). *In vitro* digestibility of CP, AA, OM, lipids, and

carbohydrates are utilized to predict PEE of diets to avoid variations in nutrient digestibility due to animal factor. The energy value obtained is also corrected by subtracting energy contributions from the compounds originating from endogenous synthesis of protein and lipids throughout the GIT. In this system, the energy values for feeds are expressed as Feed units (FU), which are calculated from the PPE values of each nutrient in the diet. One FU for growing pigs is equivalent to 7.38 MJ PPE. The following equation is used to predict the energy values for growing pigs in this system.

$$FU = (9.9 \times RDCP + 31.7 \times RDCF + 11.7 \times EDC + 7.0 \times FERMC – 2.8 \times EIDMi) / 7.38$$

where FU is expressed on a DM basis and other components are in g/kg DM; RDCP equals to *in vitro* ileal digestible CP, RDCF equals to calculated ileal digestible fat, EDC equals to *in vitro* ileal digestible carbohydrates; FERMC equals to fermentable carbohydrates; EIDMi equals to enzyme – undigested ileal DM.

2.7.6 Comparison of Energy Systems

Energy system is capable of ranking feed ingredients. Therefore, selection of energy systems has a significant impact on the hierarchy among feed ingredients (Noblet et al., 1994; Rijnen et al., 2004; Noblet, 2007). It has been suggested that the DE and ME systems may overestimate energy values of ingredients high in protein and fiber, but underestimate energy values of ingredients high in lipid and starch (Noblet et al., 1994; Noblet, 2007) because the efficiency of ME utilization for NE differs significantly among nutrients (Noblet et al., 1994; Noblet and van Milgen, 2013). These conclusions are clearly demonstrated in Table 2.3. For example, considering that NE value is actually available energy to pigs, energy values of animal fat, corn, wheat, and barley (ingredients contain high levels of starch or fat) are relatively

underestimated with DE and ME systems, whereas DE and ME system relatively overestimate the energy values of wheat bran, distiller dried grains, SBM, and canola meal (ingredients contain high levels of fiber or protein).

Also, comparison of energy values in corn (high in starch), SBM (high in protein), and camelina cake (high in fat, protein, and fiber) in our previous study (Kim et al., 2017) clearly showed the superiority of NE system in terms of its capability to rank feed ingredients. The SBM and camelina cake had significantly higher DE content compared to corn, whereas no difference was observed among ingredients with the ME system. However, with NE system, the energy value was the highest for corn and the least for SBM with the value for camelina cake being intermediate.

Table 2.3. Relative DE, ME, and NE values of ingredients for growing pigs[1,2]

	DE	ME	NE	NE/ME, %
Animal fat	243	252	300	90
Corn	103	105	112	80
Wheat	101	102	106	78
Barley	94	94	96	77
Reference diet	*100*	*100*	*100*	*75*
Pea	101	100	98	73
Wheat bran	68	67	63	71
Distillers dried grains	82	80	71	67
Soybean meal	107	102	82	60
Canola meal	84	81	64	60

[1]Adapted from Sauvant et al. (2004).

[2]Within each system, values are expressed as percentages of the energy values of a diet containing 68% wheat, 16% soybean meal, 2.5% fat, 5% wheat bran, 5% peas, and 4% minerals and vitamins. The DE, ME, and NE contents of the reference diet are 3,277, 3,145, and 2,376 kcal/kg, respectively

The quality of a nutritional evaluation system may be determined by its ability to predict the performance of the animals. Performance should be diet-independent (or specific effects of

nutrients) because several ingredients containing wide range of nutrients contents can be used for least cost diet formulation. With respect to energy evaluation systems, the superiority of NE system has been demonstrated in several performance studies (Quiniou and Noblet, 2012; Velayudehan and Nyachoti, 2016). In their studies, the relationship between the energy system and energy cost of BW gain was confirmed and it has been concluded that the NE system is a better predictor of performance than DE or ME systems. In other words, the NE value is the most satisfactory estimate of the energy value of feeds.

2.8 CONCLUSION

Ingested dietary energy is digested, absorbed, and metabolized by pigs and utilized for maintenance of various physiological functions within the body and development of organs and body tissues. Accurate estimation of available energy in feed ingredients and energy requirement of pigs are critical to ensure optimal performance of pigs along with cost effective pork production. Several energy evaluation systems (DE, ME, NE, or PPE system) have been developed and used to determine the amounts of actually available energy to pigs in feed ingredients and diets. Among these systems, the NE system is believed to be superior to the DE and ME systems because the DE and ME systems are not able to correct energy losses as HI during digestion, absorption, and metabolism of nutrients, and therefore tend to overestimate the energy values of ingredients high in protein and fiber but underestimate energy values of ingredients high in lipid and starch. Also, NE value is the only value in which energy requirement of pigs and the supply of dietary energy are expressed on the same basis regardless of diet compositions, consequently, provides a better prediction of pig performance. However, there is also a concern against the use of NE system because more accurate descriptions of

available energy in diets increase the dependency on individual pigs, which likely results in variations in absolute NE values of diets. Therefore, factors affecting NE values, including measurements of FHP, diet composition, physical activity, temperature, physiological status, genotype and gender, and health status should be carefully considered when NE contents of ingredients and diets are determined.

The NE content of diets and feed ingredients fed to pigs is commonly determined by the CS and IC methods, and prediction equations. The IC method is highly preferable for NE determination due to its accuracy and relatively short experimental period although it involves an expensive experimental instrument and there is a concern that physical activity of pigs is likely limited in chamber during measurement of HP. Moreover, the NE contents can be easily estimated using prediction equations without direct measurement if chemical composition of the ingredient is analyzed. Although several prediction equations are available for estimation of NE contents in ingredients and have been demonstrated to be accurate in terms of dietary energy evaluation, there is a possibility that the NE contents of several coproducts, which are widely used in swine diets, may not agree with predicted values. In the future, therefore, more studies are required for the comparison of NE value of ingredient estimated by the prediction equations and directly determined by the IC method. Also, additional validation studies for the NE system in swine diets formulated with variety feed ingredients, especially high fiber diets, will be useful to accelerate the adoption of NE system as a standard practice.

CHAPTER 3

HYPOTHESES AND OBJECTIVES

The studies in this dissertation tested the following hypotheses:

1. Net energy contents of ingredients determined using the IC method would differ from predicted values using prediction equations.
2. Net energy contents of ingredients determined by the difference method would be influenced by nutrient composition of the basal diet.
3. The presence of antinutritional factor in a feed ingredient would affect the NE content of diet, therefore additivity of dietary NE in complete diets.
4. Physiological status (i.e., age) of pigs would have impacts on dietary NE contents.

The overall objectives were to 1) evaluate the energy contents of selected coproducts, and 2) to determine the effects of factors that may affect NE of diets and ingredients fed to pigs.

The specific objectives were:

1. To determine the DE, ME, and NE contents of hemp hulls, processed hemp hull products, camelina cake, canola meal, and high-protein sunflower meal using the IC method and to compare directly determined NE with predicted NE using prediction equations published in NRC (2012).
2. To investigate the effects of dietary glucosinolates originating from camelina cake on energy values of diets and additivity of energy in complete diets.
3. To determine the effects of nutrient composition of basal diets (i.e., AA and P contents) on NE values of feed ingredients.

4. To investigate the effects of age of weaned pigs on dietary NE values and to evaluate responses of weaned pigs to increasing dietary NE concentrations.

CHAPTER 4

MANUSCRIPT I: Net energy of hemp hulls and processed hemp hull products fed to growing pigs and the comparison of net energy determined via indirect calorimetry and calculated from prediction equations

J. W. Kim, and C. M. Nyachoti

Department of Animal Science, University of Manitoba, Winnipeg, MB R3T 2N2, Canada

Published: Journal of Animal Science. 2017. 95: 2649-2657

doi:10.2527/jas.2016.1255

4.1 ABSTRACT

An experiment was conducted to determine the net energy (NE) of hemp hulls (HH), extruded HH (EHH), and a blended product of HH with pea (HHP) fed to growing pigs using indirect calorimetry (IC) and to determine effects of dietary fiber on heat production (HP) and fasting HP (FHP). Twenty-four growing barrows with an average initial BW of 22.9 ± 1.75 kg were individually housed in adjustable metabolism crates. Pigs were randomly allotted to 1 of 4 dietary treatments with 6 replicates per treatment. A corn–soybean meal basal diet was prepared. Three additional diets were formulated to contain a constant ratio of corn and soybean meal and each of the test ingredients. Pigs were fed experimental diets for 16 d including 10 d for adaptation and 6 d for total collection of feces and urine to determine digestible energy (DE) and metabolizable energy (ME) of experimental diets. Pigs were then moved into IC chambers to determine HP and FHP. The apparent total tract digestibility (ATTD) of DM was greater ($P < 0.01$) in the HHP diet than in the HH and EHH diets but less ($P < 0.01$) than in the basal diet. Similarly, the ATTD of GE in the basal diet was greater ($P < 0.01$) compared with the HH, EHH, and HHP diets. The DE, ME, and NE of the basal diet were greater ($P < 0.01$) than those of the HH, EHH, and HHP diets. No significant differences were observed for the HP (on average, 1,904 kcal/kg DM) and FHP (on average, 1,320 kcal/kg DM) among treatments. However, the retained energy of pigs fed the basal diet (1,763 kcal/kg DM) was greater ($P < 0.05$) than for those fed the HH (1,501 kcal/kg DM) and HHP (1,482 kcal/kg DM) diets. The NE:ME ratio tended to be greater ($P \leq 0.10$) for the basal diet (0.85) than for the HH (0.82), EHH (0.82), and HHP (0.83) diets. The NE of HH, EHH, and HHP determined by the IC method were 2,375, 2,320, and 2,399 kcal/kg DM, respectively, whereas values calculated using published prediction equations were 2,308, 2,161, and 2,278 kcal/kg DM, respectively. However there was no

difference between determined and predicted values. In conclusion, the NE of HH, EHH, and HHP determined using the IC method were 2,375, 2,320, and 2,399 kcal/kg DM, respectively, and these values were 2.9, 7.1, and 5.2% greater, respectively, than the predicted values, although no difference was observed between determined and predicted values. However, the HP values observed for the basal diet and the diets containing high dietary fiber in the form of HH, EHH, or HHP were similar.

Key words: heat production, hemp hulls, indirect calorimetry, net energy, pig

4.2 INTRODUCTION

Hemp (*Cannabis sativa* L.) is an annual herbaceous plant belonging to the Cannabinaceae family and has been planted for fiber and medicine production for many years (Russo and Reggiani, 2015). The production of hemp hulls (HH), a coproduct of dehulled hempseed, has gradually increased due to increased human consumption of shelled hempseed in North America and in many European countries (Callaway, 2004). Due to its high protein and oil contents, HH has potential as a protein and energy source for swine diets. Moreover, the nutritive value could be enhanced through processing such as extrusion or blending with other feed ingredients. Indeed, extruded and blended feed ingredients have been shown to have a higher nutritive value for swine and poultry compared with raw feed ingredients (Kiarie and Nyachoti, 2007; Jha et al., 2013).

Among the available energy evaluating systems, the net energy (NE) system provides the most accurate estimates of the dietary energy utilizable for pigs (Noblet, 2007; Velayudhan et al., 2015b), especially for high-fiber feeds and ingredients. Hemp hulls and HH-containing

feedstuffs contain relatively high dietary fiber contents, which necessitates the need to determine their NE value to facilitate their effective utilization as energy sources in swine diets (Velayudhan et al., 2015b). However, inconsistent results regarding effects of dietary fiber on heat production (HP) have been reported, although HP is the major component for estimation of NE (Noblet and van Milgen, 2013).

Therefore, this experiment was conducted 1) to determine the NE of HH and HH-containing products including extruded HH (EHH) and a blended product of HH with pea (HHP) fed to growing pigs using either indirect calorimetry (IC) or prediction equations and 2) to test the hypothesis that increased dietary fiber in the form of HH, EHH, or HHP added to a corn–soybean meal (SBM) diet will increase HP when fed to growing pigs.

4.3 MATERIALS AND METHODS

The experimental protocol used in the present study was reviewed and approved by the University of Manitoba Animal Care Committee, and pigs were cared for according to the guidelines of the Canadian Council on Animal Care (2009).

4.3.1 Experimental Animals and Diets

The HH, EHH, and HHP evaluated in the present study were obtained from a local supplier (Starlite Colony, Starbuck, MB, Canada). The HHP contained 30% pea and 70% HH, which were blended and co-milled through a hammer mill.

Twenty-four growing barrows ([Yorkshire × Landrace] × Duroc) with an average initial BW of 22.9 kg (SD 1.75) were obtained from Glenlea Swine Research Unit, University of Manitoba (Winnipeg, Canada). Pigs were individually housed for 16 d in adjustable metabolism

crates (1.80 by 0.60 m) with smooth, transparent plastic sides and plastic-covered expanded metal sheet flooring in a temperature-controlled room (22 ± 2℃). A corn–SBM basal diet was formulated to meet or exceed energy and nutrient requirements of growing pigs (NRC, 2012; Table 4.1). Three additional diets were formulated by replacing 30% of corn and SBM with the test ingredients (HH, EHH, and HHP), with the corn:SBM ratio kept constant (Velayudhan et al., 2015a).

Table 4.1. Composition and nutrient content of experimental diets (as-fed basis)

	Dietary treatment[1]			
Item	Basal	HH	EHH	HHP
Ingredients, %				
Corn	67.50	46.34	46.34	46.34
Soybean meal	28.20	19.36	19.36	19.36
Hemp hulls	-	30.00	-	-
Extruded hemp hulls	-	-	30.00	-
Hemp hulls + pea	-	-	-	30.00
Vegetable oil	0.84	0.84	0.84	0.84
Lys-HCl	0.06	0.06	0.06	0.06
Limestone	1.00	1.00	1.00	1.00
Monocalcium phosphate	0.90	0.90	0.90	0.90
Salt	0.50	0.50	0.50	0.50
Vitamin-mineral premix[2]	1.00	1.00	1.00	1.00
Analyzed composition				
DM, %	90.0	90.5	90.6	89.8
GE, kcal/kg	3,958	4,332	4,316	4,200
CP, %	18.0	19.2	19.3	18.7
Ether extract, %	5.8	10.5	10.2	7.0
Starch, %	43.1	26.1	30.6	31.6
Ash, %	4.3	4.8	4.6	4.7
NDF, %	10.6	24.2	22.2	18.3
ADF, %	3.5	12.2	10.5	11.1
Ca, %	0.83	0.86	0.78	0.79
P, %	0.60	0.66	0.69	0.67

[1]Basal = corn-SBM basal diet; HH = a diet containing basal and hemp hulls at a 70:30 ratio; EHH = a diet containing basal and extruded hemp hulls at a 70:30 ratio; HHP = a diet containing basal and blended product of HH with pea at a 70:30 ratio.

[2] Supplied the following per kilogram of finished feed: 2,000 IU vitamin A, 200 IU vitamin D, 40 IU vitamin E, 2 mg vitamin K, 350 mg choline, 14 mg pantothenic acid, 7 mg riboflavin, 1 mg folic acid, 21 mg niacin, 1.5 mg thiamin, 2.5 mg vitamin B6, 70 µg biotin, 20 µg vitamin B12, 10 mg Cu, 110 mg Zn, 120 mg Fe, 10 mg Mn, 0.4 mg I, and 0.3 mg Se.

4.3.2 Experimental Design and Procedure

The experiment was conducted in 2 consecutive periods (12 pigs per period) using the same facility and similar experimental conditions and procedures because only 3 IC chambers were available. Pigs were randomly allotted to 1 of 4 experimental diets in a completely randomized design with 3 replicates per diet (per period).

Pigs were fed their assigned diets at 550 kcal ME/kg $BW^{0.60}$ per day based on BW on d 1, 5, and 10, which was close to ad libitum intake. During the experiment, pigs were fed at 0830 h and were trained to consume their daily feed allowance within 1 h. Pigs had free access to water via a low-pressure nipple drinker throughout the experimental period. Pigs were fed experimental diets for 16 d including 10 d for adaptation to feed and environmental conditions (metabolism crates and temperature inside room) and 6 d for total collection of feces and urine. During the last 6 d of each feeding period, total fecal and urine collection was performed for the determination of digestible energy (DE) and metabolizable energy (ME) of test ingredients (HH, EHH, and HHP) as previously described by Woyengo et al. (2010). Briefly, in the morning (0830 h) of the first day of the collection period (d 11), each pig received 5 g of ferric oxide as an indigestible marker mixed in 100 g of assigned feed. The remaining portion of feed was provided after all the marked feed was consumed. Fecal collection was initiated when the marker appeared in feces. In the morning (0830 h) of d 16, pigs were offered 100 g of marked feed as described above, and fecal collection was terminated when the marker appeared in feces. Feces were collected once daily in the morning and were stored at −20°C. Urine collection was initiated at 0830 h on d 11 and ceased at 0830 h on d 16. Urine was collected once daily in the morning (in jugs containing 20 mL of 3 *N* HCl to minimize N losses) and weighed. A subsample (10% of the total weight) was obtained, filtered through glass wool, and stored at −20°C.

On d 16, 3 pigs each were transferred to the calorimetry chambers (1.22 by 0.61 by 0.91 m; Columbus Instruments, Columbus, OH) to measure HP and fasting HP (FHP) based on O_2 consumption, CO_2 production, and urinary N excretion. Pigs were brought into the calorimetric chambers within 1 h of consuming their daily feed allowance, and HP was continuously measured for 24 h (fed state) followed by 12 h (fasting state) of FHP measurement. The following sets of 3 pigs were moved into the calorimetry chambers every 2 d (d 18, 20, and 22). Pigs had unlimited access to fresh water in the chambers at all times, and urine voided during the 24- and 12-h periods was collected separately, weighed, subsampled, and stored at −20°C until required for N analysis. The experimental temperature inside the chamber was maintained at 22℃ ± 1℃, and personnel movement in the chamber room was limited to measure HP and FHP under calm conditions. After d 16 (at the end of collection day), pigs were fed their assigned experimental diets until they were moved into the IC.

4.3.3 Sample Preparation and Chemical Analyses

Fecal samples were oven-dried at 50℃ for 5 d and were finely ground before chemical analysis. Urine samples from metabolism crates and calorimetry chambers were thawed and pooled separately for each pig, sieved through cotton gauze, and filtered with glass wool. The DM content of diets, ingredients, and feces was determined according to the Association of Official Analytical Chemists (method 925.09; AOAC, 1990) and the GE content of diets, ingredients, feces, and urine was estimated using an adiabatic bomb calorimeter (model 6400; Parr Instrument, Moline, IL), which had been calibrated using benzoic acid as a standard. Nitrogen content of diets, ingredients, feces, and urine was determined using the combustion method (method 990.03; AOAC, 1990) using the LECO N analyzer (model CNS-2000; LECO

Corp., St. Joseph, MI), and CP was calculated as N × 6.25. Ether extract (EE) was measured for diets and ingredients after hexane extraction (method 920.39; AOAC, 1990) in an extraction apparatus. The ADF and NDF contents in diets and ingredients were determined according to the method of Goering and van Soest (1970). Ash content of diets and ingredients was determined according to the AOAC (method 942.05; AOAC, 1990). Starch content in diets and ingredients was measured using an assay kit (Megazyme Total Starch assay kit; Megazyme International Ltd., Wicklow, Ireland). Nonstarch polysaccharide (NSP) contents in test ingredients were analyzed using GLC (component neutral sugars) using an SP-2340 column and a Varian CP3380 gas chromatograph (Varian Inc., Palo Alto, CA) and by colorimetry (uronic acids) using a Biochrom Ultrospec 50 (Biochrom Ltd., Cambridge, England) and the procedure described by Englyst and Cummings (1988) with minor modifications (Slominski and Campbell, 1990).

To determine the GE of urine, approximately 0.5 g of cellulose was dried at 103℃ for 24 h, 2 mL of urine sample was added over it, and the weight of the final mixture was recorded. The urine–cellulose mixture along with a sample of pure cellulose were dried in an oven at 50℃ for 24 h and then weighed for estimation of urine DM. The GE of the dried urine–cellulose mixture and pure cellulose were determined using an adiabatic bomb calorimeter as described above, from which the GE of urine samples were calculated by the difference method (Fleischer et al., 1981).

4.3.4 Calculations

The apparent total tract digestibility (ATTD) of DM, energy, and CP and N retention (%) were calculated as described by Woyengo et al. (2010).

The HP and FHP (Brouwer, 1965), retained energy (RE; Noblet et al., 1994), DMI, and

NE values (Noblet et al., 1994) were calculated using the following equations:

$$HP = 3.87 \times O_2 + 1.20 \times CO_2 - 1.43 \times \text{urinary N excretion},$$

in which HP is in kilocalories, O_2 is oxygen consumption in liters, CO_2 is carbon dioxide production in liters, and urinary N excretion is total urinary N excretion in grams. The FHP was also calculated using same equation for HP.

$$RE = ME - HP,$$

where RE, ME, and HP are in kilocalories per day.

$$DMI = \text{feed intake} \times \text{DM contents of feed}$$

in which DMI and feed intake are in kilograms, and DM contents of feed is a percentage.

$$NE = (RE + FHP)/DMI,$$

in which NE is in kilocalories per kilogram DM, RE and FHP are in kilocalories per day, and DMI is in kilograms. The NE of the experimental diets and test ingredients were calculated according to the equations established by Noblet et al. (1994):

$$NE = 0.843 \times DE - 463,$$

$$NE = 0.700 \times DE + 1.61 \times \%\ EE + 0.48 \times \%\ \text{starch} - 0.91 \times \%\ CP - 0.87 \times \%\ ADF,$$

$$NE = 0.870 \times ME - 442,$$

$$NE = 0.726 \times ME + 1.33 \times \%\ EE + 0.39 \times \%\ \text{starch} - 0.62 \times \%\ CP - 0.83 \times \%\ ADF,$$

in which NE, DE, and ME are in kilocalories per kilogram DM, EE is ether extract in % DM, ST is starch in % DM, and ADF is in % DM. The average value of 4 prediction equations was used for the predicted NE.

The DE, ME, and NE of test ingredients (HH, EHH, and HHP) were calculated according to the study of Velayudhan et al. (2015a), who reported that constant corn:SBM ratio showed the least difference between the determined and predicted NE among other formulation

techniques (i.e., simple substitution, constant corn:SBM ratio, and constant CP). The DE, ME, and NE of corn and SBM (constant ratio) and NRC (2012) value of canola oil (8,759, 8,384, and 7,554 kcal/kg for DE, ME, and NE, respectively) were used to calculate the energy values of test ingredients. The energy values of test ingredients were calculated as follows:

$$DE_{\text{test ingredients}} \text{ (kcal/kg DM)} = (DE_{\text{test diet}} - 0.657 \times DE_{\text{corn and SBM}} - 0.0084 \times DE_{\text{oil}})/ 0.3,$$

The ME and NE of the test ingredients were calculated using the same equation as with DE, but ME and NE replaced DE.

4.3.5 Statistical Analysis

All data were analyzed using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). The individual pig was considered the experimental unit. Effects of diet and period were included in the model for statistical analysis. However, the effect of period was not significant in this study; therefore, it was not included in the final model. The LSMEANS procedure was used to calculate mean values, and the PDIFF option of SAS was used to separate means. A probability of $P < 0.05$ was considered significant, whereas $0.05 < P \leq 0.10$ was considered a tendency.

4.4 RESULTS

All pigs adapted well to their respective diets and environmental conditions, remained healthy and readily consumed their daily feed allowance throughout the experimental period.

4.4.1 Chemical Composition of Hemp Hulls and Processed Hemp Hull Products

The analyzed nutrients and GE of HH, EHH, and HHP are presented in Table 4.2.

Compared to EHH and HHP, HH contains more GE, EE, NDF, and ADF. In addition, NSP were greater in HH than in EHH and HHP with a larger proportion of this being in the form of insoluble NSP. The analyzed nutrients and GE contents of the experimental diets were in good agreement with the formulated values (Table 4.1).

4.4.2 ATTD of DM, GE, and CP and N balance

The ATTD of DM was greater ($P < 0.01$) in the HHP diet than in the HH and EHH diets but less ($P < 0.01$) than in the basal diet (Table 4.3). Similarly, the ATTD of GE in the basal diet was greater ($P < 0.01$) compared with the HH, EHH, and HHP diets. The ATTD of CP was less ($P < 0.01$) in the EHH diet than in the basal, HH, and HHP diets, but there were no differences among the basal, HH, and HHP diets.

Nitrogen intake was less ($P < 0.01$) from the basal diet than from the HH and HHP diets, but no difference was observed between the basal and HHP diets (Table 4.4). There were no differences in urinary N excretion among the experimental diets. Fecal N output was greater ($P < 0.01$) for pigs fed the EHH diet than for those fed the other diets. However, no differences were observed for N retention (%) and daily N retention among the diets.

4.4.3 Net Energy of Hemp Hulls and Processed Hemp Hull Products

The energy balance of pigs and DE, ME, and NE of experimental diets and test ingredients are presented in Table 4.5. The DE, ME, and NE of the basal diet were greater ($P < 0.01$) than those of the HH, EHH, and HHP diets. No differences were observed for the HP and FHP among treatments. However, the RE of pigs fed the basal diet was greater ($P < 0.05$) than for those fed the HH and HHP diets. The NE:ME and NE:DE ratios, indicators of efficiency of

NE utilization, tended to be greater ($P \leq 0.10$) for the basal diet than for the HH, EHH, and HHP diets. The DE of HH (3,433 kcal/kg DM) was greater ($P < 0.01$) than that of the EHH (3,196 kcal/kg DM), with an intermediate value for HHP (3,339 kcal/kg DM). No differences were observed for the ME and NE of HH, EHH, and HHP. The NE of HH, EHH, and HHP determined by the IC method were 2,375, 2,320, and 2,399 kcal/kg DM, respectively, whereas values calculated using published prediction equations were 2,308, 2,161, and 2,278 kcal/kg DM, respectively. However, there was no difference between predicted and determined NE of HH, EHH, and HHP, although the NE of HH, EHH, and HHP obtained with prediction equations were 2.9, 7.1, and 5.2% less, respectively, compared with the values determined using the IC method (Table 4.6).

Table 4.2. Analyzed nutrient composition of ingredients (as-fed basis)

	Ingredients[1,2]		
Item	HH	EHH	HHP
DM, %	92.8	94.8	91.3
CP, %	21.9	21.8	19.2
Ether extract, %	23.5	16.5	14.5
Starch, %	1.9	7.1	10.9
Ca, %	0.15	0.13	0.13
P, %	0.37	0.52	0.50
NDF, %	57.1	48.8	36.8
ADF, %	37.9	31.4	22.8
Total NSP[3],%	30.8	30.5	24.6
Insoluble NSP	30.2	28.0	23.3
Soluble NSP	0.6	2.5	1.3

[1]HH = hemp hulls; EHH = extruded hemp hulls; HHP = blended product of hemp hulls with pea.

[2]The HHP contained 30% pea and 70% HH, which were blended and co-milled through a hammer mill.

[3]NSP = non-starch polysaccharide.

Table 4.3. Apparent total tract digestibility (ATTD) of DM, GE and CP of experimental diets fed to growing pigs[1]

	Dietary treatment[2]					
Item	Basal	HH	EHH	HHP	SEM	*P*-value
ATTD, %						
DM	85.6[a]	75.9[c]	75.3[c]	77.3[b]	0.30	<0.001
GE	84.3[a]	75.6[bc]	74.8[c]	76.5[b]	0.36	<0.001
CP	82.9[a]	81.1[a]	78.4[b]	81.6[a]	0.61	<0.001

[a–c]Means without a common superscript within a row differ ($P < 0.05$).

[1]Each value represents the mean of 6 observations.

[2]Basal = corn–soybean meal basal diet; HH = hemp hulls (a diet containing basal and HH at a 70:30 ratio); EHH = extruded HH (a diet containing basal and EHH at a 70:30 ratio); HHP = blended product of HH with pea (a diet containing basal and HHP at a 70:30 ratio).

Table 4.4. Nitrogen (N) balance of pigs fed experimental diets[1]

	Dietary treatment[2]					
Item	Basal	HH	EHH	HHP	SEM	*P*-value
Nitrogen balance						
N intake, g/d	32.7[c]	37.5[a]	36.8[ab]	34.4[bc]	0.64	<0.001
N output in feces, g/d	5.6[c]	7.1[b]	7.9[a]	6.3[c]	0.19	0.001
N excretion in urine, g/d	6.7	8.5	6.5	5.6	0.77	0.099
N retained, %	62.4	58.5	60.9	65.2	1.93	0.126
N retained, g/d	20.4	21.9	22.4	22.4	0.73	0.204

[a–c]Means without a common superscript within a row differ ($P < 0.05$).

[1]Each value represents the mean of 6 observations.

[2]Basal = corn–soybean meal basal diet; HH = hemp hulls (a diet containing basal and HH at a 70:30 ratio); EHH = extruded HH (a diet containing basal and EHH at a 70:30 ratio); HHP = blended product of HH with pea (a diet containing basal and HHP at a 70:30 ratio).

Table 4.5. Energy balance in growing pigs and energy values of experimental diets, hemp hulls, and processed hemp hull products determined by the indirect calorimetry method[1]

	Dietary treatment[2]					
Item	Basal	HH	EHH	HHP	SEM	*P*-value
Energy value, kcal/kg DM						
DE	3,706[a]	3,621[b]	3,564[b]	3,581[b]	16.8	<0.001
ME	3,569[a]	3,454[b]	3,446[b]	3,457[b]	20.1	0.001
HP[3]	1,806	1,953	1,880	1,975	59.7	0.210
FHP[4]	1,285	1,335	1,262	1,398	52.2	0.291
RE[5]	1,763[a]	1,501[b]	1,566[ab]	1,482[b]	63.6	0.020
Determined NE[6]	3,048[a]	2,836[b]	2,828[b]	2,880[b]	32.0	<0.001
Efficiencies of NE						
NE/ME	0.85	0.82	0.82	0.83	0.009	0.052
NE/DE	0.82	0.78	0.79	0.80	0.010	0.058
Energy value of hemp hull-products, kcal/kg DM[7]						
DE	-	3,433[a]	3,196[b]	3,339[ab]	53.5	0.009
ME	-	3,219	3,078	3,258	67.9	0.179
Determined NE	-	2,375	2,320	2,399	108.2	0.862
Predicted NE[8]		2,308	2,161	2,278	41.4	0.058

[a,b]Means without a common superscript within a row differ ($P < 0.05$).

[1]Each value represents the mean of 6 observations.

[2]Basal = corn-SBM basal diet; HH = a diet containing basal and hemp hulls at a 70:30 ratio; EHH = a diet containing basal and extruded hemp hulls at a 70:30 ratio; HHP = a diet containing basal and blended product of HH and pea at a 70:30 ratio.

[3]Heat production = (3.87 × O_2 + 1.20 × CO_2 − 1.43 × urinary N)/DMI.

[4]Fasting heat production = (3.87 × O_2 + 1.20 × CO_2 − 1.43 × urinary N)/DMI.

[5]Retained energy = (ME intake − HP)/DMI.

[6]Net energy = (RE + FHP)/DMI.

[7]Energy value of test ingredients were calculated using the difference method by subtracting the energy contribution of the basal diet from the energy value of the diets containing 30% of test ingredients.

[8] The average of 4 predicted NE from Noblet et al. (1994), where 1) NE = 0.843 × DE − 463 (NE of HH, HHP, and EHH were 2,431, 2,232, and 2,352 kcal/kg DM, respectively); 2) NE = 0.700 × DE + 1.61 × % ether extract + 0.48 × % starch − 0.91 × % CP − 0.87 × % ADF (NE of HH, HHP, and EHH were 2,248, 2,082, and 2,157 kcal/kg DM, respectively); 3) NE = 0.870 × ME − 442 (NE of HH, HHP, and EHH were 2,358, 2,270, and 2,392 kcal/kg DM, respectively); and 4) NE = 0.726 × ME + 1.33 × % ether extract + 0.39 × % starch − 0.62 × % CP − 0.83 × % ADF (NE of HH, HHP, and EHH were 2,194, 2,126, and 2,209 kcal/kg DM, respectively).

Table 4.6. Comparison of determined and predicted NE of hemp hulls, and processed hemp hull products fed to growing pigs

	NE of ingredient[1]				
Item	Determined	Predicted	SEM	*P*-value	Percentage difference[2]
HH	2,375	2,308	94.7	0.624	2.9
EHH	2,320	2,161	71.1	0.133	7.1
HHP	2,399	2,278	58.9	0.175	5.2

[1]HH = hemp hulls; EHH = extruded hemp hulls; HHP = blended product of hemp hulls with pea.

[2]Percentage difference was computed as [(determined NE – predicted NE)/(determined NE + predicted NE)/2] × 100.

4.5 DISCUSSION

Hemp has been reconsidered a profitable industrial crop for food in North America and in many European countries during the last decade due to health benefits from its high level of dietary fiber and essential fatty acids (Callaway, 2004). Food products related to hempseed have become more available for the general public in these countries although the potential for human consumption has not yet entered mass markets (Callaway, 2004). Consequently, the production of HH, a coproduct of shelled hempseed, has gradually increased along with the increase in consumption of shelled hempseed. Nutritionists have been looking for alternatives to conventional feed ingredients to reduce feed costs and to establish feeding strategies for the production of nutrient-fortified final products (i. e. n-3 fatty acids fortified eggs and pork) that may satisfy consumer demand (Eastwood et al., 2009). Increased n-3 fatty acid deposition in eggs (Gakhar et al., 2012; Goldberg et al., 2012) and tissues (Mustafa et al., 1999; Gibb et al, 2005) as a result of the inclusion of hemp seed and oil in the diets has been reported.

For efficient utilization of feed ingredients, it is essential to match energy and nutrient requirements of the animal with amount of available energy and nutrients in the diet. However, to our knowledge, no information exists for the nutritive and energy values of HH for swine and poultry although one study reported AA digestibility in hempseed cake fed to growing pigs (Presto et al., 2011). According to the nutrient composition estimated in this study, HH have a relatively high level of dietary fiber, most of which is insoluble in water with very little soluble dietary fiber (32.5 vs. 0.7%). Hemp hulls also contain 24% EE, and field pea is a legume seed containing around 22% CP and 43% starch (NRC, 2012). Blending HH with pea, therefore, allows the mixing of macronutrients and improving potential handling problems caused by the released oil during grinding of HH. In addition, application of the extrusion technique to the HH

may have positive effects on nutritive value, because extrusion has been reported to improve energy and nutrient digestibility due to the disruption of the cell wall structure and the inactivation of heat-labile antinutritional factors (Golian et al., 2007; Kiarie and Nyachoti, 2007; Ayoade et al., 2012b). Energy concentrations of high-fiber feeds are often overestimated when it is determined with either the DE or ME system (Noblet et al., 1994). Because HH, EHH, and HHP contain high levels of dietary fiber, NE of these ingredients fed to growing pigs needs to be determined for their effective utilization in formulating swine diets. The present study was, therefore, conducted to determine the NE of HH, EHH, and HHP fed to growing pigs using the IC method.

The DE and ME of the HH, EHH, and HHP diets was less than those of the basal diet, which is most likely due to the fact that 30% of the basal diet was replaced by the test ingredients containing high fiber. It has been reported that the dietary level of NDF was negatively correlated with the DM and energy digestibilities (Le Goff and Noblet, 2001). The reduced DM and energy digestibilities of the HH, EHH, and HHP diets compared with the basal diet observed in the current study further support the reduced DE and ME of diets containing HH, EHH, and HHP. The reduced CP digestibility of the EHH diet compared with the HH diet was unexpected, because it has been reported that extrusion of ingredients may enhance denaturation of proteins in diets or ingredients and expose more peptide bonds to enzymatic hydrolysis (Ayoade et al., 2012b). Kiarie and Nyachoti (2007) reported increased AA digestibility in coextruded peas and full-fat canola compared with the raw materials, whereas a contradictory result was observed in the study of Golian et al. (2007), who evaluated the effect of extrusion on AA digestibility in canola seed. This inconsistent result may be associated with the extrusion temperature. Under the influence of heat, overheating may destroy digestible nutrients, especially AA, and lead to

formation of Maillard reaction compounds, which are not biologically available (Fontaine et al., 2007).

The ME:DE ratio measured in this study (0.96; average value for the 4 experimental diets) was comparable to previously reported values of common swine diets. Noblet and van Milgen (2004) reported that the ME:DE ratio of complete diets would be around 0.96 in most circumstances.

We hypothesized that pigs fed test ingredients would have greater HP compared with those fed the basal diet because diets containing test ingredients have high level of dietary fiber. However, a similar HP among treatments (1,904 kcal/kg DM; average value of 1,806, 1,953, 1,975, and 1,880 kcal/kg DM) was observed in this experiment. In some studies, significant increases in HP were observed with increased inclusion level of dietary fiber (Noblet et al., 1989; Jørgensen et al., 1996; Ramonet et al., 2000), whereas similar (Ayoade et al., 2012a; Heo et al., 2014) or decreased HP values (Jaworski et al., 2016) were observed in other studies. The possible reason for similar or even decreased HP may be related to a reduction in physical activities of pigs or the changes in overall metabolism caused by the addition of dietary fiber (Schrama et al., 1998). Furthermore, different characteristics of dietary fiber used in previous studies (i.e., insoluble vs. soluble or highly fermentable vs. poorly fermentable) may lead to the inconsistent results of HP value from pigs.

Many studies have reported increased weight of the gastrointestinal tract with increasing level of dietary fiber (Nyachoti et al., 2000; Agyekum et al., 2012) and a positive correlation between FHP and size or weight of gastrointestinal tract (van Milgen et al., 1998). Consequently, the FHP or NE required for maintenance increases when pigs consume highly fibrous diets. However, in the current study, a similar FHP value was observed among treatments, which may

be a result of a relatively short feeding period. The experimental diets were provided for 3 wk including adaptation, collection, and chamber period, and this experimental period may not be sufficient to cause significant changes in weight and size of visceral organs. Similar results were also observed by Jaworski et al. (2016), who reported that inclusion of 15 or 30% of wheat bran to corn–SBM diets did not affect the FHP of pigs. However, the average FHP value of 1,320 kcal/kg DM observed in the present study is within the range of values for FHP obtained in similar studies conducted in the same facility (Ayoade et al., 2012a; Heo et al., 2014; Velayudhan et al., 2015a) and comparable to that obtained by Noblet et al. (1994) if 0.60 is used as the exponent (179 kcal/$BW^{0.60}$ for 30 kg pigs; equal to 1,378 kcal/kg of DM).

The observed tendency for greater NE:DE and NE:ME ratios in the basal diet than in the other diets might have been due to different energetic efficiencies of dietary components in each experimental diet. Noblet (2007) reported that dietary lipids and starch had greater NE:DE and NE:ME ratios compared with dietary fiber because the energetic efficiency of lipids (90%) and starch (82%) are greater than for dietary fiber (60%). Indeed, the basal diet used in the current study contained less NDF and ADF contents and greater dietary starch concentration compared with the HH, EHH, and HHP diets. The average NE:ME ratio of 0.83 for the 4 experimental diets was similar to the value reported by Velayudhan et al. (2015a). However, this value was slightly higher than that reported by Noblet et al. (1994), who suggested that efficiency of ME utilization for NE was approximately 0.74 to 0.75% for conventional swine diets. This discrepancy could be attributed to differences in genetic background of experimental animals (Kil et al., 2013). For example, Large White barrows were used in the study by Noblet et al. (1994), whereas the current study and that of Velayudhan et al. (2015a) used 3-way-crossbred pigs ([Yorkshire × Landrace] × Duroc). In addition, the genetic improvement in low residual feed intake might have

contributed to the greater NE:ME ratio because pigs have been genetically selected to gain more lean tissue with improved G:F (Patience et al., 2015).

No difference was observed between the determined and predicted NE of test ingredients. The determined NE was, on average, 5.1% greater than the predicted NE, which indicates that prediction equations well predict the NE of ingredients. However, not considering the physical activity of pigs during FHP estimation can explain the less NE of test ingredients obtained with prediction equations compared with the values obtained with the IC method, although they were not significantly different (van Milgen and Noblet, 2003). The NE values obtained with both methods have shown to be similar in some studies (Ayoade et al., 2012a); however, others have reported differences between values obtained with prediction equations and those determined using the IC method (Heo et al., 2014; Velayudhan et al., 2015a). Differences in feeding strategies, experimental conditions, genetics, and BW of pigs, which influence energy expenditure, growth, and body composition, may lead to variation in the NE values of diets and ingredients among experiments (Boisen and Verstegen, 1998). In addition, it should be noted that prediction equations for NE were derived using complete diets, not ingredients (Noblet et al., 1994), which may generate inevitable or inherent variations when energy values calculated from prediction equation and determined using the IC method are compared.

In conclusion, the NE of HH, EHH, and HHP determined using the IC method were 2,375, 2,320, and 2,399 kcal/kg DM, respectively, and these values were 2.9, 7.1, and 5.2% higher, respectively, compared with those obtained with prediction equations (2,308, 2,161, and 2,278 kcal/kg DM, respectively). However, no difference was observed between determined and predicted values, which suggests that prediction equations well predict the NE of ingredients. The HP values observed for the basal diet and the diets containing high dietary fiber in the form

of HH, EHH, or HHP were similar.

CHAPTER 5

MANUSCRIPT II: Digestible, metabolizable, and net energy of camelina cake fed to growing pigs and additivity of energy in mixed diets

J. W. Kim, B. Koo, and C. M. Nyachoti

Department of Animal Science, University of Manitoba, Winnipeg, MB, Canada R3T 2N2

Published: Journal of Animal Science. 2017. 95:4037-4044

doi:10.2527/jas2017.1759

5.1 ABSTRACT

This experiment was conducted to determine the digestible energy (DE), metabolizable energy (ME), and net energy (NE) contents of camelina cake (CC) and to test the hypothesis that dietary glucosinolates originating from CC will affect the additivity of energy in mixed diets containing different inclusion levels of corn, soybean meal (SBM), and CC. A total of 30 growing barrows ([Yorkshire × Landrace] × Duroc) with a mean BW of 16.8 kg (SD 1.4) were randomly allotted to 1 of 5 treatments with 6 replicates per treatment. Pigs were fed experimental diets for 16 d, including 10 d for adaptation and 6 d for total collection of feces and urine. The 5 experimental diets consisted of 3 corn-based diets to determine the DE, ME, and NE of the 3 ingredients (corn, SBM, and CC) and 2 mixed diets to test the additivity of DE, ME, and NE. The corn diet contained 97.52% corn; the SBM diet contained 67.52% corn and 30.0% SBM; the CC diet contained 67.52% corn and 30.0% CC; the Mixed diet 1 contained 67.52% corn, 20.0% SBM, and 10.0% CC; and the Mixed diet 2 contained 67.52% corn, 10.0% SBM, and 20.0% CC. Vitamins and minerals were included in the diets to meet or exceed the requirements for growing pigs (NRC, 2012). Pigs were fed their assigned diets at 550 kcal ME/kg $BW^{0.60}$ per day on the basis of BW on d 1, 5, and 10, which was close to ad libitum intake. Pigs had free access to water. Determined DE, ME, and NE contents of corn were 3,348, 3,254, and 2,579 kcal/kg, respectively; those of SBM were 3,626, 3,405, and 2,129 kcal/kg, respectively; and those of CC were 3,755, 3,465, and 2,383 kcal/kg, respectively. No differences between the predicted and determined DE, ME, and NE were observed in the 2 mixed diets. In conclusion, DE, ME, and calculated NE content of CC fed to growing pigs were 3,755, 3,465, and 2,383 kcal/kg (as-fed basis), respectively. In addition, additivity of DE, ME, and calculated NE was observed in the mixed diets containing corn, SBM, and CC, which indicates that dietary glucosinolates originating from

up to 30% of CC inclusion do not affect DE, ME, and calculated NE of diets.

Key words: additivity, camelina cake, net energy, pig

5.2 INTRODUCTION

The supply of nutritionally adequate feeds in the right amounts to the pig is essential for optimal pig production. To achieve this, accurate estimation and good understanding of the availability of nutrients and energy in each feed ingredient are required. Estimation of the energy value of feed ingredients is especially important because pigs tend to consume feed until their energy requirements are fulfilled and, therefore, the concentration of dietary energy affects the voluntary feed intake of pigs (Nyachoti et al., 2004).

Camelina (*Camelina sativa*), a member of Brassicaceae family, is a fast-growing short-season crop grown in temperate regions for oil production (Kiarie et al., 2016). Camelina cake (CC), a coproduct of the seed crushing industry, generally contains high CP (30%) and fat (10 to 30%) contents (Woyengo et al., 2016). Although CC is a good source of protein and energy for swine diets, there may be a limitation on its utilization due to antinutritional factors such as high glucosinolate concentrations, which may lead to endocrine disturbance and reduced growth performance (Ahlin et al., 1994). Glucosinolate levels should be maintained in a range of 1.5 to 2.0 μmol/g of swine diets to avoid its negative effects on pigs' performance (Tripathi and Mishra, 2007). However, there is a paucity of information about effects of dietary glucosinolates originating from CC on energy values of diets. We hypothesized that levels of dietary glucosinolates originating from different inclusion levels of CC will affect the additivity of energy values in mixed diets containing multiple ingredients. Therefore, the objectives of this

study were to determine the digestible energy (DE), metabolizable energy (ME), and net energy (NE) of CC and to test the hypothesis that dietary glucosinolates originating from CC will affect the additivity of energy values in mixed diets containing multiple ingredients with different concentrations of glucosinolates originating from CC.

5.3 MATERIALS AND METHODS

The experimental protocol used in the present study was reviewed and approved by the University of Manitoba Animal Care Committee, and pigs were cared for according to the guidelines of the Canadian Council on Animal Care (2009).

5.3.1 Experimental Animals and Diets

The CC fed in the current study was obtained from Alberta Agriculture and Forestry (Edmonton, AB, Canada). The camelina seed was from Midas variety grown in Yorktown, SK, Canada, in the summer of 2014. The seed was pressed at Ag Discovery Place, University of Alberta (Edmonton, AB, Canada) using a Reinartz press (model D22941; Reinartz, Bargteheide, Germany). Cake temperature on exiting the press barrel was 55°C.

Thirty growing barrows ([Yorkshire × Landrace] × Duroc) with a mean initial BW of 16.8 kg (SD 1.37) were acquired from the Glenlea Swine Research Unit, University of Manitoba (Winnipeg, MB, Canada). Pigs were housed in adjustable metabolism cages (1.8 by 0.6 m) equipped with a feeder and a nipple drinker in a temperature-controlled room (22 ± 2°C). Five experimental diets, including 3 corn-based diets to determine the DE, ME, and NE content of corn, soybean meal (SBM), and CC and 2 mixed diets containing different inclusion levels of ingredients to test the additivity of DE, ME, and NE, were formulated (Table 5.1). The basal diet

contained 97.52% corn (as-fed basis); the SBM diet contained 67.52% corn and 30.0% SBM (as-fed basis); the CC diet contained 67.52% corn and 30.0% CC (as-fed basis); the Mixed diet 1 contained 67.52% corn, 20.0% SBM, and 10.0% CC (as-fed basis); and the Mixed diet 2 contained 67.52% corn, 10.0% SBM, and 20.0% CC (as-fed basis). Vitamins and minerals were included in the diets to meet or exceed the requirements for growing pigs (NRC, 2012).

Table 5.1. Composition and nutrient content of experimental diets, as-fed basis

	Diet[1]				
Item	Corn	SBM	CC	Mixed diet 1	Mixed diet 2
Ingredients, %					
Corn	97.52	67.52	67.52	67.52	67.52
Soybean meal, 46 % CP	–	30.00	–	20.00	10.00
Camelina cake	–	–	30.00	10.00	20.00
Limestone	1.00	1.00	1.00	1.00	1.00
Monocalcium phosphate	0.70	0.70	0.70	0.70	0.70
Salt	0.50	0.50	0.50	0.50	0.50
Vitamin-mineral premix[2]	0.28	0.28	0.28	0.28	0.28
Analyzed composition					
DM, %	85.0	86.9	87.9	87.4	88.0
GE, kcal/kg	3,729	3,857	4,079	3,932	3,976
CP, %	8.0	20.0	15.6	18.5	16.3
Ether extract, %	3.9	3.4	7.4	4.6	5.2
Starch, %	56.9	40.4	38.5	38.3	37.2
Ash, %	3.0	5.0	4.8	4.7	4.2
NDF, %	7.9	7.7	19.3	9.7	11.9
ADF, %	2.7	3.9	7.0	4.8	5.1
Ca, %	0.51	0.65	0.73	0.59	0.55
P, %	0.46	0.61	0.70	0.66	0.57
Glucosinolates, µmol/g	0.00	0.00	8.40	2.80	5.60

[1]The corn diet contained 97.52% corn; the soybean meal (SBM) diet contained 67.52% corn and 30.0% SBM; the camelina cake (CC) diet contained 67.52% corn and 30.0% CC; the Mixed diet 1 contained 67.52% corn, 20.0% SBM, and 10.0% CC; and the Mixed diet 2 contained 67.52% corn, 10.0% SBM, and 20.0% CC.

[2]Provided the following quantities of vitamins and minerals per kilogram of a complete diet: 2,000 IU vitamin A, 200 IU vitamin D, 40 IU vitamin E, 2 mg vitamin K, 350 mg choline, 14 mg pantothenic acid, 7 mg riboflavin, 1 mg folic acid, 21 mg niacin, 1.5 mg thiamin, 2.5 mg vitamin B6, 70 µg biotin, 20 µg vitamin B12, 10 mg Cu, 110 mg Zn, 120 mg Fe, 10 mg Mn, 0.4 mg I, and 0.3 mg Se.

5.3.2 Experimental Design and Procedure

Pigs were allotted to 1 of 5 experimental diets in a completely randomized design with 6 replicates per diet. Pigs were fed their assigned diets at 550 kcal ME/kg $BW^{0.60}$ per day on the basis of BW on d 1, 5, and 10, which was assumed to be close to ad libitum intake (Noblet et al., 1994). Water was freely available via a low-pressure nipple drinker during the experimental periods. Pigs were fed experimental diets for 16 d, including 10 d for adaptation to the experimental environment and diets and 6 d for total collection. During the last 6 d of the feeding period, total, but separate, collection of feces and urine was conducted as previously described by Kim and Nyachoti (2017). Briefly, 5 g of ferric oxide (as an indigestible marker) was added to 100 g of feed that was fed in the morning on d 11 and d 16 to mark the beginning and the end, respectively, of fecal collections. The remaining portion of the morning feed was offered after all the marked feed was consumed. Feces were collected once daily in the morning, weighed, and stored frozen at −20°C. Urine collection was initiated on d 11 at 0830 h and ceased on d 16 at 0830 h. Urine was also collected once daily in the morning (in jugs containing 20 mL of 3 *N* HCl to minimize N losses). The collected urine was weighed, and a 10% subsample was filtered through glass wool and stored at −20°C.

5.3.3 Sample Preparation and Chemical Analyses

Fecal samples were oven-dried at 50℃ for 5 d, weighed, and pooled on a pig basis. Samples of ingredients and diets were ground through a 1-mm screen in a Wiley mill (Thomas Scientific, Swedesboro, NJ) before chemical analysis, whereas fecal samples were finely ground in a coffee grinder (Smart Grind; Applica Consumer Products, Inc., Miami Lakes, FL). Urine samples were thawed, sieved through cotton gauze, and filtered with glass wool. All samples

were analyzed in duplicate. Diets and ingredients were analyzed for DM, GE, CP, ether extract (EE), starch, ash, NDF, ADF, Ca, and P. Ingredients were also analyzed for AA. The CC sample was further analyzed for glucosinolate concentrations. Fecal and urine samples were analyzed for DM and GE. The GE of urine was determined as previously described by Kim and Nyachoti (2017). Briefly, 2 mL of each sample was mixed with 0.5 g of cellulose and the weight of the resulting mixture was recorded. The urine–cellulose mixture along with a sample of pure cellulose was again dried in an oven at 50°C for 24 h and then weighed for determination of DM of urine. The GE content was then determined on the dried urine–cellulose mixtures and pure cellulose, from which the GE of urine samples were calculated by the difference method.

The DM content was determined according to Association of Official Analytical Chemists (1990) method 925.09 and the GE was measured using an adiabatic bomb calorimeter (model 6400; Parr Instrument Co., Moline, IL), which had been calibrated using benzoic acid as a standard. Nitrogen was determined by the combustion method (method 990.03; AOAC, 1990) using the LECO N analyzer (model CNS-2000; LECO Corp., St. Joseph, MI), and CP was calculated as N × 6.25. The EE content was determined after hexane extraction (method 920.39; AOAC, 1990) in an extraction apparatus. Amino acid contents were determined according to Association of Official Analytical Chemists (1990) method 982.30. Briefly, 100 mg of each sample was digested in 4 mL of 6 *M* HCl for 24 h at 110°C followed by neutralization with 4 mL of 25% (wt/vol) NaOH and was cooled to room temperature. The mixture was then equalized to a 50-mL volume with sodium citrate buffer (pH 2.2) and analyzed using an AA analyzer (Sykam GmbH, Fürstenfeldbruck, Germany). For analysis of sulfur-containing AA (Met and Cys), samples were subjected to performic acid oxidation before acid hydrolysis. Tryptophan was not determined. Samples for analysis of Ca and P were ashed for 12 h and digested according to

Association of Official Analytical Chemists (1990) method 985.01 and read on a Varian inductively coupled plasma mass spectrometer (Varian Inc., Palo Alto, CA). The ADF and NDF contents were determined according to the method of Goering and van Soest (1970). Ash content was determined according to Association of Official Analytical Chemists (1990) method 942.05. Total starch was determined using an assay kit (Megazyme Total Starch assay kit; Megazyme International Ltd, Bray, Co. Wicklow, Ireland). Glucosinolate concentrations were determined using gas–liquid chromatography as described by Slominski and Campbell (1987).

5.3.4 Calculations

The apparent total tract digestibility, apparent energy retention, and DE and ME contents were calculated using standard procedures (Adeola, 2001). The DE and ME in the corn diet were divided by 0.9752 to determine the DE and ME in corn. The contributions of DE and ME from corn to the SBM and CC diets were then calculated and subtracted from the total DE and ME of these diets, and the DE and ME of SBM and CC were calculated by difference (Adeola, 2001). The NE of the diets and ingredients were calculated according to the equations established by Noblet et al. (1994):

$$NE = 0.700 \times DE + 1.61 \times EE + 0.48 \times \text{starch} - 0.91 \times CP - 0.87 \times ADF \text{ and}$$

$$NE = 0.726 \times ME + 1.33 \times EE + 0.39 \times \text{starch} - 0.62 \times CP - 0.83 \times ADF,$$

in which NE, DE, and ME are expressed in kilocalories per kilogram DM; EE is expressed in grams per kilogram DM; ST is starch expressed in grams per kilogram DM; and ADF is expressed in grams per kilogram DM.

Determined DE, ME, and NE of corn, SBM, and CC were used to calculate the predicted DE, ME, and NE contents of the 2 mixed diets according to the following equation:

$DE_P = (DE_C \times \text{inclusion level}_C) + (DE_{SBM} \times \text{inclusion level}_{SBM}) + (DE_{CC} \times \text{inclusion level}_{CC})$,

in which DE_P is the predicted DE of a mixed diet; DE_C, DE_{SBM}, and DE_{CC} are the determined DE of corn, SBM, and CC, respectively (kcal/kg); and inclusion level_C, inclusion level_{SBM}, and inclusion level_{CC} are inclusion level (%) of corn, SBM, and CC in the mixed diet, respectively. The ME and NE of the mixed diets were predicted using the same equation as with DE but with ME and NE replacing DE.

5.3.5 Statistical Analysis

All data were analyzed using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC) with the individual pig as the experimental unit. Homogeneity of the variances among treatments was tested using the UNIVARIATE procedure, and this procedure was also used for identifying outliers, but no outliers were observed. Diet was the fixed effect and replicate was the random effect. The LSMEANS procedure was used to calculate mean values, and the PDIFF option of SAS was used to separate means. The *t* test was applied to test the null hypothesis that the difference between the determined and predicted DE, ME, and NE contents for mixed diets is equal to 0. A probability of $P < 0.05$ was considered significant.

5.4 RESULTS AND DISCUSSION

5.4.1 Chemical Composition

The analyzed composition of ingredients used in the current study is presented in Table 5.2. The chemical composition of corn and SBM is in agreement with the values reported by the NRC (2012) and Kahindi et al. (2014). The CC had GE, CP, and EE contents of 5,046 kcal/kg,

33.2%, and 15.4%, respectively. The Lys, Met, and Thr contents in CC were 1.6, 0.3, and 1.4%, respectively. The most abundant indispensable AA were Arg, Leu, Lys, and Val, whereas the Met and Cys were the least abundant AA. The CP content (33.2%) was within the range of values (31.5 to 35.4%) previously reported (Pekel et al., 2009; Almeida et al., 2013; Kahindi et al., 2014) for CC. However, the EE content of CC (15.4%) was slightly less than the 18.5% reported by Almeida et al. (2013) but greater than the 11.0% reported by Kahindi et al. (2014), which may be explained by the fact that variation in residual oil concentration of the oilseed coproduct is associated with the differences in processing conditions (Kahindi et al., 2014). The GE content of CC (5,046 kcal/kg) was greater than the 4,810 kcal/kg reported by Kahindi et al. (2014) but less than the 5,126 kcal/kg reported by Almeida et al. (2013). The CC containing a high level of EE appears to have greater GE content compared with the CC containing less EE content, and consequently, different DE, ME, and NE of CC are expected from batch to batch. The NDF content of CC (40.4%) was similar to the value (41%) reported by Aziza et al. (2010) and the 34.9% reported by Woyengo et al. (2016) but greater than the 22.0 to 29.2% reported by Almeida et al. (2013) and Kahindi et al. (2014). The dietary fiber level in coproducts of oilseeds varies with seed variety and residual oil content, which is the possible reason for the differences in NDF content among studies. The total glucosinolate concentration in CC was 28.04 µmol/g, most of which were 9-methylsulfinylnonyl (5.77 µmol/g), 10-methylsulfinyldecyl (17.18 µmol/g), and 11-methylsulfinylundecyl (4.15 µmol/g), which was similar to the value (33.7 µmol/g) reported by Kahindi et al. (2014) and within the range (26.6 to 42.3 µmol/g) reported by Almeida et al. (2013). Differences in environmental conditions and the type of soil in which the camelina was grown may lead to variation in glucosinolate concentrations among seeds. Biosynthesis of glucosinolates is closely related to the concentration of sulfur-containing AA in camelina seeds,

Table 5.2. Analyzed nutrient composition of ingredients, as-fed basis

	Ingredient		
Item	Corn	Soybean meal	Camelina cake
DM, %	84.5	90.3	93.0
GE, kcal/kg	3,811	4,261	5,046
CP, %	8.2	46.1	33.2
Ether extract, %	3.8	2.1	15.4
Starch, %	58.3	0.6	2.2
Ash, %	1.1	6.3	4.2
NDF, %	7.6	8.6	40.4
ADF, %	2.4	6.7	20.1
Ca, %	0.01	0.26	0.31
P, %	0.34	0.74	0.82
Indispensable AA, %			
Arg	0.40	3.48	2.72
His	0.26	1.46	0.90
Ile	0.25	1.94	1.14
Leu	1.01	3.75	2.22
Lys	0.27	2.89	1.60
Met	0.11	0.47	0.29
Phe	0.40	2.41	1.32
Thr	0.29	1.82	1.37
Val	0.38	2.07	1.62
Dispensable AA, %			
Ala	0.66	2.30	1.72
Asp	0.58	5.60	2.98
Cys	0.13	0.48	0.41
Glu	1.49	8.41	5.42
Gly	0.30	1.91	1.68
Pro	0.61	1.87	1.45
Ser	0.45	2.67	1.78
Tyr	0.27	1.46	0.86
Total glucosinolates, µmol/g			28.04
9-Methylsulfinylnonyl	–	–	5.77
10-Methylsulfinyldecyl	–	–	17.18
11-Methylsulfinylundecyl	–	–	4.15
Indol	–	–	0.37
Alkenyl	–	–	0.57

which is dependent on the soil (e.g., sulfur concentration) where cultivars are grown (Schuster and Friedt, 1998).

5.4.2 Digestible Energy, ME, and NE of Camelina Cake and Additivity of Energy in Mixed Diets

All pigs remained healthy throughout the experimental period and readily consumed their daily feed allowance. The GE intake and fecal and urinary GE output of the pigs fed the corn diet were less ($P < 0.05$) compared with pigs fed the other diets (Table 5.3). However, pigs fed the corn diet had greater ($P < 0.05$) apparent total tract digestibility of GE and DM and energy retention (%) than pigs fed the CC, Mixed diet 1, and Mixed diet 2 containing different inclusion levels of CC, which may be caused by the increased dietary fiber as a result of the addition of CC in the diets. It has been reported that dietary NDF level is negatively correlated with DM and GE digestibility (Le Goff and Noblet, 2001; Kim and Nyachoti, 2017). However, because of greater GE content in CC, the DE of the corn diet (3,265 kcal/kg) was less ($P < 0.05$) than that of CC (3,387 kcal/kg), whereas ME did not differ among experimental diets. The ME:DE ratio of the corn diet was greater ($P < 0.05$) than that of the CC diet and Mixed diet 2, but the ME:DE ratio of the SBM, CC, Mixed diet 1, and Mixed diet 2 were not different, which is most likely due to less CP content in the corn diet compared with the other diets. It has been suggested that the ME:DE ratio of diets linearly decrease with increasing level of dietary protein (Noblet and van Milgen, 2004; NRC, 2012).

Table 5.3. Apparent total tract digestibility (ATTD) of DM and GE, energy retention, and DE, ME, and NE of experimental diets (as-fed basis)[1]

	Diet[2]						
Item	Corn	SBM	CC	Mixed diet 1	Mixed diet 2	SEM	*P*-value
GE intake, kcal/day	2,352[b]	3,181[a]	2,778[a]	3,045[a]	2,988[a]	101.1	<0.001
Fecal GE output, kcal/day	292[b]	419[a]	470[a]	465[a]	487[a]	19.9	<0.001
Urinary GE output, kcal/day	58[b]	107[a]	102[a]	92[a]	101[a]	7.2	<0.001
ATTD of DM, %	88.5[a]	87.2[ab]	83.5[c]	85.1[bc]	84.4[c]	0.55	<0.001
ATTD of GE, %	87.5[a]	86.8[ab]	83.0[c]	84.7[bc]	83.7[c]	0.60	<0.001
Energy retention, %	85.1[a]	83.4[ab]	79.3[c]	81.7[bc]	80.3[c]	0.63	<0.001
Diet DE, kcal/kg	3,265[b]	3,348[ab]	3,387[a]	3,332[ab]	3,327[ab]	23.4	0.020
Diet DE, kcal/kg DM	3,841	3,853	3,853	3,812	3,780	26.9	0.277
Diet ME, kcal/kg	3,173	3,218	3,236	3,212	3,193	24.7	0.440
Diet ME, kcal/kg DM	3,733	3,704	3,682	3,676	3,628	28.4	0.148
Diet NE[3], kcal/kg	2,507[a]	2,381[c]	2,458[ab]	2,385[c]	2,393[bc]	16.9	<0.001
Diet NE[3], kcal/kg DM	2,959[a]	2,739[b]	2,796[b]	2,728[b]	2,720[b]	19.4	<0.001
ME:DE	0.972[a]	0.961[ab]	0.956[b]	0.964[ab]	0.960[b]	0.0026	0.003
NE:ME	0.790[a]	0.740[d]	0.760[b]	0.742[d]	0.750[c]	0.0010	<0.001

[a–d]Means without a common superscript within a row differ ($P < 0.05$).

[1]Each value represents the mean of 6 replicates per treatment.

[2]Corn = corn diet; SBM = corn + 30% soybean meal; CC = corn + 30% camelina cake; Mixed diet 1 = corn + 20% soybean meal + 10% camelina cake; Mixed diet 2 = corn + 10% soybean meal + 20% camelina cake.

[3]The average of 2 calculated NE from Noblet et al. (1994), where 1) NE = 0.700 × DE + 1.61 × EE + 0.48 × starch – 0.91 × CP − 0.87 × ADF; and 2) NE = 0.726 × ME + 1.33 × EE + 0.39 × starch – 0.62 × CP − 0.83 × ADF; energy is expressed in kcal/kg DM and other nutrients are expressed in g/kg DM.

The corn diet had the greatest ($P < 0.05$) NE content and NE:ME ratio among dietary treatments. The diets containing 20 or 30% CC (i.e., Mixed diet 2 and CC diet) had a greater ($P < 0.05$) NE content and NE:ME ratio than those containing 20 or 30% SBM (i.e., Mixed diet 1 and SBM diet), although the ME content of the diets and the ME:DE ratio were not different. These results are mostly related to the differences in chemical composition among the diets and, therefore, different coefficients of EE, CP, and ADF for NE prediction equation used for calculation (Noblet et al., 1994). Noblet (2007) suggested that dietary lipids and starch have greater NE:DE and NE:ME ratios compared with dietary protein and fiber because energetic efficiency of lipid (90%) and starch (82%) are greater than for protein (60%) and fiber (60%). Indeed, greater EE and less CP content in the Mixed diet 2 and the CC diet compared with the SBM diet and the Mixed diet 1 led to greater dietary NE and efficiency of NE.

Energy values of corn, SBM, and CC fed to growing pigs are presented in Table 5.4. The determined DE, ME, and NE contents of corn were 3,348, 3,254, and 2,579 kg/kg, respectively, and those of SBM were 3,626, 3,405, and 2,129 kcal/kg, respectively. These values are in good accordance with values from the NRC (2012). The GE content of CC was 5,046 kcal/kg and the DE, ME and NE were 3,755, 3,465, and 2,383 kcal/kg, respectively. Kahindi et al. (2014) reported that the DE, ME, and calculated NE contents of CC were 3,882, 3,593, and 2,262 kcal/kg, respectively. These values are slightly greater for DE and ME and less for NE compared with the values determined in the current study. The differences in chemical composition of CC used in the current and previous studies may result in different DE, ME, and NE. The CC used in the present study contained 40.4% NDF and 20.1% ADF, whereas NDF and ADF contents of CC used in the study of Kahindi et al. (2014) were 29.2 and 18.8%, respectively. In addition, the different BW of pigs used in the study of Kahindi et al. (2014) and in the current study (80.2 vs.

16.8 kg) may also contribute to the different energy values of CC because it has been reported that energy digestibility increases with increasing BW (Noblet and van Milgen, 2013). Among the available energy evaluating systems, the NE system provides the most accurate estimate of the dietary energy available to the animal (Velayudhan et al., 2015a). Comparison of energy values in corn, SBM, and CC determined in the current study clearly indicates the superiority of NE system. The SBM and CC had greater ($P < 0.05$) DE content than did corn, whereas no difference was observed among ingredients with the ME system. However, with the NE system, the available energy for the pig was the greatest ($P < 0.05$) for corn and the least ($P < 0.05$) for SBM, with the value for CC being intermediate. Considering that the NE system is superior to the DE and ME systems regarding prediction of actual energy cost required for BW gain, the use of NE system appears to have obvious advantages in optimal pig production with reduced feed costs (Noblet, 2007; Oresanya et al., 2008; Kil et al., 2013). Moreover, this advantage is more apparent with the diet containing several different feed ingredients, especially those high in dietary fiber (Patience and Beaulieu, 2005; de Lange, 2008).

Table 5.4. Digestible, metabolizable, and net energy of corn, soybean meal, and camelina cake fed to growing pigs (as-fed basis)[1]

	Ingredient				
Item	Corn	Soybean meal	Camelina cake	SEM	*P*-value
As-fed basis, kcal/kg					
GE	3,811	4,261	5,046		
DE	3,348[b]	3,626[a]	3,755[a]	57.4	<.001
ME	3,254	3,405	3,465	68.0	0.111
NE[2]	2,579[a]	2,129[c]	2,383[b]	44.1	<.001
DM basis, kcal/kg					
GE	4,511	4,721	5,426		
DE	3,963	4,017	4,038	62.3	0.686
ME	3,852	3,772	3,726	73.9	0.493
NE[2]	3,053[a]	2,359[c]	2,562[b]	47.9	<.001

[a–c]Means within a row with different superscripts differ ($P < 0.05$).

[1]Each value represents the mean of 6 observations.

[2]The average of 2 calculated NE from Noblet et al. (1994), where 1) NE = 0.700 × DE + 1.61 × EE + 0.48 × starch – 0.91 × CP − 0.87 × ADF; and 2) NE = 0.726 × ME + 1.33 × EE + 0.39 × starch – 0.62 × CP − 0.83 × ADF; energy is expressed in kilocalories per kilogram DM and other nutrients are expressed in grams per kilogram DM.

Camelina seed and its coproducts such as camelina meal and cake can be a potential alternative feed ingredient for swine diets to supply dietary energy due to its high fat content. However, inclusion of these ingredients in animal feeds may be limited due to some secondary plant metabolites called glucosinolates, which possibly reduce palatability of diet and growth performance of animals, especially monogastric animals (Tripathi and Mishra, 2007; Pekel et al., 2009). The detrimental effects of dietary glucosinolates on the performance of animals are most likely related to the drastic endocrine disturbance induced by glucosinolates (Ahlin et al., 1994). Therefore, it has been suggested that the levels of glucosinolates should be maintained in a range of 1.5 to 2.0 µmol/g of swine diets to avoid its negative effect on performance of pigs (Tripathi and Mishra, 2007; Canola Council of Canada, 2015). According to this safety inclusion level, we hypothesized that energy values determined with 30% of CC would be underestimated because of the high level of dietary glucosinolates in the test diet and, therefore, would not show additivity of energy in the mixed diets containing 10 or 20% of CC, which, therefore, have less glucosinolates (calculated glucosinolate concentrations in the diets containing 10, 20, and 30% of CC were 2.8, 5.6, and 8.4 µmol/g, respectively). Contrary to our hypothesis, however, no differences between the predicted and determined DE, ME, and NE were observed in the 2 mixed diets (Table 5.5). Differences between predicted and determined DE, ME, and NE values for the Mixed diet 1 were 29, 13, and 20 kcal/kg (as-fed basis), respectively. Respective values for the Mixed diet 2 were 47, 38, and 38 kcal/kg (as-fed basis). These results clearly show the additivity of DE, ME, and NE of corn, SBM, and CC in the mixed diets containing 2 different concentrations of dietary glucosinolates originating from CC. Matthäus and Zubr (2000) have suggested that enzymatic hydrolysis of the glucosinolates in camelina seed may produce only nonvolatile isothiocyanates because of long carbon chains in its residue. In addition to this,

camelina does not contain progoitrin, which can be converted into toxic goitrin. Therefore, the adverse effect of glucosinolates in CC may be less than that in rapeseed and rapeseed coproducts (Matthäus and Zubr, 2000). However, it should be noted that the NE values of experimental diets and ingredients were predicted from prediction equations using DE and ME values rather than direct determination of NE using indirect calorimetry for measurement of heat production (HP) by pigs. Dietary glucosinolates may affect HP by increasing production of thyroid hormones, which are involved in energy metabolism and HP within body, and metabolic activity in the liver and kidneys for detoxification of glucosinolates, which possibly affect the NE values of feed ingredients and diets and, therefore, additivity of NE values in mixed diets containing different level of dietary glucosinolates. Therefore, further study will be required to determine the effect of dietary glucosinolates originating from CC on HP of pigs for testing additivity of NE in mixed diets containing CC.

In conclusion, DE, ME, and calculated NE content of CC fed to growing pigs were 3,755, 3,465, and 2,383 kcal/kg (as-fed basis), respectively. In addition, additivity of DE, ME, and calculated NE was observed in the mixed diets containing corn, SBM, and CC, which indicates that dietary glucosinolates originating from up to 30% of CC inclusion do not affect DE, ME, and calculated NE of diets.

Table 5.5. Determined and predicted DE, ME, and NE contents of mixed diets containing different inclusion level of corn, soybean meal, and camelina cake, as-fed basis[1]

	Mixed diet 1[2]				Mixed diet 2[2]			
Item	Determined	Predicted	Difference[3]	SE	Determined	Predicted	Difference[3]	SE
Energy content, kcal/kg								
GE	3,932	3,930	2		3,976	4,008	-32	
DE	3,332	3,361	-29	23	3,327	3,374	-47	26
ME	3,212	3,225	-13	20	3,193	3,231	-38	29
NE[4]	2,385	2,405	-20	15	2,393	2,431	-38	19

[1]Each value represents the mean of 6 observations.

[2]Mixed diet 1 = corn + 20% soybean meal + 10% camelina cake; Mixed diet 2 = corn + 10% soybean meal + 20% camelina cake.

[3]Difference is calculated by subtracting predicted energy values from determined values.

[4]The average of 2 calculated NE from Noblet et al. (1994), where 1) NE = 0.700 × DE + 1.61 × EE + 0.48 × starch – 0.91 × CP − 0.87 × ADF; and 2) NE = 0.726 × ME + 1.33 × EE + 0.39 × starch – 0.62 × CP − 0.83 × ADF; energy is expressed in kilocalories per kilogram DM and other nutrients are expressed in grams per kilogram DM.

CHAPTER 6

MANUSCRIPT III: Net energy content of canola meal fed to growing pigs and effect of experimental methodology on energy values

J. W. Kim, B. Koo, and C. M. Nyachoti

Department of Animal Science, University of Manitoba, Winnipeg, MB, Canada R3T 2N2

Published: Journal of Animal Science. 2018.96:1441-1452

doi:10.1093/jas/sky039

6.1 ABSTRACT

An experiment was conducted to determine the digestible energy (DE), metabolizable energy (ME), and net energy (NE) contents of canola meal (CM) and to investigate the effects of basal diet [corn diet vs. corn-soybean meal (SBM) diet] and methodology (difference method vs. regression method) on energy values of CM. Thirty-six growing barrows (20.8 ± 1.0 kg initial body weight [BW]) were individually housed in metabolism crates and randomly allotted to one of six dietary treatments to give six replicates per treatment. The six experimental diets included a corn diet, a corn-SBM diet, a corn diet with 15 or 30% of CM, and a corn-SBM diet with 15 or 30% of CM. The DE, ME, and NE of CM were determined using the corn diet or the corn-SBM diet as a basal diet. In each basal diet, two additional diets containing 15 or 30% of CM were formulated to compare the determined energy values by the difference method and estimated energy values from the regression method. Feeding level was set at 550 kcal ME/kg $BW^{0.6}$ per day. Pigs were fed experimental diets for 16 d including 10 d for adaptation and 6 d for total collection of feces and urine. Pigs were then moved into indirect calorimetry chambers to determine 24 h heat production (HP) and 12 h fasting HP. The DE, ME, and NE of CM determined by the difference method were within the 95% confidence intervals estimated for the DE, ME, and NE of CM by the regression method regardless of the basal diets used, which indicates that the difference and regression methods give equivalent DE, ME, and NE of CM. However, when the goodness of fit for the linear model was compared, the r^2 of the regression analysis from the corn-SBM diet (0.78) was relatively greater than that from corn diet (0.40). The estimated NE of CM by the prediction equations generated by either the corn diet or corn-SBM diets were 2,096 kcal/kg and 1,960 kcal/kg (as-fed basis), respectively, whereas those values determined by the difference method were 2,233 kcal/kg and 2,106 kcal/kg (as-fed basis),

respectively. In conclusion, the NE of CM determined in the current study was, on average, 2,099 kcal/kg (as-fed basis). The difference and regression methods do not give different NE value of CM fed to growing pigs. Although the NE values of CM determined using either the corn diet or the corn-SBM diet were not different, the greater r^2 of the regression analysis from the corn-SBM diet than that from the corn diet suggests that the corn-SBM diet is a more appropriate basal diet for NE determination of ingredients.

Key words: canola meal, heat production, indirect calorimetry, net energy, pig

6.2 INTRODUCTION

Energy values of fibrous or high protein ingredients are often overestimated by digestible energy (DE) and metabolizable energy (ME) systems (Noblet et al., 1994). Canola meal (CM), a coproduct of the canola seed crushing industry, is widely used as a protein source but also contains relatively high levels of fiber (Bell, 1993). Therefore, the energy value of CM needs to be evaluated using net energy (NE) system to provide a more accurate estimate of the energy available for pigs (Noblet, 2007). However, there is limited information on NE value of CM fed to growing pigs.

The regression method and prediction equations are most often used to determine NE of ingredients (Noblet et al., 1993a). Recently, however, several studies reported that the difference and regression methods, and the prediction equations give equivalent NE of ingredients (Heo et al., 2014; Jaworski et al., 2016; Kim and Nyachoti, 2017). In addition, balanced diets that meet nutrient requirements of pigs for supporting optimal growth and lean tissue gain have been suggested for determination of NE of diets (Noblet et al., 1993). For this reason, a corn diet, the

commonly used basal diet for DE and ME determination, may be inappropriate for NE determination due to insufficient nutrients for pigs (Noblet and van Milgen, 2013), whereas, a corn-soybean meal (SBM) diet that meets the nutrient requirements appears to be more suitable as a basal diet. However, no difference in NE of SBM determined using the corn basal and corn-SBM basal diets has been reported (Liu et al., 2014) and the effect of basal diet needs to be investigated further. To our knowledge, NE values of CM determined by the difference method and the regression method using different basal diets have not been compared yet.

Therefore, the first objective of this experiment was to determine DE, ME, and NE of CM. The second objective was to investigate the effects of basal diet (corn diet vs. corn-SBM diet) and methodology (difference method vs. regression method) on energy values of CM.

6.3 MATERIALS AND METHODS

The experimental protocol used in the current study was reviewed and approved by the University of Manitoba Animal Care Committee, and pigs were handled in accordance with the guidelines described by the Canadian Council on Animal Care (2009).

6.3.1 Animals, Housing, and Experimental Diets

Thirty-six growing barrows [(Yorkshire × Landrace) × Duroc] with an average initial BW of 20.8 ± 1.0 kg (mean ± SD) were obtained from the Glenlea Swine Research Unit, University of Manitoba. All pigs were individually housed in adjustable metabolism crates (1.8 × 0.6 m) in a temperature-controlled room (22 ± 1℃) throughout the experiment. The metabolism crates had smooth transparent plastic sides and plastic-covered expanded metal sheet flooring with a screen underneath for fecal collection and a stainless steel urine tray underneath

the fecal screen, which allowed for the total, but separate, collection of feces and urine from each pig.

Corn, SBM, and CM used in this experiment were acquired from the Glenlea Research Station, University of Manitoba (Table 6.1). Six experimental diets, including a corn diet, a corn-SBM diet, a corn diet with 15 or 30% of CM, and a corn-SBM diet with 15 or 30% of CM were formulated (Table 6.2). The DE, ME, and NE of CM were determined using the corn diet or the corn-SBM diet as a basal diet. In each basal diet (i.e., corn diet, corn-SBM diet), two additional diets containing 15 or 30% of CM were formulated to compare the determined energy values using the difference method and estimated energy values from the regression method. To formulate the diets containing 15 or 30% of CM in a corn diet, 15 or 30% of corn was replaced with the same amount of CM, whereas two additional diets were formulated by mixing 15 or 30% CM with 85 or 70% of the corn-SBM diet, respectively. All experimental diets were supplemented with vitamins and minerals to meet or exceed the requirements of growing pigs (NRC, 2012). The corn-SBM diet was over-formulated compared to the expected nutrient requirements for 25–50 kg pigs to ensure that the corn-SBM diets containing 15 or 30% of CM met current requirement estimates for standardized ileal digestible indispensable AA, standardized total tract digestible P, vitamins, and minerals (NRC, 2012).

Table 6.1. Analyzed nutrient composition of ingredients (as-fed basis)

	Ingredients		
Item	Corn	Soybean meal	Canola meal
DM, %	85.9	88.3	90.9
GE, kcal/kg	3,870	4,129	4,346
CP, %	8.1	44.9	37.8
Ether extract, %	3.2	1.4	2.6
Starch, %	65.5	1.0	0.9
NDF, %	8.6	6.9	24.3
ADF, %	3.1	6.6	19.1
Ca, %	0.01	0.48	0.66
P, %	0.30	0.70	1.13

Table 6.2. Ingredient composition and calculated and analyzed nutrient composition of experimental diets (as-fed basis)

	Corn-basal diet[1]			Corn-SBM-basal diet[1]		
Item	Basal	15% CM	30% CM	Basal	15% CM	30% CM
Ingredients, %						
Corn	97.160	82.160	67.160	68.460	58.191	47.922
Soybean meal	-	-	-	28.200	23.970	19.740
Canola meal	-	15.000	30.000	-	15.000	30.000
Vegetable oil	-	-	-	1.000	0.850	0.700
Lys-HCl	-	-	-	0.100	0.085	0.070
Limestone	1.000	1.000	1.000	1.000	0.850	0.700
Monocalcium phosphate	1.300	1.300	1.300	0.700	0.595	0.490
Salt	0.400	0.400	0.400	0.400	0.340	0.280
Vitamin-mineral premix[2]	0.140	0.140	0.140	0.140	0.119	0.098
Calculated composition[3]						
ME, kcal/kg	3,327	3,265	3,203	3,386	3,330	3,274
SID[4] Lys, %	0.195	0.396	0.596	0.925	1.017	1.109
SID[4] Met, %	0.146	0.214	0.281	0.259	0.310	0.361
Analyzed composition						
DM, %	86.4	86.8	87.1	87.0	87.2	87.4
GE, kcal/kg	3,760	3,821	3,883	3,905	3,942	3,996
CP, %	7.8	12.1	16.3	18.7	20.9	23.7
Ether extract, %	3.0	3.0	2.8	3.3	3.1	3.0
Starch, %	60.7	51.6	40.3	43.0	37.7	31.5
NDF, %	8.2	10.6	12.4	8.5	10.0	12.4
ADF, %	3.1	5.3	7.5	3.9	5.5	8.2
Ca, %	0.63	0.54	0.70	0.53	0.60	0.57
P, %	0.54	0.64	0.77	0.50	0.63	0.71

[1]SBM = soybean meal; CM = canola meal.

[2]Supplied the following per kilogram of finished feed: vitamin A, 2,000 IU; vitamin D, 200 IU; vitamin E, 40 IU; vitamin K, 2 mg; choline, 350 mg; pantothenic acid, 14 mg; riboflavin, 7 mg; folic acid, 1 mg; niacin, 21 mg; thiamin, 1.5 mg; vitamin B_6, 2.5 mg; biotin, 70 µg; vitamin B_{12}, 20 µg; Cu, 10 mg; Zn, 110 mg; Fe, 120 mg; Mn, 10 mg; I, 0.4 mg; Se, 0.3 mg.

[3]Calculated from NRC (2012) values.

[4]SID = standardized ileal digestible.

6.3.2 Experimental Design and Procedure

This experiment was conducted in two consecutive periods (18 pigs per period) using the same facility and similar experimental conditions and procedures because only 3 indirect calorimetry chambers were available for measuring heat production (HP) of pigs at the same time. Pigs were assigned to one of six experimental diets in a completely randomized design with three replicates per diet (per period).

Feed intake was set at 550 kcal ME/kg $BW^{0.60}$ per day based on BW of pigs on days 1, 5, and 10, which has been reported to be close to ad libitum intake (Noblet et al., 1994). During the experiment, pigs were fed at 0800 h once daily and trained to consume their daily feed allowance within 1 h after feeding. Pigs were given ad libitum access to water via a low-pressure nipple drinker throughout the experimental period. Each experimental period lasted 16 d with the initial 10 d being the adaptation period to the experimental conditions and diets. During the last 6 d of each experimental period, total, but separate, fecal and urine collections were conducted for the determination of DE and ME as previously described by Kim and Nyachoti (2017). From day 11 to 16, feces were collected once daily in the morning, weighed, and stored at −20°C. Urine collection commenced on day 11 at 0800 h and terminated on day 16 at 0800 h. Urine was also collected once daily in the morning (in jugs containing 20 mL of 3 *N* HCl to minimize nitrogen losses). The collected urine was weighed and a 5% subsample was filtered through glass wool, and stored at −20°C.

Before and after each period, the accuracy of indirect calorimetry chambers was validated by burning ethanol in a chamber system, based on the stoichiometric equation of ethanol burning. The ratio of CO_2 production to O_2 consumption in the burning ethanol yields a respiratory quotient (RQ) of 0.667 (Benedict and Tompkins, 1916). Acceptable range of RQ was

set from 0.640 up to 0.690 and no accuracy problem was observed during the two experimental periods. On day 16, three pigs each were transferred to the indirect calorimetry chambers (1.22 × 0.61 × 0.91 m; Columbus Instruments, Columbus, OH) from the metabolic crates to measure HP and fasting HP (FHP) based on O_2 consumption, CO_2 production, and urinary nitrogen (N) excretion. Pigs were moved into the calorimetric chambers within 1 h after daily feed allowance was provided and all consumed, and HP was measured continuously for 24 h (fed-state) followed by 12 h (fasting-state) of FHP measurement. The next sets of three pigs were moved to the indirect calorimetric chambers every 2 days (days 18, 20, 22, 24, and 26). Pigs were allotted to one of three indirect calorimetry chambers in a randomized complete block design using the chamber as a blocking factor to avoid possible confounding effects. Pigs had free access to fresh water via a nipple drinker in the chambers, and urine voided during the HP and FHP measurement was collected separately, weighed, subsampled, and stored at −20°C. The experimental temperature inside the chamber was maintained at 22℃ ± 1℃. Personnel movement in the chamber room was limited, except for the regular check and urine collection, to measure HP and FHP under calm conditions. After the day 16 (at the end of collection day), pigs were fed their assigned experimental diets until they were transferred into the indirect calorimetry chambers.

6.3.3 Sample Preparation and Chemical Analyses

Diet and ingredients samples were ground through a 1-mm screen in a Thomas Wiley mill model 4 (Labwrench, Midland, ON, Canada) and thoroughly mixed before chemical analysis. Fecal samples were dried in a forced-air drying oven at 60℃ for 5 days and finely ground before chemical analysis. Urine samples from metabolism crates and indirect calorimetry

chambers were thawed and pooled separately for each pig, filtered through glass wool, and transferred into a plastic bottle. Diet and ingredient samples were analyzed in duplicate for dry matter (DM), gross energy (GE), crude protein (CP; N × 6.25), ether extract (EE), starch, ash, neutral detergent fiber (NDF), acid detergent fiber (ADF), calcium (Ca), and phosphorus (P), whereas fecal and urine samples were analyzed in duplicate for DM, GE, and CP. The DM content was determined according to AOAC (method 934.01; 2006) and the GE was estimated using an adiabatic bomb calorimeter (model 6400; Parr Instrument, Moline, IL), which had been calibrated using benzoic acid as a standard. Nitrogen concentration was measured by the combustion method (method 990.03; AOAC, 2006) using the LECO N analyzer (model CNS2000; LECO Corp., St. Joseph, MI), and CP was calculated as N × 6.25. Ether extract was determined after hexane extraction (method 920.39A; AOAC, 2006) in an extraction apparatus. The ADF and NDF contents were determined according to the method of Goering and Van Soest (1970). Ash content was determined according to AOAC (method 942.05; 2006). To analyze Ca and P, ashed sample was digested according to AOAC (method 985.01; 2006) and read on a Varian inductively coupled plasma mass spectrometer (Varian Inc., Palo Alto, CA). Starch content was determined using an assay kit (Megazyme Total Starch assay kit; Megazyme International Ltd, Wicklow, Ireland).

To determine the GE of urine, 0.5 g of cellulose was dried at 103℃ for 24 h and 2 mL of urine sample were mixed with it, and the final weight of the resulting mixture was recorded. The urine-cellulose mixture along with a sample of pure cellulose were dried in an oven at 50℃ for 24 h and then weighed to estimate DM content of urine. The GE of the dried urine-cellulose mixture and cellulose itself were determined using an adiabatic bomb calorimeter as described above, from which the GE of urine samples were calculated by the difference method (Fleischer

et al., 1981).

6.3.4 Calculations

The apparent total tract digestibility (ATTD) of DM, GE, and CP was calculated as described by Woyengo et al. (2010) and N balance of pigs was calculated according to Kim and Nyachoti (2017).

The HP and FHP (Brouwer, 1965), retained energy (RE; Noblet et al., 1994), and NE values (Noblet et al., 1994) were calculated using the following equations:

$$HP = 3.866 \times O_2 + 1.200 \times CO_2 - 1.431 \times \text{urinary N excretion},$$

where HP is in kilocalories, O_2 is oxygen consumption in liters, CO_2 is carbon dioxide production in liters, and urinary N excretion is total urinary N excretion in grams. The FHP was also calculated using the same equation for HP.

$$RE = ME - HP,$$

where RE, ME, and HP are in kilocalories per day.

Retained energy as protein (RE_P) was calculated as N retention (g) × 6.25 × 5.68 (kcal/g) according to Ewan (2001). Retained energy as lipid (RE_L) was calculated as the difference between RE and RE_P (Labussière et al., 2009).

$$NE = (RE + FHP)/DMI,$$

where NE is in kilocalories per kilogram DM, RE and FHP are in kilocalories per day, and DMI is in kilograms.

The RQ was calculated with the ratio of CO_2 production to O_2 consumption (Noblet et al., 2001).

After the energy values of each experimental diet were determined, the DE, ME, and NE

of CM were calculated using the difference method (Adeola, 2001). The DE, ME, and NE in the corn diet were divided by 0.9716 to determine the DE, ME, and NE of corn. The contributions of DE, ME, and NE from corn to the diet containing 15 or 30% of CM were then calculated and subtracted from the total DE, ME, and NE of these diets, and the DE, ME, and NE of CM were calculated by difference (Adeola, 2001). However, DE, ME, and NE of CM determined by the corn-SBM diet was calculated by subtracting the energy contribution of the corn-SBM diet from the energy content of the diets containing 15 or 30% of CM (Adeola, 2001).

6.3.5 Statistical Analysis

Homogeneity of variances was verified using the UNIVARIATE procedure (SAS Inst. Inc., Cary, NC). No outlier was observed for all measurements. All data were analyzed using the MIXED procedure of SAS. The individual pig was considered the experimental unit. The initial model included diet and period as fixed effect, and pig as random effect for statistical analysis. However, the effect of period was not significant in this study; therefore, it was excluded in the final model. The LSMEANS procedure was used to calculate mean values and the PDIFF option was used to separate means. Orthogonal polynomial contrasts were used to determine linear and quadratic effects of inclusion level of CM. Regression equations to estimate the DE, ME, and NE of CM were generated using the REG procedure in SAS (Noblet et al., 1993a). The dependent variables in the three prediction equations (in each basal diet) were dietary DE, ME, or NE, kcal/kg (as-fed basis), respectively, and the independent variable was CM inclusion in the diet, % (as-fed basis). The DE, ME, and NE of CM were then predicted by solving the regression equations with 100% of CM inclusion. The energy values of CM determined by either the difference or regression method was compared according to Jaworski et al. (2016). The CLB and

AIC statement in SAS were used to determine the 95% confidence levels and Akaike's information criterion for the regression coefficients used to estimate the DE, ME, and NE of CM, respectively. The DE, ME, and NE of CM determined by the difference method was considered not different from the respective values of CM estimated by the regression method if the values were within the 95% confidence interval for the DE, ME, and NE of CM estimated by the regression method. Probability of $P < 0.05$ was considered significant, whereas $0.05 < P \leq 0.10$ was considered a tendency.

6.4 RESULTS

All pigs adapted well to their experimental diets and conditions, remained healthy and readily consumed their daily feed allowance throughout the experimental period.

6.4.1 Energy and Nutrient Digestibility and Nitrogen Balance

The ATTD of DM and GE decreased (linear, $P < 0.01$) with increasing inclusion level of CM in the corn and corn-SBM diets (Table 6.3). Also, decreased (linear, $P < 0.01$) ATTD of CP was observed for the corn-SBM diets as inclusion level of CM increased. However, inclusion of CM did not affect the ATTD of CP in the corn diet. The N intake (g), fecal N excretion (g), retained N (g) by pigs linearly increased ($P < 0.01$) as CM inclusion increased in the corn and corn-SBM diets (Table 6.3). Urinary N excretion (g) linearly increased ($P < 0.05$) for pigs fed corn diets containing increasing inclusion level of CM, but no difference was observed in pigs fed corn-SBM diets with increasing inclusion level of CM. Retained N (%) by pigs fed the corn diets linearly increased ($P < 0.01$) as CM inclusion increased in the diets. There was a tendency for a quadratic decrease ($P \leq 0.10$) in the retained N (%) as CM inclusion increased in the corn

and corn-SBM diets.

6.4.2 Energy Balance and Energy Values of Experimental Diets

The energy balance and DE, ME, and NE of experimental diets are presented in Table 6.4. The DE and ME of the corn and corn-SBM diets decreased (linear, $P < 0.01$) as dietary inclusion level of CM increased. The HP (kcal/kg $BW^{0.6}$) of pigs fed the corn diets increased (linear, $P < 0.01$), whereas that of pigs fed the corn-SBM diets was not affected by increasing dietary inclusion level of CM. Fasting HP of pigs fed the corn and corn-SBM diets was not affected by increasing CM content in the diets. Total RE tended to decrease (linear, $P \leq 0.10$) from 2,154 to 2,176 and 1,922 kcal/kg DM with increasing CM content in the corn diets, likewise, that of pigs decreased (linear, $P < 0.01$) from 2,320 to 2,147 and 1,946 kcal/kg DM with increasing CM in the corn-SBM diets. The RE_P increased (linear, $P < 0.01$), whereas RE_L decreased (linear, $P < 0.01$) as increasing CM was included in the corn and corn-SBM diets. The NE of diets decreased (linear, $P < 0.01$) from 3,131 to 3,034 and 2,894 kcal/kg with increasing inclusion level of CM in the corn diets, whereas NE of diets decreased (linear, $P < 0.01$) as CM inclusion increased in the corn-SBM diets (3,293, 3,185, and 2,962 kcal/kg DM for diets containing 0, 15, and 30% CM, respectively). The fed-state RQ of pigs fed the corn diets tended to increase (linear, $P \leq 0.10$), but a linear decrease ($P < 0.01$) was observed for pigs fed the corn-SBM diets with increasing inclusion level of CM. The NE:DE and NE:ME were not affected by CM inclusion, but the ME:DE decreased (linear, $P < 0.01$) as CM inclusion increased in the corn diets. However, the NE:DE and NE:ME decreased (linear, $P < 0.05$) but the ME:DE tended to decrease (linear, $P \leq 0.10$) as CM inclusion increased in the corn-SBM diets.

6.4.3 Energy Values of Corn and Canola Meal

The DE, ME, and NE of corn fed to growing pigs were 3,406, 3,275, 2,845 kcal/kg, as-fed basis, respectively (Table 6.5). Linear regression analyses were used to estimate the relationship between energy contents and dietary CM. The prediction equations estimated using the corn diet are presented in Table 6.6. The intercept, slope, and r^2 of the prediction equation for dietary DE were 3,311 ($P < 0.01$), −6.0 ($P < 0.01$), and 0.87, whereas respective values for dietary ME were 3,186 ($P < 0.01$), −6.7 ($P < 0.01$), and 0.87 respectively. The prediction equation for dietary NE had an intercept equal to 2,711 ($P < 0.01$) and a slope estimate of −6.2 ($P < 0.01$) with r^2 of 0.40. The equations derived using the corn-SBM diet are also presented in Table 6.6. The intercept, slope, and r2 of the prediction equation for dietary DE were 3,475 ($P < 0.01$), −7.6 ($P < 0.01$), and 0.80, whereas respective values for dietary ME were 3,316 ($P < 0.01$), −7.7 ($P < 0.01$), and 0.81 respectively. The prediction equation for dietary NE had an intercept equal to 2,882 ($P < 0.01$) and a slope estimate of −9.2 ($P < 0.01$) with r^2 of 0.78. The estimated DE, ME, and NE of CM by the prediction equations generated by either the corn diet or corn-SBM diets were 2,709, 2,515, and 2,096 kcal/kg or 2,717, 2,543, and 1,960 kcal/kg (as-fed basis), respectively (Table 6.7), whereas those values determined by the difference method were 2,818, 2,646, and 2,233 kcal/kg or 2,782, 2,629, and 2,106 kcal/kg (as-fed basis), respectively. There were, however, no differences in the energy contents of CM fed to growing pigs when determined using either the corn diet or corn-SBM diet (Table 6.8).

Table 6.3. Apparent total tract digestibility (ATTD) of DM, GE, and CP and N balance of experimental diets fed to growing pigs[1]

Item	Corn-based diet[2]				*P*-value		Corn-SBM-based diet[2]				*P*-value	
	Basal	15% CM	30% CM	SEM	Linear	Quadratic	Basal	15% CM	30% CM	SEM	Linear	Quadratic
ATTD, %												
DM	89.4	85.7	81.6	0.29	<0.001	0.699	89.7	86.4	81.9	0.47	<0.001	0.289
GE	88.0	84.4	80.6	0.33	<0.001	0.883	88.8	85.6	81.1	0.51	<0.001	0.285
CP	77.6	76.7	75.8	0.84	0.147	0.991	87.9	85.2	80.8	0.87	<0.001	0.416
N balance, g/d												
N intake	13.0	20.8	29.0	0.32	<0.001	0.577	31.1	37.1	42.8	0.57	<0.001	0.860
Fecal excretion	2.9	4.8	7.0	0.18	<0.001	0.582	3.8	5.5	8.2	0.38	<0.001	0.282
Urinary excretion	6.1	6.5	8.2	0.64	0.042	0.395	10.4	9.5	12.1	0.97	0.246	0.168
N retained	4.0	9.5	13.8	0.56	<0.001	0.402	17.0	22.1	22.6	1.06	0.002	0.088
N retained, %	30.2	45.7	47.8	3.02	<0.001	0.090	54.4	59.7	52.7	2.69	0.661	0.083

[1]Each value represents the mean of 6 observations.
[2]SBM = soybean meal; CM = canola meal.

Table 6.4. Energy balance in growing pigs and energy values of experimental diets[1]

Item	Corn-based diet[2]				*P*-value		Corn-SBM-based diet[2]				*P*-value	
	Basal	15% CM	30% CM	SEM	Linear	Quadratic	Basal	15% CM	30% CM	SEM	Linear	Quadratic
Energy value, kcal/kg DM												
DE	3,831	3,716	3,593	14.4	<0.001	0.831	3,984	3,872	3,707	23.1	<0.001	0.356
ME	3,683	3,566	3,422	15.9	<0.001	0.479	3,799	3,693	3,517	22.7	<0.001	0.224
HP[3]	1,529	1,391	1,500	72.4	0.780	0.185	1,479	1,546	1,571	60.6	0.297	0.782
HP[4], kcal/kg $BW^{0.6}$	138	158	165	5.5	0.003	0.356	166	168	169	4.6	0.646	0.943
Total RE[5]	2,154	2,176	1,922	78.2	0.053	0.172	2,320	2,147	1,946	67.2	0.001	0.864
As protein	140	337	491	19.8	<0.001	0.402	602	786	801	37.7	0.002	0.088
As lipid	2,014	1,839	1,431	76.3	<0.001	0.231	1,718	1,361	1,144	71.8	<0.001	0.439
FHP[6]	977	858	971	49.9	0.943	0.077	973	1,038	1,016	56.8	0.595	0.543
NE[7]	3,131	3,034	2,894	47.2	0.003	0.717	3,293	3,185	2,962	28.0	<0.001	0.114
Respiratory quotient												
Fed state	0.98	1.08	1.05	0.024	0.052	0.068	1.02	1.00	0.99	0.008	0.004	0.814
Fasting state	0.75	0.78	0.75	0.015	0.885	0.219	0.74	0.74	0.73	0.005	0.772	0.164
Energy utilization												
ME/DE	0.961	0.960	0.952	0.0021	0.009	0.268	0.953	0.954	0.949	0.0016	0.056	0.213
NE/DE	0.817	0.816	0.805	0.0114	0.474	0.725	0.826	0.823	0.799	0.0067	0.011	0.254
NE/ME	0.850	0.851	0.846	0.0116	0.788	0.853	0.867	0.863	0.842	0.0069	0.025	0.366

[1]Each value represents the mean of 6 observations.

[2]SBM = soybean meal; CM = canola meal.

[3]Heat production = $(3.87 \times O_2 + 1.20 \times CO_2 - 1.43 \times$ urinary N)/DMI.

[4]Heat production = Daily HP, kcal/kg $BW^{0.6}$.

[5]Retained energy = (ME intake − HP)/DMI; Retained energy as protein was calculated according to Ewan (2001) as N retention (g) × 6.25 × 5.68 (kcal/g). Whereas, retained energy as lipid was calculated as the difference between total retained energy and retained energy as protein.

[6]Fasting heat production = (3.87 × O_2 + 1.20 × CO_2 − 1.43 × urinary N)/DMI.
[7]Net energy = (RE + FHP)/DMI.

Table 6.5. Energy content of corn fed to growing pigs determined using a corn-basal diet[1]

	Corn	
Item	Mean	Standard deviation
As-fed basis		
DE, kcal/kg	3,406	29.0
ME, kcal/kg	3,275	19.8
NE, kcal/kg	2,845	66.9
DM basis		
DE, kcal/kg	3,965	33.8
ME, kcal/kg	3,812	23.0
NE, kcal/kg	3,241	77.9

[1]Each value represents the mean of 6 observations.

Table 6.6. Regression coefficient used for estimating DE, ME, and NE (kcal/kg) of canola meal (CM) from two different basal diets (as-fed basis)[1]

Dependent variable	Prediction equation	SE		*P*-value		r^2	RMSE	AIC[2]
		Intercept	Slope	Intercept	Slope			
Corn-based basal diet								
Dietary DE	3310.9 − 6.022 × (CM inclusion, %)	11.03	0.57	<0.001	<0.001	0.87	29.6	123
Dietary ME	3186.0 − 6.711 × (CM inclusion, %)	12.46	0.64	<0.001	<0.001	0.87	33.4	128
Dietary NE	2711.1 − 6.150 × (CM inclusion, %)	36.40	1.88	<0.001	0.005	0.40	97.7	167
Corn-SBM-based basal diet[3]								
Dietary DE	3475.0 – 7.583 × (CM inclusion, %)	18.34	0.95	<0.001	<0.001	0.80	49.2	142
Dietary ME	3315.9 – 7.733 × (CM inclusion, %)	18.29	0.94	<0.001	<0.001	0.81	49.1	142
Dietary NE	2882.3 – 9.228 × (CM inclusion, %)	23.45	1.21	<0.001	<0.001	0.78	62.9	151

[1]Data were subjected to linear regression analysis with the percent inclusion of canola meal as the independent variable and the DE, ME, or NE of the diet (kcal/kg) as the dependent variable. The regression coefficients indicate the change in the DE, ME, or NE of the diets for each percentage point change of canola meal included in the diet; therefore, the coefficient multiplied by 100 is equal to the DE, ME, or NE of canola meal.

[2]AIC = Akaike's information criterion.

[3]SBM = soybean meal.

Table 6.7. Energy content of canola meal determined by the difference and regression methods

Item	Corn-basal diet		95 % Confidence interval	Corn-SBM basal diet[1]		95 % Confidence interval
	Difference method[2]	Regression method		Difference method[2]	Regression method	
As-fed basis						
DE, kcal/kg	2,818	2,709	2,594 – 2,824	2,782	2,717	2,525 – 2,908
ME, kcal/kg	2,646	2,515	2,384 – 2,647	2,629	2,543	2,353 – 2,732
NE, kcal/kg	2,233	2,096	1,717 – 2,475	2,106	1,960	1,716 – 2,203
DM basis						
DE, kcal/kg	3,100	2,980	2,854 – 3,107	3,061	2,989	2,778 – 3,199
ME, kcal/kg	2,910	2,767	2,623 – 2,911	2,892	2,798	2,589 – 3,006
NE, kcal/kg	2,457	2,306	1,889 – 2,723	2,317	2,156	1,888 – 2,424

[1]SBM = soybean meal.

[2]The values are the mean DE, ME, and NE of canola meal determined using the difference method for the 2 diets containing 15 or 30% canola meal.

Table 6.8. Energy content of canola meal determined using corn-based and corn-soybean meal-based basal diets

	Energy content of canola meal[1]			
Item	Corn	Corn-soybean meal	SEM	*P*-value
As-fed basis				
DE, kcal/kg	2,818	2,782	64.2	0.695
ME, kcal/kg	2,646	2,629	69.4	0.864
NE, kcal/kg	2,233	2,106	136.6	0.520
DM basis				
DE, kcal/kg	3,100	3,061	70.6	0.695
ME, kcal/kg	2,910	2,892	76.3	0.864
NE, kcal/kg	2,457	2,317	150.3	0.520

[1]The values are the mean DE, ME, and NE of canola meal determined using the difference method for the 2 diets containing 15 or 30% canola meal.

6.5 DISCUSSION

6.5.1 Chemical Composition of Ingredients and Diets

Canola meal, a coproduct of canola seed crushing industry for oil extraction via a combination of mechanical press and solvent extraction (Bell, 1993), is widely used as a protein source in swine diets (Canola Council of Canada, 2015). The GE, CP, and EE of the CM used in the current study were 4,346 kcal/kg, 37.8%, and 2.6%, respectively, similar to average values of 4,332 kcal/kg, 37.5%, 3.2% reported by NRC (2012), respectively. The NDF (24.3%) and ADF (19.1%) contents were slightly less than the values reported by Woyengo et al. (2010) but greater than those reported by Liu et al. (2016), which could be explained by differences in varieties, growing conditions in which the canola seeds were grown, and processing conditions during the oil extraction among the experiments (Bell, 1993). The nutrient composition of the corn and SBM used in this experiment is in accordance with values previously reported (NRC, 2012; Kim et al., 2017). Generally, CM contains greater concentration of fiber compared to SBM because, unlike SBM, canola hulls remain with the meal after processing. Also, as a protein source, it contains high levels of protein. Energy value of high fiber and protein diets is often overestimated when determined with either the DE or ME system (Noblet et al., 1994), which necessitates the determination of the NE value in CM to facilitate its effective utilization.

The analyzed nutrient and energy composition of experimental diets were similar to the values calculated from the summation of the ingredient contributions. The GE, CP, NDF, and ADF contents increased whereas starch content decreased as CM inclusion increased in each basal diet.

6.5.2 Energy and Nutrient Digestibility and Nitrogen Balance

Linear reduction in ATTD of DM and GE was observed as CM inclusion increased in each basal diet, which is most likely caused by the addition of CM resulting in increased dietary fiber content as observed in other studies (Velayudhan and Nyachoti, 2017). In addition, the hull fraction of canola seed is difficult to be digested by digestive system of pigs because half of ADF in CM is lignified (Slominski et al., 2012). Also, the linear reduction in ATTD of CP in corn-SBM diets with increasing CM inclusion is possibly explained by the CP digestibility of CM, similar to corn but less than SBM (NRC, 2012).

The corn diet used in the current study contained insufficient N and AA for protein deposition, and therefore a linear increase in daily N retention (g) and N retention rate (%) by pigs fed the corn diets with increasing CM was expected. Indeed, there was a linear effect of CM inclusion on those of pigs fed the corn diets with increasing inclusion of CM. On the other hand, the corn-SBM diet was formulated to meet or exceed the nutrient requirements of growing pigs (25–50 kg) to support their optimal growth. However, linear increase in N retention (g) of pigs fed these diets with increasing CM inclusion was also observed, which may be attributed to insufficient AA and N intake caused by limited feed intake. Although the feeding level at 550 kcal ME/kg $BW^{0.60}$ has been considered ad libitum feed intake (Noblet et al., 1994), other factors such as experimental conditions and genetic background may affect the feed intake of pigs. Indeed, a greater feeding level was proposed by Zhang et al. (2014) who reported that 573 kcal ME/kg $BW^{0.6}$ was close to the voluntary feed intake for growing pigs.

6.5.3 Energy Balance of Pigs and Energy Values of Experimental Diets

The observation that DE, ME, and NE of the corn and corn-SBM diets linearly decreased as CM inclusion increased is closely related to the increased dietary fiber content as a result of CM inclusion. This observation is further supported by the linear decrease in ATTD of DM and GE. In the current study, HP by pigs linearly increased from 138.0 to 157.9 and 165.1 kcal/kg $BW^{0.6}$ as pigs were fed diets containing increasing amounts of CM, which is possibly related to the increased dietary CP due to the addition of CM. The addition of CM to the corn diet resulted in decreased dietary starch content but increased dietary CP content, which has greater heat increment per unit of energy associated with metabolic utilization of energy than that of starch (Noblet et al., 1994; Le Bellego et al., 2001; Noblet et al., 2001). Moreover, the increased dietary fiber is also the possible reason for increased HP by pigs fed the corn diets containing increasing amounts of CM. Due to the low efficiency of utilization of ME from dietary fiber, an increase in dietary fiber often leads to an increase in HP by pigs (Noblet et al., 1989; Jørgensen et al., 1996; Rijnen et al., 2003). However, the effect of dietary fiber on HP needs to be investigated further. In some studies, similar (Ayoade et al., 2012a; Heo et al., 2014) or even decreased HP values (Le Goff et al., 2002; Jaworski et al., 2016) have been reported due to the increase in dietary fiber content. This inconsistent response to dietary fiber may partly contribute to no effect on HP by pigs fed the corn-SBM diets containing increasing CM inclusion. Also, the addition of CM increased dietary CP to less extent when added to the corn-SBM diet (increase by 2% of dietary CP per 15% CM inclusion) than when added to the corn diet (increase in 4% of dietary CP per 15% CM inclusion), which may have contributed to the failure to detect a linear effect on HP by pigs fed the corn-SBM diets with increasing CM inclusion.

Increase in size and weight of the visceral organs due to the consumption of increased dietary fiber has been reported (Nyachoti et al., 2000; Agyekum et al., 2012), which possibly results in increased FHP or NE required for maintenance of pigs because the gastrointestinal tract of pigs may account for about 30% of FHP (van Milgen et al., 1998). However, in the current study, no differences in FHP were observed among dietary treatments, which may be a consequence of a relatively short duration of feeding experimental diets. This feeding duration (i.e., 21 d) may be insufficient to induce the enlargement of gastrointestinal tract (Agyekum et al., 2012).

The RQ is typically slightly greater than 1.0 during the fed-state and less than 1.0 during the fasting-state (Agyekum et al., 2016). As expected, the RQ of pigs measured in the fed-state was around 1.0, whereas that of pigs in fasting-state was 0.75. The observation that fed-state RQ of pigs fed the corn-SBM diets linearly decreased from 1.02 to 1.00 and 0.99 with increasing CM inclusion indicates that metabolism of non-carbohydrate is linearly increasing in pigs (Liu et al., 2014). However, the fed-state RQ of pigs fed the corn diets with increasing CM inclusion was not affected in the current experiment. The reason for this observation is unclear because carbohydrate metabolism in the body would also decrease by replacing corn with CM as observed in pigs fed the corn-SBM diets with increasing CM inclusion.

6.5.4 Energy Values of Corn and Canola Meal

The DE and ME of feed ingredients fed to pigs are most often determined by either the difference method or the regression method (Adeola, 2001) and the energy values determined by these two methods have been reported to be similar (Bolarinwa and Adeola, 2016; Jaworski et al., 2016). Whereas, for the NE determination, the regression method and the prediction equations

are most widely used (Noblet et al., 1993a; Noblet et al., 1994; NRC, 2012). However, the difference method has also been recently used to determine NE value of feed ingredients and no difference between determined NE using indirect calorimetry and predicted NE from prediction equations was also reported (Ayoade et al., 2012a; Heo et al., 2014; Velayudhan et al., 2015b; Kim and Nyachoti, 2017). Also, according to the study of Jaworski et al. (2016), the NE of wheat bran determined by the difference method was not different from that estimated from the regression method. Likewise, in the current experiment, the DE, ME, and NE of CM determined by the difference method were within the 95% confidence intervals estimated for the DE, ME, and NE of CM by the linear regression method regardless of the basal diets used, which indicates that the two different methods (i.e., difference method, regression method) give equivalent values for DE, ME, and NE of CM fed to growing pigs.

The efficiency of ME utilization of a diet is dependent on final utilization of dietary energy in pigs (i.e., deposition as protein vs. deposition as lipid). If the energy is deposited as protein, the efficiency of ME utilization of the diet will be less than when it is deposited as lipid because the efficiency of ME utilization for protein deposition is about 60% whereas that for lipid deposition is approximately 80% (Noblet and van Milgen 2013), which may have significant effect on NE of the diet. Therefore, it has been suggested that the NE of diet should be determined based on balanced diets that meet nutrient requirements of pigs for supporting optimal growth and lean tissue gain (Noblet et al., 1993a, Noblet and van Milgen, 2013). For this reason, a corn diet, the most commonly used basal diet for DE and ME determination, may be inappropriate for NE determination due to the fact that this diet normally contains insufficient dietary AA and N, consequently reducing growth performance and affecting the NE of the diet. Contrary to our hypothesis, however, the NE of CM determined using either the corn diet (2,233

kcal/kg) or the corn-SBM diet (2,106 kcal/kg) was not different. Similar to our observation, Liu et al. (2014) reported that there were no differences in NE values of SBM determined using either the corn diet or the corn-SBM diet. When the goodness of fit for the linear model was compared, however, the coefficient of determination (i.e., r^2) of the regression analysis from the corn-SBM diet ($r^2 = 0.78$) was relatively greater than that from corn diet ($r^2 = 0.40$), which may indicate a better fit to the linear model. This result is likely caused by less variation in NE of CM determined using the corn-SBM diet than in the corn diet as affected by increasing inclusion level of CM in the diets. In other words, the HP and RE by pigs fed the balanced diets (i.e., corn-SBM diet with increasing inclusion level of CM) that meet the nutrient requirements was less variable compared to those of pigs fed unbalanced diets (i.e., corn diet with increasing inclusion level of CM), thereby reducing the variation in the NE value of diets and feed ingredient.

The values for DE and ME of CM fed to growing pigs obtained in the current study are within the range of previously published values (Woyengo et al., 2010; NRC, 2012; Heo et al., 2013). However, to our knowledge, the NE of CM from *Brassica napus* black, the most widely used variety for swine diet formulation, has not been reported yet. The NE of CM from *Brassica napus* yellow and *Brassica juncea* yellow were recently reported at 2,103 and 2,342 kcal/kg, respectively, by Heo et al. (2014), which is comparable to the NE value obtained in the current study (2,099 kcal/kg; an average of 2,233, 2,096, 2,106, and 1,960 determined by two methods using two different basal diets). This value is also similar to the published value from NRC (2012) calculated from the prediction equations based on chemical composition of CM. The similar NE values between *Brassica napus* black and yellow (2,099 kcal/kg vs. 2,103 kcal/kg) could be attributed to the similar chemical composition between two varieties, whereas greater NDF and less CP content of the *Brassica napus* black used in this experiment than in *Brassica juncea*

yellow used in the study of Heo et al. (2013) may have led to a slightly less NE value (2,099 kcal/kg vs. 2,342 kcal/kg). The values for DE and ME of corn fed to growing pigs determined in the current experiment are in close agreement with previously published values (NRC, 2012; Kim et al., 2017). Also, determined NE of corn (2,845 kcal/kg) is in accordance with the value (2,785 kcal/kg) reported by Liu et al. (2014), but is greater than the value (1,780 kcal/kg) reported by Kil et al. (2013). The reason for this discrepancy is mainly due to the difference in methodology among the experiments. The indirect calorimetry method was used to determine HP, FHP, and RE of pigs in the study of Liu et al. (2014) and the current study, whereas the comparative slaughter method was used to measure the RE of pigs in the study of Kil et al. (2013). In addition to this, experimental conditions such as feeding level (restricted vs. ad libitum), housing condition (group vs. individual), and genetic background may also affect the NE of diets and ingredients among experiments (Noblet and van Milgen, 2013; Kiarie et al., 2015).

In conclusion, the NE of CM determined in the current study was, on average, 2,099 kcal/kg, as-fed basis. The difference and regression methods do not give different NE value of CM fed to growing pigs. Furthermore, the NE values of CM determined using a basal diet based on either corn or corn-SBM were not different. However, when the goodness of fit for the linear model was compared, the r^2 of the regression analysis from the corn-SBM diet (balanced diet) was relatively greater than that from the corn diet (unbalanced diet), which suggests that the basal diet that meets the nutrient requirements of pigs is more suitable for NE determination of feed ingredients than basal diet that is nutritionally unbalanced.

CHAPTER 7

MANUSCRIPT IV: Pigs weighing less than 20 kg are unable to adjust feed intake in response to dietary net energy concentration regardless of diet composition

J. W. Kim, B. Koo, and C. M. Nyachoti

Department of Animal Science, University of Manitoba, Winnipeg, MB, Canada R3T 2N2

Submitted: Animal Feed Science and Technology, 2018

7.1 ABSTRACT

This study was conducted to investigate the effects of increasing dietary net energy (NE) concentrations adjusted by either dietary fat or fiber content on growth performance and energy intake of weaned pigs. Sixty barrows (8.40 ± 0.91 kg) were randomly allotted to 1 of 5 dietary treatments based on initial body weight with 12 replicates per treatment. The experimental diets were formulated to contain increasing NE concentrations (i.e. 9.9, 10.3, and 10.7 MJ NE/kg) by adjusting either dietary fat or fiber content. First, a control diet was formulated to meet the energy requirement of 7-11 kg weaned pigs (10.3 MJ NE/kg). Then, two diets containing 0.4 MJ NE/kg less or greater than the control diet were prepared by replacing corn starch with vegetable oil in the control diet. Dietary NE concentrations of last two diets were also formulated to contain 0.4 MJ NE/kg less or greater than the control diet by adjusting the inclusion level of plant-based ingredients in the control diet. Dietary NE concentrations of these two diets were primarily controlled by dietary fiber content but fat content was maintained. Results indicated that increasing dietary NE concentrations had no effects on average daily feed intake, average daily gain, and gain to feed ratio of weaned pigs regardless of diet composition. During week 1 to 3, the apparent total tract digestibility (ATTD) of dry matter (DM), gross energy (GE), crude protein, and neutral detergent fiber (NDF) of the diets were not affected by increasing dietary NE concentrations adjusted by dietary fat content. However, the ATTD of DM, GE, fat, and NDF of the diets linearly increased ($P < 0.05$) during week 1 to 3 as dietary NE concentrations increased by adjusting dietary fiber content. Also, no differences were observed in digestible energy (DE) and NE intake of pigs fed diets containing increasing dietary NE concentrations during week 1, regardless of diet composition. However, DE and NE intake increased (linear, $P < 0.01$) with increasing dietary NE concentrations adjusted by dietary fiber content during week 2 and 3.

Similarly, a tendency for a linear increase in DE and NE intake was observed during week 2 and 3 when dietary NE concentrations were adjusted by dietary fat content. In conclusion, weaned pigs may not be able to adjust feed intake in response to dietary NE concentration ranging from 9.9 to 10.7 MJ/kg, which are slightly less and greater than NE requirement of weaned pigs.

Key words: digestibility, energy intake, growth performance, net energy, weaned pig

7.2 INTRODUCTION

Estimation of voluntary feed intake (FI) and the associated energy intake of pigs play an important role in pork production because pigs have a tendency to consume feed until their energy requirements are fulfilled, which in turn influences growth performance and feed efficiency (Nyachoti et al., 2004; Li and Patience, 2017). Feed intake of growing-finishing pigs is affected by various factors such as environmental conditions, health status, genotypes, and diet composition (Nyachoti et al., 2004). Among them, dietary energy concentration is considered the first determinant of FI (Henry, 1985). As available dietary energy concentration decreases, pigs will attempt to maintain constant daily energy intake by consuming more feed, until physical FI capacity or other environmental factors restrict FI (Beaulieu et al., 2009; Quiniou and Noblet, 2012). For these reasons, the ability of young pigs (less than 20 kg), which have an immature gut system and limited physical gut capacity, to adjust FI in response to wide range of dietary energy concentration has known to be limited (Black et al., 1986).

However, inconsistent results have been reported regarding the response of weaned pigs to dietary energy concentration. For example, Oresanya et al. (2008) reported that daily net energy (NE) intake of weaned pigs [i.e. average daily FI (ADFI) × dietary NE concentration]

linearly increased as dietary NE concentration increased from 9.2 to 10.1 MJ NE/kg, whereas Pedersen et al. (2016) found that daily NE intake of weaned pigs fed a diet containing 9.6 MJ NE/kg was similar to those fed a diet containing 10.3 MJ NE/kg due to an increase in FI. However, to date, no research has evaluated the effects of increasing dietary NE concentration ranging from slightly less to slightly greater than the NE requirement (i.e. from 9.9 to 10.7 MJ/kg) on growth performance and energy intake of weaned pigs.

An adjustment of dietary NE concentration can be achieved by adding feed ingredients rich in fat or those high in fiber (Quiniou and Noblet, 2012; Acosta et al., 2017). As an attempt to reduce dietary NE concentration, the inclusion of fibrous feed ingredients will result in an increase in diet bulkiness, which may in turn limit the FI by piglets due to their limited physical gut capacity (Black et al., 1986). Whereas, a reduction in dietary fat content to decrease dietary NE concentration may not cause bulkiness problem compared to the fiber addition to the diet. Therefore, it was hypothesized that response of piglets to increasing dietary NE concentration would be different when dietary NE concentration was adjusted by either adding dietary fiber or fat content. However, in previous studies dietary fat content was primarily adjusted to increase dietary NE concentration (Oresanya et al., 2008; Pedersen et al., 2016) and this needs to be further investigated to determine whether dietary composition affects the ability of weaned pigs to adjust FI in response to dietary NE concentration.

Thus, the current study was conducted to test the hypothesis that response of piglets to increasing dietary NE concentration will be different when dietary NE concentration was adjusted by either dietary fiber or fat content.

7.3 MATERIALS AND METHODS

The experimental protocol was reviewed and approved by the University of Manitoba Animal Care Committee, and pigs were handled according to the guidelines of the Canadian Council on Animal Care (2009).

7.3.1 Animals, Housing, and Experimental Design

Sixty piglets [(Yorkshire × Landrace) × Duroc; barrow] that had been weaned for 1 week [body weight (BW); 8.40 ± 0.91 kg] were obtained from the Glenlea Swine Research Unit at the University of Manitoba (Winnipeg, MB, Canada). Pigs were individually weighed and randomly allotted via randomized complete block design to the 1 of 5 dietary treatments based on initial BW with 12 replicates per treatment. Pens (1.2 by 1.5 m) had plastic-covered expanded metal flooring, and each was equipped with a stainless-steel feeder and a low-pressure nipple drinker. Pigs had unlimited access to feed and water during the 21-d experiment. Room temperature was maintained at 28 ± 1℃ during the first week and then reduced by 1.5℃ per week thereafter.

7.3.2 Experimental Diets and Procedure

Dietary NE concentration of experimental diets was calculated based on NE values of feed ingredients reported in NRC (2012). Five experimental diets were formulated to contain increasing NE concentrations (i.e. 9.9, 10.3, and 10.7 MJ NE/kg) by adjusting either dietary fat or fiber content (Table 7.1). First, a control diet was formulated to meet the energy and nutrients requirements of weaned pigs (10.3 MJ NE/kg; NRC, 2012). Then, two diets containing 0.4 MJ NE/kg less or greater than the control diet were prepared by replacing corn starch with vegetable oil in the control diet to adjust NE concentration primarily with dietary fat content. Dietary NE concentrations of last two diets were also formulated to contain 0.4 MJ NE/kg less or greater

than the control diet by replacing the plant-based ingredients in the control diet. Dietary NE concentrations of these two diets were primarily controlled by dietary fiber content but fat content was maintained. All nutrients and energy in the control diet (10.3 MJ NE/kg) were included to meet or exceed the NRC recommendations. The concentration of standardized ileal digestible (SID) lysine was maintained at 1.19 g/MJ NE in all diets. Other SID amino acids (AA) were formulated relative to the SID lysine concentration with the ratios in agreement with the ideal protein ratio for this BW class of pig (NRC, 2012). This ensured that the AA supply was not a limiting factor for growth. Standardized total tract digestible phosphorus (P) was 0.33 g/MJ NE in all diets. Titanium dioxide (TiO_2; Sigma Chemical Company, St. Louis, MO) was added at 0.3 % to the experimental diets as an indigestible index to determine apparent total tract digestibility (ATTD) of energy and nutrients.

The BW of pigs and feed disappearance were recorded weekly to determine average daily gain (ADG) and ADFI, and gain to feed ratio (G:F) was calculated using these two variables. Freshly voided feces were collected from each pig from 0800 to 1800 h by grab sampling over a 2-d period at the end of each week (i.e. d 6 and 7, d 13 and 14, and d 20 and 21 for week 1, 2, and 3, respectively) and analyzed to determine digestible energy (DE) and NE concentrations, and digestibility of nutrients in experimental diets according to the index method. Samples were pooled and frozen at −20°C until required for further analyses.

Table 7.1. Ingredient composition of experimental diets (as-fed basis)

		Diet				
		By fat		Control	By fiber	
Ingredient, %	MJ/kg NE[1]:	9.9	10.7	10.3	9.9	10.7
Corn		49.17	47.72	48.44	40.22	57.26
Soybean meal		15.00	15.00	15.00	10.00	27.00
Canola meal		15.00	15.00	15.00	20.00	-
Flaxseed meal		5.00	5.00	5.00	8.00	-
Corn DDGS[2]		-	-	-	6.50	-
Oat hulls		-	-	-	1.00	-
Fish meal		3.00	3.00	3.00	3.00	3.00
Dried whey		7.50	7.50	7.50	7.50	7.50
Vegetable oil		-	4.00	2.00	1.40	2.28
Cornstarch		3.00	-	1.50	0.27	-
Limestone		0.83	0.98	0.90	0.75	1.10
Monocalcium phosphate		0.23	0.35	0.30	0.15	0.43
Salt		0.40	0.40	0.40	0.40	0.40
Vitamin-mineral premix[3]		0.18	0.18	0.18	0.18	0.18
Lysine-HCl		0.30	0.42	0.36	0.31	0.35
$_{DL}$-Methionine		0.03	0.05	0.04	-	0.09
$_{L}$-Threonine		0.06	0.10	0.08	0.02	0.11
Titanium dioxide		0.30	0.30	0.30	0.30	0.30

[1]NE = net energy.

[2]DDGS = distillers' dried grains with solubles.

[3]Supplied, per kilogram of diet, 8,250 IU vitamins A, 825 IU vitamin D_3, 40 IU vitamin E, 4 mg vitamin K, 2 mg thiamine, 10 mg riboflavin, 15 mg calcium pantothenic acid, 500 mg choline, 35 mg niacin, 4.5 mg pyridoxine, 25 µg vitamin B_{12}, 200 µg biotin, 2 mg folic acid, 15 mg Cu (copper sulfate), 0.21 mg I (as potassium iodine), 100 mg Fe (ferrous sulfate), 20 mg Mn (manganese oxide), 0.15 mg Se (sodium selenite), and 100 mg Zn (zinc oxide).

7.3.3 Sample Preparation and Chemical Analyses

Diet and ingredient samples were ground through a 1-mm screen in a Thomas Wiley mill model 4 (Labwrench, Midland, ON, Canada) and thoroughly mixed before chemical analysis. Fecal samples were dried in a forced-air drying oven at 60℃ for 5 d and finely ground in a coffee grinder (CBG5 Smart Grind; Applica Consumer Products, Inc., Shelton, CT) before chemical analysis. Diet and ingredient samples were analyzed in duplicate for dry matter (DM), gross energy (GE), crude protein (CP; nitrogen × 6.25), crude fat, starch, ash, neutral detergent fiber (NDF), acid detergent fiber (ADF), calcium, and P, whereas fecal samples were analyzed in duplicate for DM, GE, CP, crude fat, and NDF. The TiO_2 concentrations in diets and fecal samples were further analyzed in duplicate. The DM was measured according to AOAC (method 934.01; 2006) and the GE was determined using an isoperibol bomb calorimeter (model 6400; Parr Instrument, Moline, IL), which had been calibrated using benzoic acid as a standard. Nitrogen was determined by the combustion method (method 990.03; AOAC, 2006) using the LECO nitrogen analyzer (model CNS-2000; LECO Corp., St. Joseph, MI). The crude fat was determined after hexane extraction (method 920.39A; AOAC, 2006) in an extraction apparatus. The ADF and NDF contents were determined according to the method of Goering and Van Soest (1970). Ash content was analyzed according to AOAC (method 942.05; 2006). For analyses of Ca and P, ashed sample was digested according to AOAC (method 985.01; 2006) and read on a Varian inductively coupled plasma mass spectrometer (Varian Inc., Palo Alto, CA). Starch content was analyzed using an assay kit (Megazyme Total Starch assay kit; Megazyme International Ltd, Wicklow, Ireland).

7.3.4 Calculations

The ATTD was calculated using the following equation:

$$ATTD\ (\%) = \{1 - [(T_d/T_f) \times (N_f/N_d)]\} \times 100,$$

in which N_f is the nutrient concentration in feces (%), N_d is the nutrient concentration in diet (%), T_d is the TiO_2 concentration in diet (%), and T_f is the TiO_2 concentration in feces (%).

The NE concentration of the experimental diets was calculated according to the following equation established by Noblet et al. (1994) based on measured DE concentration and chemical composition:

$$NE = 0.700 \times DE + 1.61 \times \text{crude fat} + 0.48 \times \text{starch} - 0.91 \times CP - 0.87 \times ADF,$$

in which NE and DE are in kilocalories per kilogram DM; crude fat, starch, CP, and ADF are in g/kg DM. Other equations published in NRC (2012) were not applicable because dietary ME could not be calculated due to the absence of urine collection.

7.3.5 Statistical Analysis

The UNIVARIATE procedure (SAS Inst. Inc., Cary, NC) was used to verify homogeneity of variances. All data were analyzed as a randomized complete block design using the MIXED procedure of SAS with the individual pig as the experimental unit. The LSMEANS procedure was used to calculate mean values and the PDIFF option was used to separate means. Orthogonal polynomial contrasts were used to determine linear and quadratic effects of increasing dietary NE concentration. The data from the control diet (10.3 MJ NE/kg) were used for both contrast tests (when dietary NE concentration was adjusted by either dietary fiber or fat content). A probability of $P < 0.05$ was considered significant whereas $0.05 \leq P < 0.10$ was considered a tendency.

7.4 RESULTS

7.4.1 Chemical Composition of Ingredients and Experimental Diets

The chemical composition of plant-based ingredients used in the current study (Table 7.2) was within the ranges reported by NRC (2012). Also, the chemical composition of experimental diets was in good agreement with expected values calculated based on the analyzed composition of the ingredients and their contribution to the diets (Table 7.3). If the analyzed and calculated chemical compositions are similar, the corresponding estimated nutritive values are assumed to be reliable. As expected, dietary fat content of the diets formulated to contain 9.9 and 10.7 MJ NE/kg, adjusted by replacing corn starch with vegetable oil in the control diet, increased from 2.8 to 6.6 %, whereas NDF and ADF contents were maintained (on average, 13.0 and 5.9 %, respectively). Also, dietary fat contents of the diets formulated to contain 9.9 and 10.7 MJ NE/kg, adjusted by replacing several plant-based ingredients in the control diet (to mainly adjust dietary fiber content), were similar (on average, 4.6 %), whereas NDF and ADF contents decreased from 17.8 to 9.5 % and from 8.4 to 3.1 %, respectively.

7.4.2 Growth Performance

All pigs adapted well to their experimental diets and conditions, remained healthy and readily consumed their assigned diets. However, a total of 3 pigs were excluded from the experiment because they became sick or lame. The death of these pigs was not considered related to the experimental treatments and no further sickness was recorded. There was no difference in initial BW among pigs allotted to the 5 dietary treatments (Table 7.4). Increasing NE concentrations of diets adjusted by either dietary fat or dietary fiber content had no effects on

ADFI, ADG, and G:F of weaned pigs during the entire experimental period.

7.4.3 Nutrient and Energy Digestibilities

During week 1 to 3, the ATTD of DM, GE, CP, and NDF of the diets fed to weaned pigs were not affected by increasing dietary NE concentrations adjusted by dietary fat content, but the ATTD of fat increased (linear, $P < 0.01$) as dietary NE concentrations adjusted by dietary fat content increased (Table 7.5). The ATTD of DM, GE, fat, and NDF of the diets fed to weaned pigs linearly increased ($P < 0.05$) during week 1 to 3 as dietary NE concentrations increased by adjusting dietary fiber content. Also, increasing dietary NE concentrations increased (linear, $P < 0.01$) the ATTD of CP during week 2 when it was adjusted by dietary fiber content.

7.4.4 Digestible and Net Energy Concentrations of Diets and Intake by Weaned Pigs

During week 1 to 3, measured DE and NE concentrations linearly increased ($P < 0.05$) as dietary NE concentrations increased by adjusting either dietary fat or fiber content (Table 7.6). During week 1, no differences were observed in DE and NE intake of pigs fed diets containing increasing dietary NE concentrations adjusted by either dietary fat or fiber content. However, the DE and NE intake increased (linear, $P < 0.01$) with increasing dietary NE concentrations adjusted by dietary fiber content during week 2 and 3. Similarly, a tendency for linear increases in DE and NE intake was observed during week 2 and 3 when dietary NE concentrations were adjusted by dietary fat content.

Table 7.2. Analyzed nutrient composition of corn, soybean meal, flaxseed meal, corn distillers' dried grains with solubles (DDGS), canola meal, and oat hulls (as-fed basis)

	Ingredients					
Item, %	Corn	Soybean meal	Flaxseed meal	Corn DDGS	Canola meal	Oat hulls
Dry matter	86.4	88.5	90.6	88.9	90.0	92.3
Gross energy, MJ/kg	16.0	17.2	19.3	18.5	17.9	16.8
Crude protein	7.8	46.3	31.9	28.8	36.8	2.5
Crude fat	2.4	0.6	11.1	5.9	2.5	1.7
Starch	4.6	4.0	4.1	3.0	5.3	7.3
Neutral detergent fiber	8.2	8.5	30.8	35.0	25.6	73.5
Acid detergent fiber	3.1	5.4	17.9	15.9	18.8	39.9
Ash	1.8	7.2	4.8	6.3	7.4	2.1
Calcium	0.01	0.48	0.29	0.38	0.66	0.14
Phosphorus	0.30	0.70	0.98	0.83	0.13	0.08

Table 7.3. Energy and nutrient composition of experimental diets (as-fed basis)

		Diet				
		By fat		Control	By fiber	
Item	MJ/kg NE[1]:	9.9	10.7	10.3	9.9	10.7
Analyzed composition, %						
Gross energy, MJ/kg		16.3	17.3	16.8	17.1	16.5
Dry matter		88.6	89.2	88.7	89.2	88.2
Crude protein		21.3	21.1	20.9	23.1	20.0
Starch		31.7	28.0	29.9	23.0	34.3
Ash		7.3	6.1	6.9	7.2	5.8
Crude fat		2.8	6.6	4.6	4.8	4.5
Neutral detergent fiber		12.4	13.0	13.4	17.8	9.5
Acid detergent fiber		5.9	5.9	6.0	8.4	3.1
Calcium		0.68	0.85	0.70	0.67	0.75
Phosphorus		0.65	0.69	0.69	0.70	0.60
Calculated composition[2]						
Net energy, MJ/kg		9.9	10.7	10.3	9.9	10.7
SID[3] lysine, g/kg		11.8	12.8	12.3	11.8	12.7
SID methionine, g/kg		3.7	3.9	3.8	3.8	3.9
SID threonine, g/kg		7.2	7.6	7.4	7.4	7.7
SID tryptophan, g/kg		2.1	2.1	2.1	2.1	2.2
SID cysteine, g/kg		3.1	3.1	3.1	3.4	2.7
STTD[4] phosphorus, g/kg		3.2	3.5	3.4	3.4	3.5
SID lysine, g/MJ NE		1.19	1.19	1.19	1.19	1.19
STTD phosphorus, g/MJ NE		0.33	0.33	0.33	0.33	0.33
Calcium, g/MJ NE		0.73	0.73	0.72	0.72	0.71

[1]NE = net energy.
[2]Calculated based on NRC (2012) values of each ingredient.

[3]SID = standardized ileal digestible.
[4]STTD = standardized total tract digestible.

Table 7.4. Growth performance of weaned pigs fed experimental diets[1]

	Diet[2]						*P*-value[3]				
	NE adjusted by fat		Control	NE adjusted by fiber		Pooled		NE adjusted by fat		NE adjusted by fiber	
Item MJ/kg NE[4]:	9.9	10.7	10.3	9.9	10.7	SEM	Trt	Lin	Q	Lin	Q
Number of pigs[5]	11	12	11	12	11						
Initial body weight, kg	8.4	8.4	8.4	8.4	8.4	0.04	0.83	0.66	0.78	0.78	0.37
Average daily feed intake, g											
week 1	459	451	468	460	426	31.1	0.88	0.73	0.72	0.46	0.53
week 2	756	822	737	762	795	48.1	0.71	0.34	0.34	0.46	0.38
week 3	1,152	1,176	1,076	1,076	1,126	52.9	0.54	0.41	0.20	0.39	0.60
Overall	789	806	760	766	785	37.8	0.91	0.71	0.23	0.55	0.62
Average daily gain, g											
week 1	344	316	358	348	279	30.4	0.31	0.44	0.42	0.19	0.68
week 2	512	522	475	489	521	34.3	0.81	0.82	0.26	0.31	0.33
week 3	744	801	726	754	719	32.6	0.40	0.20	0.37	0.47	0.75
Overall	533	539	524	531	507	21.8	0.87	0.79	0.94	0.83	0.22
Gain: feed g/g											
week 1	0.75	0.70	0.75	0.75	0.64	0.039	0.20	0.40	0.52	0.09	0.31
week 2	0.67	0.64	0.65	0.64	0.66	0.023	0.87	0.50	0.82	0.42	0.98
week 3	0.66	0.69	0.66	0.71	0.64	0.029	0.55	0.40	0.84	0.07	0.82
Overall	0.69	0.68	0.69	0.70	0.65	0.018	0.27	0.58	0.59	0.10	0.20

[1]Data are least squares means.

[2]Energy concentrations in diets were adjusted by either dietary fat or dietary fiber content. Data from control diet (10.3 MJ NE/kg) were used in both orthogonal polynomial contrast tests.

[3]Trt = overall effects of treatments; Lin = linear effects of increasing NE concentrations of diets; Q = quadratic effects of increasing NE concentrations of diets.

[4]NE = net energy.

[5]A total of 3 pigs were removed from the experiment because they became sick or lame.

Table 7.5. Apparent total tract digestibility (ATTD) of dry matter, gross energy, and nutrients in diets fed to weaned pigs[1]

	Diet[2]						*P*-value[3]				
	NE adjusted by fat		Control	NE adjusted by fiber		Pooled		NE adjusted by fat		NE adjusted by fiber	
Item MJ/kg NE[4]:	9.9	10.7	10.3	9.9	10.7	SEM	Trt	Lin	Q	Lin	Q
Number of pigs[5]	11	12	11	12	11						
ATTD of dry matter											
week 1	71.1^{b}	72.5^{b}	72.8^{b}	64.3^{c}	79.2^{a}	1.63	<0.01	0.49	0.56	<0.01	0.59
week 2	74.2^{b}	73.2^{b}	71.6^{bc}	65.9^{c}	81.2^{a}	1.53	<0.01	0.57	0.18	<0.01	0.28
week 3	72.9^{b}	73.8^{b}	72.5^{b}	65.4^{c}	82.8^{a}	1.24	<0.01	0.55	0.47	<0.01	0.28
ATTD of gross energy											
week 1	68.5^{b}	71.0^{b}	69.9^{b}	62.8^{c}	77.0^{a}	1.85	<0.01	0.29	0.95	<0.01	0.98
week 2	72.9^{b}	73.3^{b}	70.8^{bc}	65.5^{c}	79.9^{a}	1.60	<0.01	0.80	0.17	<0.01	0.34
week 3	72.1^{b}	74.3^{b}	72.4^{b}	65.3^{c}	82.0^{a}	1.31	<0.01	0.16	0.52	<0.01	0.44
ATTD of fat											
week 1	17.3^{c}	50.1^{a}	45.7^{ab}	36.4^{b}	60.0^{a}	4.17	<0.01	<0.01	0.01	0.02	0.67
week 2	36.2^{c}	68.7^{a}	53.8^{b}	52.0^{b}	62.3^{ab}	3.35	<0.01	<0.01	0.72	0.03	0.38
week 3	45.2^{c}	73.5^{a}	60.2^{b}	58.5^{b}	69.6^{a}	2.12	<0.01	<0.01	0.67	<0.01	0.16
ATTD of crude protein											
week 1	60.4	63.7	63.7	61.2	67.4	2.57	0.31	0.29	0.50	0.09	0.84
week 2	67.8^{ab}	67.6^{ab}	64.9^{b}	64.6^{b}	74.3^{a}	2.04	<0.01	0.93	0.16	<0.01	0.07
week 3	65.6	67.0	65.6	64.3	71.3	2.35	0.27	0.57	0.73	0.06	0.49
ATTD of NDF[6]											
week 1	40.2^{b}	42.1^{ab}	44.3^{ab}	30.7^{c}	50.9^{a}	3.24	<0.01	0.62	0.33	<0.01	0.35
week 2	42.8^{ab}	39.7^{ab}	40.3^{ab}	34.1^{b}	52.6^{a}	3.72	0.02	0.49	0.81	<0.01	0.49
week 3	37.9^{b}	39.1^{b}	39.9^{b}	29.2^{b}	55.2^{a}	3.13	<0.01	0.77	0.69	<0.01	0.58

[a–c]Means within a row with different superscripts differ ($P < 0.05$).

[1]Data are least squares means.

[2]Energy concentrations in diets were adjusted by either dietary fat or dietary fiber content. Data from control diet (10.3 MJ NE/kg) were used in both orthogonal polynomial contrast tests.

[3]Trt = overall effects of treatments; Lin = linear effects of increasing NE concentrations of diets; Q = quadratic effects of increasing NE concentrations of diets.

[4]NE = net energy.

[5]A total of 3 pigs were removed from the experiment because they became sick or lame.
[6]NDF = neutral detergent fiber.

Table 7.6. Digestible energy (DE) and net energy (NE) of diets and DE and NE intake of weaned pigs fed experimental diets (as-fed basis)[1]

	Diet[2]						*P*-value[3]				
	NE adjusted by fat		Control	NE adjusted by fiber		Pooled		NE adjusted by fat		NE adjusted by fiber	
Item MJ/kg NE[4]:	9.9	10.7	10.3	9.9	10.7	SEM	Trt	Lin	Q	Lin	Q
Number of pigs[5]	11	12	11	12	11						
Measured DE, MJ/kg											
week 1	11.2^{cd}	12.2^{ab}	11.8^{bc}	10.7^{d}	12.6^{a}	0.32	<0.01	0.03	0.96	<0.01	0.28
week 2	11.9^{bc}	12.6^{ab}	11.9^{bc}	11.2^{d}	13.2^{a}	0.28	<0.01	0.04	0.35	<0.01	0.45
week 3	11.8^{c}	12.8^{b}	12.2^{c}	11.2^{d}	13.5^{a}	0.22	<0.01	<0.01	0.50	<0.01	0.52
Measured NE, MJ/kg											
week 1	7.6^{bc}	8.5^{a}	8.1^{ab}	7.1^{c}	9.0^{a}	0.23	<0.01	<0.01	0.96	<0.01	0.58
week 2	8.1^{bc}	8.8^{ab}	8.2^{b}	7.4^{c}	9.3^{a}	0.20	<0.01	<0.01	0.35	<0.01	0.81
week 3	8.0^{b}	9.0^{a}	8.4^{b}	7.4^{c}	9.6^{a}	0.15	<0.01	<0.01	0.46	<0.01	0.69
DE intake[6], MJ/d											
week 1	5.1	5.2	5.6	4.9	5.2	0.34	0.72	0.86	0.41	0.75	0.20
week 2	8.7^{bc}	10.4^{ab}	8.5^{c}	8.1^{c}	11.2^{a}	0.61	0.01	0.09	0.21	<0.01	0.06
week 3	13.1^{ab}	15.1^{a}	13.1^{ab}	11.7^{b}	15.1^{a}	0.76	0.02	0.10	0.13	<0.01	0.80
NE intake[7], MJ/d											
week 1	3.5	3.6	3.8	3.2	3.7	0.24	0.46	0.72	0.41	0.37	0.18
week 2	5.9^{bc}	7.3^{ab}	5.9^{bc}	5.4^{c}	7.9^{a}	0.42	<0.01	0.06	0.21	<0.01	0.02
week 3	8.9^{bc}	10.5^{a}	9.0^{ab}	7.8^{c}	10.7^{a}	0.50	<0.01	0.06	0.10	<0.01	0.84

$^{a–d}$Means within a row with different superscripts differ ($P < 0.05$).

[1]Data are least squares means.

[2]Energy concentrations in diets were adjusted by either dietary fat or dietary fiber content. Data from control diet (10.3 MJ NE/kg) were used in both orthogonal polynomial contrast tests.

[3]Trt = overall effects of treatments; Lin = linear effects of increasing NE concentrations of diets; Q = quadratic effects of increasing NE concentrations of diets.

[4]NE = net energy.

[5]A total of 3 pigs were removed from the experiment because they became sick or lame.

[6]DE intake = ADFI × measured DE.

[7]NE intake = ADFI × measured NE.

7.5 DISCUSSION

The current experiment investigated the effects of increasing dietary NE concentrations adjusted by either the dietary fat or fiber content to be slightly less and greater than the NE requirement of weaned pigs (10.3 MJ NE/kg) on growth performance and energy intake of weaned pigs, an approach never before tested in weaned pigs. It has been well known that growing-finishing pigs are capable of adjusting FI depending on dietary composition and characteristics. In most circumstances, dietary energy concentration is the first determinant of voluntary FI of pigs although other dietary factors such as AA and P are also known to determine FI of pigs (Quiniou and Noblet, 2012). As dietary energy concentration declines, pigs will increase FI to satisfy their energy requirements. However, according to Black et al. (1986), during weaning and beginning of the growing period (weighing less than 20 kg) energy intake regardless of the dietary energy concentration is the first limiting factor for growth because energy intake may not be regulated by the young pigs, which implies that the maximum capacity of the digestive tract is not reached yet. In this study, to investigate whether the lack of ability to adjust FI in response to dietary energy concentration is attributed to insufficient physical gut capacity of weaned pigs, other factors that may affect FI of pigs were precisely controlled. As reviewed by Nyachoti et al. (2004), factors that may influence the maximum FI capacity such as husbandry conditions (i.e. temperature, group size, and space allowance), genotype, and sanitary condition were carefully controlled. Especially, pigs housed under commercial conditions have shown variable ADFI due to their social hierarchy (Beaulieu et al., 2009). Therefore, pigs were individually housed to avoid competition for feeder space that may occur if they were group-housed. Also, considering that digestive system of weaned pigs is immature unlike growing-finishing pigs, the narrower range of dietary NE concentrations relative to the studies using

growing-finishing pigs was tested (Quiniou and Noblet, 2012; Lee et al., 2015).

In the current study, ADG, ADFI, and G:F of weaned pigs were not affected by increasing dietary NE concentrations adjusted by either dietary fat or dietary fiber content during the entire experimental period. Therefore, associated daily DE and NE intake of pigs, which were calculated by multiplying dietary DE and NE concentrations by ADFI, increased with increasing dietary DE and NE concentrations regardless of dietary composition. Contrary to our hypothesis that the response of weaned pigs to increasing dietary NE concentration adjusted by dietary fat content would be different from that adjusted by dietary fiber content, we failed to observe the regulation of FI in weaned pigs, which may indicate that weaned pigs weighing less than 20 kg have inability to adjust FI in response to dietary NE concentrations when dietary NE concentrations remained between 9.9 and 10.7 MJ/kg which are slightly less and greater than the NE requirement (10.3 MJ NE/kg; NRC, 2012) of weaned pigs. These results agree with those of the study by Oresanya et al. (2008) who reported that pigs fed the diets containing dietary NE concentrations ranging from 9.0 to 9.9 MJ NE/kg had similar ADFI, and consequently increased NE intake. Also, according to the study of de Souza et al. (2000), an adjustment of ADFI to dietary energy concentration was not observed in weaned pigs weighing less than 25 kg when the dietary DE concentration ranged from 13.5 to 14.6 MJ/kg (corresponding to 9.4 to 11.0 MJ NE/kg; NRC, 2012).

The ratio between the essential nutrients (e.g. AA, Ca, and P) and NE concentration in the control diet (10.3 MJ NE/kg) were maintained among the experimental diets to ensure that supply of other nutrients was not a limiting factor for growth. However, this was assumed based on the speculation that pigs are able to adjust FI according to dietary energy concentration. Therefore, with a similar ADFI among experimental treatments, a reduction in ADG would be

expected in pigs fed the diets containing 9.9 MJ NE/kg because essential nutrients concentrations in this diet were less than their requirements, and therefore insufficient to support optimal growth. Interestingly, the pigs fed the diets containing 9.9 MJ NE/kg showed similar ADG to those in other treatments. This might have been due to either the overestimation of nutrient requirements of weaned pigs (e.g. AA, Ca, and P) suggested by NRC (2012) or the underestimation of energy or nutrients availability in the feed ingredients used in this study (i.e. corn, canola meal, flaxseed meal, soybean meal, vegetable oil). However, these needs to be further investigated.

The ATTD of DM, GE, CP, and NDF was unaffected but the ATTD of fat linearly increased with increasing dietary NE concentrations adjusted by dietary fat content. This observation is in agreement with previous data reported in pigs (Jørgensen et al., 1993; Kil et al., 2010). Contribution of endogenous sources of fat to the total excreted fat in pigs fed the diet containing 9.9 MJ NE/kg is likely higher than those fed the diet containing 10.7 MJ NE/kg due to small amounts of fat in diets containing 9.9 MJ NE/kg, and therefore leads to decreased apparent digestibility of fat. A linear reduction in the ATTD of DM, GE, fat, CP, and NDF was observed as dietary NE concentration adjusted by dietary fiber content increased, which is most likely caused by the addition of several coproducts to the diets at the expense of soybean meal which contains less dietary fiber compared to the coproducts used in the current study. Indeed, many studies have reported decreased nutrient digestibilities as a consequence of adding fibrous ingredients to pig diets (Noblet and Le Goff, 2001; Koo et al., 2017; Kim et al., 2018). Interestingly, the ATTD of GE and fat of experimental diets (pooled within week) linearly increased with age of pigs from 1 to 3 week (data are not shown). This observation agrees with previous results reported by Le et al. (2016) who observed increased DM and GE digestibilities in weaned pigs from 1 to 3 weeks of age. It should be noted that most NE values in feeding

tables (e.g., NRC, 2012) available for swine diet formulation are predicted values from the prediction equations generated using growing pigs based on available energy and chemical composition of ingredients (Noblet et al., 1994; NRC, 2012). Considering that GE and nutrients digestibilities are lower in weaned pigs compared with growing pigs and the fact that NE values of feed ingredients in the feeding tables available for nursery diet formulation are predicted by the equations generated using growing pigs, careful use of NE values in the feeding tables needs to be guaranteed to accurately supply dietary energy required by weaned pigs.

In conclusion, no changes in the ADG, ADFI, and G:F of weaned pigs fed diets containing increasing dietary NE concentrations were observed in the current study, regardless of diet compositions. Therefore, daily DE and NE intake, which were calculated by multiplying dietary DE and NE concentrations by ADFI, were not maintained but linearly increased as dietary energy concentration increased. These results suggest that weaned pigs weighing less than 20 kg are unable to adjust FI in response to dietary NE concentration ranging from 9.9 to 10.7 MJ/kg which are slightly less and greater than the NE requirement of weaned pigs.

CHAPTER 8

MANUSCRIPT V: Net energy content of high-protein sunflower meal fed to growing pigs and effect of dietary phosphorus content on energy values

J. W. Kim, and C. M. Nyachoti

Department of Animal Science, University of Manitoba, Winnipeg, MB, Canada R3T 2N2

8.1 ABSTRACT

An experiment was carried out to determine digestible energy (DE), metabolizable energy (ME), and net energy (NE) of high protein sunflower meal (HP-SFM) and to compare the energy values of HP-SFM determined using either a phosphorus (P)-deficient basal diet or a P-adequate basal diet. Twenty-four growing barrows (initial body weight of 19.3 ± 1.8 kg) were randomly assigned to 1 of 4 dietary treatments with 6 replicates per treatment. Four experimental diets including two corn-soybean meal (SBM)-based basal diets containing 2 levels of standardized total tract digestible P (i.e., P-deficient basal diet and P-adequate basal diet) and the other two diets containing 30% HP-SFM with each basal diet (i.e., HP-SFM1 diet and HP-SFM 2 diet) were formulated to determine the DE, ME, and NE of HP-SFM and to compare energy values of HP-SFM determined by the difference method using two basal diets. Pigs were fed experimental diets at 550 kcal ME/kg $BW^{0.6}$ per day for 15 d including 10 d for adaptation and 5 d for total fecal and urine collections. Pigs were then moved to indirect calorimetry chambers to determine total heat production (THP) and fasting heat production (FHP). Apparent total tract digestibilities of dry matter and gross energy ware higher ($P < 0.01$) in the P-deficient and P-adequate basal diets than in the HP-SFM 1 and 2 diets. Also, a lower ($P < 0.01$) amount of N was retained in pigs fed the P-deficient basal diet compared to those fed the other diets. The THP of pigs fed the HP-SFM 1 and 2 diets was higher ($P < 0.01$) than those fed the P-deficient basal diet with the intermediate value for pigs fed the P-adequate basal diet. The retained energy (RE) as protein of pigs fed the P-deficient basal diet was lower ($P < 0.01$) but RE as lipid was higher ($P < 0.01$) than for those fed the P-adequate basal, and HP-SFM 1 and 2 diets. However, there were no differences in the FHP of pigs among dietary treatments. The DE, ME, and NE of HP-SFM determined using the P-deficient basal diet were 3,328, 3,057, and 2,062 kcal/kg, as-fed basis,

respectively, whereas, values determined using the P-adequate basal diet were 3,366, 3,037, and 2,151 kcal/kg, respectively. Although no differences were observed in the energy values, measurements found in the current study (e.g., urinary N excretion, RE as protein and lipid) may suggest that a diet containing adequate amount of P is a more suitable basal diet when the difference method is used for the calculation of NE value in feed ingredients.

Key words: indirect calorimetry, net energy, pig, phosphorus, sunflower meal

8.2 INTRODUCTION

Cost-effective coproducts have been widely used in the swine industry to reduce the total production cost due to a drastic increase in feed cost over the last decade (Woyengo et al., 2014). Sunflower meal (SFM), which is obtained after oil extraction from sunflower seeds, is one of the most widely used coproduct as a protein source in livestock feeds (Waititu et al., 2018a,b). Furthermore, advanced de-hulling technology has been developed for further processing of the de-hulled SFM to produce a high protein SFM (HP-SFM), now available for use in livestock diets.

Of the available energy systems, the net energy (NE) system provides a more accurate estimate of the dietary energy available to pigs because digestible energy (DE) and metabolizable energy (ME) systems often overestimate the energy contents of diets and ingredients high in fiber or protein (Noblet et al., 1994). Therefore, the NE value should be used to express the energy content of HP-SFM to accurately supply available dietary energy to pigs. However, there has been no information on NE value of HP-SFM fed to growing pigs.

The difference method has often been used to determine energy contents of feed

ingredients (Velayudhan et al., 2015b; Kim et al., 2017, 2018) because some ingredients cannot be fed alone due to low palatability and anti-nutritional factors present in some ingredients (Kong and Adeola, 2014). This method requires a basal diet and a test diet in which a portion of the basal diet is replaced with the test ingredient to calculate energy contents of the test ingredient. Depending on the nutrient composition of the basal diet and the test ingredient, diets can be nutritionally unbalanced or deficient in specific nutrients. This may significantly affect the absolute NE value of the test ingredient because this diet cannot fully support optimal growth and lean tissue gain of pigs (Noblet et al., 1993).

In a previous study, we found that a basal diet that is nutritionally unbalanced with respect to amino acids (AA) content may be inappropriate to determine NE value of feed ingredients (Kim et al., 2018). Thus, it is prudent to suppose that content of other essential nutrients, specifically phosphorus (P), in a basal diet may also affect the absolute NE value of feed ingredients determined by the difference method because P also plays important roles in the body, thereby affecting growth and development of pigs. Indeed, insufficient P supply led to a reduction in feed intake (FI) and body weight gain (Baker et al., 2013), and an increase in urinary nitrogen (N) excretion due to reduced protein deposition (Varley et al., 2011). However, dietary P contents had no effects on digestibility of AA, fat, dry matter (DM), and gross energy (GE) (Johnston et al., 2004).

Based on these observations, it can be speculated that DE of the ingredient determined using P-deficient and P-adequate basal diets would not be different because digestibility of energy and nutrients are not affected by dietary P content, but ME would be affected because of increased urinary N excretion, therefore increased urinary energy loss. The NE of ingredient would also be influenced due to an increase in lipid deposition rather than protein deposition

(Noblet and van Milgen, 2013). Therefore, the objectives of the current study were 1) to determine DE, ME, and NE of HP-SFM and 2) to compare the energy values of HP-SFM determined using either the P-deficient basal diet or the P-adequate basal diet.

8.3 MATERIALS AND METHODS

The experimental protocol used in this experiment was reviewed and approved by the University of Manitoba Animal Care Committee, and pigs were handled according to the guidelines described by the Canadian Council on Animal Care (2009).

8.3.1 Animals, Housing, and Experimental Diets

Twenty-four growing barrows [initial body weight (BW) of 19.3 ± 1.8 kg] that were the offspring of Tempo boars mated to TN70 females (Topigs Norsvin, Winnipeg, MB, Canada) were used in the current study. All pigs were individually housed in adjustable metabolism crates (1.8 by 0.6 m) located in a temperature-controlled room (24 ± 1°C). The metabolism crates were equipped with smooth transparent plastic sides and plastic-covered expanded metal sheet flooring with a screen underneath for fecal collection and a stainless-steel urine tray underneath the fecal screen, which allowed for the total, but separate, collection of feces and urine from each pig.

The corn and soybean meal (SBM) samples used in the current study were obtained from the Glenlea Research Station, University of Manitoba, and HP-SFM was acquired from Bunge Global Innovation, Spain (Table 8.1). The sunflower was grown in Bulgaria. Four experimental diets including two corn-SBM-based basal diets containing 2 levels of standardized total tract digestible (STTD) P [i.e., P-deficient basal diet (formulated without inorganic P inclusion), and

P-adequate basal diet (formulated with inorganic P inclusion)] and the other two diets containing 30% HP-SFM with each basal diet were formulated (Table 8.2). To formulate the diets containing 30% of HP-SFM in each basal diet, 30% of each basal diet was replaced with the same amount of HP-SFM. Then, the DE, ME, and NE of HP-SFM were determined by the difference method (Adeola, 2001) using the P-deficient or P-adequate diet as a basal diet. Amino acids, vitamins, and minerals except P were included in the experimental diets to meet or exceed the requirements for growing pigs (NRC, 2012).

Table 8.1. Analyzed nutrient composition of corn, soybean meal, and high protein sunflower meal (HP-SFM), as-fed basis

	Ingredient		
Item	Corn	Soybean meal	HP-SFM
Dry matter, %	89.3	91.0	92.7
Gross energy, kcal/kg	3,908	4,219	4,205
Crude protein, %	8.3	48.0	48.5
Ether extract, %	3.1	1.0	0.9
Neutral detergent fiber, %	9.8	6.7	13.1
Acid detergent fiber, %	2.8	5.3	9.0
Ash, %	0.9	6.2	9.4
Calcium, %	0.01	0.43	0.43
Phosphorus, %	0.21	0.72	1.87

Table 8.2. Ingredient and nutrient composition of experimental diets, as-fed basis

Item, %	Diets[1]			
	P-deficient basal	P-adequate basal	HP-SFM 1	HP-SFM 2
Ingredients				
Corn	68.460	68.460	47.922	47.922
Soybean meal	28.200	28.200	19.740	19.740
HP-SFM[2]	-	-	30.000	30.000
Vegetable oil	1.000	1.000	0.700	0.700
Corn starch	0.700	-	0.490	-
Lysine-HCl	0.100	0.100	0.070	0.070
Limestone	1.000	1.000	0.700	0.700
Monocalcium phosphate	-	0.700	-	0.490
Salt	0.400	0.400	0.280	0.280
Vitamin-mineral premix[3]	0.140	0.140	0.098	0.098
Calculated composition, %				
Metabolizable energy, kcal/kg	3,414	3,386	3,360	3,285
SID[4] lysine	0.98	0.98	1.09	1.09
SID[4] methionine	0.28	0.28	0.40	0.40
STTD[5] phosphorus	0.16	0.30	0.22	0.32
Analyzed composition, %				
Dry matter	88.6	88.9	90.1	90.1
Gross energy, kcal/kg	3,949	3,914	4,027	4,004
Crude protein	19.2	19.2	27.4	26.9
Ether extract	2.7	2.8	2.7	2.6
Neutral detergent fiber	8.7	8.2	9.6	9.9
Acid detergent fiber	3.3	3.2	4.8	4.9
Ash	3.2	4.0	5.1	5.7
Calcium	0.41	0.67	0.42	0.59
Phosphorus	0.33	0.48	0.81	0.90

[1]P-deficient basal = a corn-soybean meal-based basal diet without inorganic phosphorus inclusion; P-adequate basal = a corn-soybean meal-based basal diet with inorganic phosphorus inclusion; HP-SFM 1 = a diet containing 70% of P-deficient basal and 30% of high-protein sunflower meal; HP-SFM 2 = a diet containing a 70% of P-adequate basal and 30% of high-protein sunflower meal.

[2]HP-SFM = high protein sunflower meal.

[3]Supplied the following per kilogram of finished feed: vitamin A, 2,000 IU; vitamin D, 200 IU; vitamin E, 40 IU; vitamin K, 2 mg; choline, 350 mg; pantothenic acid, 14 mg; riboflavin, 7 mg; folic acid, 1 mg; niacin, 21 mg; thiamin, 1.5 mg; vitamin B6, 2.5 mg; biotin, 70 µg; vitamin B12, 20 µg; Cu, 10 mg; Zn, 110 mg; Fe, 120 mg; Mn, 10 mg; I, 0.4 mg; Se, 0.3 mg.

[4]SID = standardized ileal digestible.

[5]STTD = standardized total tract digestible. The values for corn, SBM, monocalcium phosphate were adapted from NRC (2012), whereas the value for HP-SFM was adapted from Lee et al., (2018; unpublished).

8.3.2 Experimental Design and Procedure

The current study was carried out in two consecutive periods (12 pigs per period) using similar experimental conditions and procedures and same facility because only 3 indirect calorimetry chambers were available for the measurement of heat production (HP) at the same time. Pigs were randomly allotted to 1 of 4 dietary treatments with 3 replicates per treatment (per period).

Pigs were fed their experimental diets at 550 kcal ME/kg $BW^{0.60}$ per day on the basis of BW on day 1, 5, and 10, which has been reported to be close to ad libitum intake (Noblet et al., 1994). During the study, experimental diets were provided to pigs at 0900 h once daily and pigs were trained to consume their daily feed allowance within 1 h after feeding. Pigs had free access to water via a low-pressure nipple drinker throughout the study. In each experimental period, pigs were fed experimental diets for 15 days, including 10 days of adaptation period to the experimental condition and diet. Total collection of feces and urine were performed separately from day 11 to 15 to determine DE and ME as previously described by Kim and Nyachoti (2017). Feces were collected once daily in the morning, weighed, and immediately stored at –20℃. Urine collection was initiated on day 11 at 0900 h and terminated on day 16 at 0900 h. Urine excretion was also collected once daily in plastic container containing 20 mL of 3 *N* HCl to minimize nitrogen (**N**) losses. The collected urine was weighed and a 5% aliquot was stored at −20℃. At the end of each collection period, urine samples were thawed, and thoroughly mixed, and a sub-sample was obtained by filtering with glass wool.

Before and after each experimental period, the accuracy of indirect calorimetry chambers was tested by burning ethanol in an indirect calorimetry system, based on the stoichiometric equation of ethanol burning. Theoretically, the ratio of CO_2 produced to O_2

consumed due to ethanol burning results in a respiratory quotient (RQ) of 0.667 (Benedict and Tompkins, 1916). The RQ value from 0.640 to 0.690 was considered acceptable range and no accuracy problem was observed during the entire experiments (average volume of O_2 consumed and CO_2 produced were 590 and 381 ml/kg/hr, respectively). On day 16, 3 pigs each were transferred to the indirect calorimetry chambers (1.22 × 0.61 × 0.91 m; Columbus Instruments, Columbus, OH) from the metabolism crates within 1 h of consuming their daily feed allowance. Then, the HP by pigs was calculated continuously for 24 h (fed-state) followed by 12 h (fasting-state) of fasting HP (FHP) based on O_2 consumption, CO_2 production, and urinary N excretion. The next sets of 3 pigs were moved to the indirect calorimetric chambers every 2 days (i.e., on day 18, 20, and 22) for measuring HP and FHP. Pigs were assigned to one of three indirect calorimetry chambers in a randomized complete block design using the chamber as a blocking factor to avoid possible confounding effects. Pigs had unlimited access to water via a nipple drinker during the measurement of HP and FHP, and urine voided during the measurements of HP (24 h) and FHP (12 h) was collected separately, weighed, sub-sampled, and stored at −20°C until required for N analysis. The temperature inside the chamber was maintained at 22℃ ± 1℃ using an air conditioner. Personnel movement in the chamber room was limited, except for the regular check and urine collection, to avoid distressing pigs during the measurements of HP and FHP.

8.3.3 Sample Preparation and Chemical Analyses

Within pig, collected fecal samples were pooled and dried in a forced-air drying oven at 60℃ for 5 days and finely ground before chemical analysis. Urine samples from metabolism crates and indirect calorimetry chambers were thawed, filtered through glass wool, and

transferred separately into a plastic vial for each pig. Diet and ingredients samples were ground through a 1-mm screen in a Thomas Wiley mill model 4 (Labwrench, Midland, ON, Canada) and thoroughly mixed before chemical analysis. Samples of diets and ingredients were analyzed in duplicate for DM, GE, crude protein (CP; N × 6.25), ether extract (EE), neutral detergent fiber (NDF), acid detergent fiber (ADF), ash, calcium (Ca), and P, whereas fecal and urine samples were analyzed in duplicate for DM, GE, and CP. Dry matter (method 934.01), EE (method 920.39A), and ash (942.05) contents were determined according to the Association of Official Analytical Chemists (AOAC, 2006). Gross energy content was measured using an isoperibol bomb calorimeter (model 6400; Parr Instrument, Moline, IL), which had been calibrated using benzoic acid as a standard. Nitrogen concentration was determined by the combustion method (984.13A-D; AOAC, 2006) using the LECO N analyzer (model CNS-2000; LECO Corp., St. Joseph, MI), and the N content was used to calculate the CP content (N × 6.25). The ADF and NDF contents were determined according to the method of Goering and van Soest (1970) using heat stable α-amylase (Sigma number A3306; Sigma-Aldrich, St. Louis, MO) and sodium sulfite. The Ca (method 968.08) and P (method 946.06) concentrations were determined according to the AOAC (2006) and read on a Varian inductively coupled plasma mass spectrometer (Varian Inc., Palo Alto, CA).

The GE content of urine was determined as described by (Kim and Nyachoti, 2017). Briefly, 0.5 g of cellulose was dried in a drying oven at 103℃ for 24 h, and then 2 mL of urine sample were added to dried cellulose, and the final weight of the resulting mixture was recorded. The urine-cellulose mixture and a sample of pure cellulose were dried at 50℃ for 24 h to determine dried urine content. The GE of the dried urine-cellulose mixture and cellulose itself were determined using an isoperibol bomb calorimeter as described above, from which the GE of

urine samples were calculated by the difference method.

8.3.4 Calculations

The apparent total tract digestibility (ATTD) of DM, GE, and CP, and N retention rate of pigs were calculated by the total collection method using the following equations:

$$ATTD\ (\%) = 100 \times [(CI - CO_{feces}) / CI],$$

where CI is the component intake (g) and CO_{feces} is the component output in feces (g).

$$\text{Nitrogen retention}\ (\%) = 100 \times [(NI - NO_{feces} - NO_{urine}) / NI],$$

where NI is the N intake (g), and NO_{feces} and NO_{urine} are the N output in feces and urine (g), respectively.

The HP and FHP (Brouwer, 1965), retained energy (RE; Noblet et al., 1994), and NE content (Noblet et al., 1994) were calculated using the following equations:

$$HP = 3.866 \times O_2 + 1.200 \times CO_2 - 1.431 \times \text{urinary N excretion},$$

where HP is in kilocalories, O_2 is oxygen consumption in liters, CO_2 is carbon dioxide production in liters, and urinary N excretion is total urinary N excretion in grams. The FHP was also calculated using the same equation for HP.

$$RE = ME - HP,$$

where RE, ME, and HP are in kilocalories per day.

Retained energy as protein (RE_P) was calculated from N retention by pigs (N × 6.25 × 5.68, kcal/g) according to Ewan (2001). Retained energy as lipid (RE_L) was calculated as the difference between RE and RE_P (Labussière et al., 2009).

$$DE = (GE_i - GE_f) / DMI,$$

$$ME = (GE_i - GE_f - GE_u) / DMI,$$

NE = (RE + FHP) / DMI,

where DE, ME, and NE are the DE, ME and NE contents of a diet in kcal/kg of DM, respectively. The GE_i, GE_f, and, GE_u are the total GE intake, total fecal GE output, and total urinary GE output in kilocalories, respectively. The RE and FHP are in kilocalories per day, and DMI is DM intake in kilograms.

The RQ was calculated as the ratio of CO_2 produced to O_2 consumed (Noblet et al., 2001).

The DE, ME, and NE of HP-SFM were calculated by subtracting the energy contribution of the basal diet (i.e., P-deficient basal diet, P-adequate basal diet) from the energy content of the diets containing 30% of HP-SFM.

8.3.5 Statistical Analysis

All data were analyzed using the MIXED procedure of SAS, and the individual pig served as the experimental unit. The initial model included diet and period as the fixed effect, and pig as the random effect for statistical analysis. However, the effect of period was not significant in this study; therefore, it was excluded in the final model. The LSMEANS procedure was used to calculate mean values and the PDIFF option with the Tukey's adjustment was used to separate means. Differences were considered significant at $P < 0.05$.

8.4 RESULTS

8.4.1 Apparent Total Tract Digestibility of Dry Matter, Gross Energy, and Crude Protein and Nitrogen Utilization of Diets

The ATTD of DM and GE was higher ($P < 0.01$) in the P-deficient and P-adequate basal

diets than in the HP-SFM 1 and 2 diets (Table 8.3). However, the ATTD of CP was not affected by dietary treatment.

Nitrogen intake (g) and fecal N excretion (g) of pigs fed the P-deficient and P-adequate basal diets was lower ($P < 0.01$) compared with those fed the HP-SFM 1 and 2 diets. The urinary N excretion (g) was lower ($P < 0.01$) in pigs fed the P-adequate basal diet than those fed the P-deficient basal diet and the HP-SFM 1 and 2 diets. However, a lower ($P < 0.01$) amount of N (g) was retained in pigs fed the P-deficient basal diet compared to those fed the other diets. Pigs fed the P-adequate basal diet had higher ($P < 0.01$) N retention (%) than those fed the other diets.

8.4.2 Energy Utilization and Content of Experimental Diets

The energy utilization of experimental diets fed to growing pigs and energy values of experimental diets are presented in Table 8.4. The DE of the P-deficient basal diet was higher ($P < 0.05$) than that of the HP-SFM 2 diet, however, that of P-adequate basal and HP-SFM 1 diet were not different. A higher ($P < 0.01$) ME value was observed in the P-deficient and P-adequate basal diets than in the HP-SFM 1 and 2 diets. The THP of pigs fed the HP-SFM 1 and 2 diets was higher ($P < 0.01$) than those fed the P-deficient basal diet with the intermediate value for pigs fed the P-adequate diet. The total RE was higher ($P < 0.01$) in pigs fed the P-deficient and P-adequate basal diets than in those fed the HP-SFM 1 and 2 diets. The RE_P of pigs fed the P-deficient basal diet was lower ($P < 0.01$) but RE_L was higher ($P < 0.01$) than those fed the P-adequate basal, and HP-SFM 1 and 2 diets. However, there was no difference in the FHP of pigs among the dietary treatments. The NE of the P-deficient and P-adequate diets was higher ($P < 0.01$) than that of the HP-SFM 1 and 2 diets.

The P-adequate basal diet had a higher ($P < 0.01$) ME:DE ratio compared to the HP-

SFM 1 and 2 diets, with the P-deficient basal diet being intermediate. In addition, the NE:DE ratio of the P-deficient basal diet was higher ($P < 0.01$) compared with that of the HP-SFM 1 and 2 diets. Similarly, a higher ($P < 0.01$) NE:ME ratio was observed in the P-deficient basal diet than in the HP-SFM 2 diet.

8.4.3 Energy Values of High Protein Sunflower Meal

The DE, ME, and NE of HP-SFM determined using the P-deficient basal diet were 3,328, 3,057, and 2,062 kcal/kg, as-fed basis, respectively, whereas, values determined using the P-adequate basal diet were 3,366, 3,037, and 2,151 kcal/kg, respectively (Table 8.5). However, there was no differences in the energy values between the values determined using either the P-deficient basal diet or the P-adequate basal diet.

Table 8.3. Apparent total tract digestibility (ATTD) of dry matter (DM), gross energy (GE), and crude protein (CP) and nitrogen (N) balance of experimental diets fed to growing pigs[1]

Item	Dietary treatment[2]				SEM	*P*-value
	P-deficient basal	P-adequate basal	HP-SFM 1	HP-SFM 2		
ATTD, %						
DM	88.7[a]	89.1[a]	85.0[b]	85.1[b]	0.45	<0.001
GE	87.4[a]	87.8[a]	85.1[b]	85.3[b]	0.54	0.003
CP	86.8	87.9	85.8	85.9	0.90	0.333
N balance, g/d						
N intake	33.0[b]	32.8[b]	47.1[a]	46.9[a]	0.95	<0.001
Fecal excretion	4.3[b]	4.0[b]	6.7[a]	6.7[a]	0.46	<0.001
Urinary excretion	14.2[a]	6.9[b]	17.0[a]	16.2[a]	1.11	<0.001
N retained	14.5[b]	21.9[a]	23.4[a]	24.1[a]	1.50	0.001
N retained, %	43.9[b]	66.8[a]	49.6[b]	51.2[b]	2.88	0.005

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

[1]Each value represents the mean of 6 observations.

[2]P-deficient basal = a corn-soybean meal-based basal diet without inorganic phosphorus inclusion; P-adequate basal = a corn-soybean meal-based basal diet with inorganic phosphorus inclusion; HP-SFM 1 = a diet containing 70% of P-deficient basal and 30% of high-protein sunflower meal; HP-SFM 2 = a diet containing a 70% of P-adequate basal and 30% of high-protein sunflower meal.

Table 8.4. Energy utilization of experimental diets fed to growing pigs and energy values of experimental diets[1]

Item	Dietary treatment[2]				SEM	*P*-value
	P-deficient basal	P-adequate basal	HP-SFM 1	HP-SFM 2		
Energy value, kcal/kg DM						
DE	3,897[a]	3,864[ab]	3,805[ab]	3,794[b]	24.2	0.021
ME	3,701[a]	3,707[a]	3,580[b]	3,578[b]	24.0	0.001
THP[3]	1,791[b]	1,937[ab]	2,111[a]	2,102[a]	58.3	0.004
Total RE[4]	1,900[a]	1,773[a]	1,475[b]	1,473[b]	65.5	<0.001
As protein	489[b]	778[a]	805[a]	841[a]	55.0	0.001
As lipid	1,411[a]	995[b]	670[b]	632[b]	93.6	<0.001
FHP[5]	1,208	1,269	1,368	1,352	50.7	0.130
NE[6]	3,108[a]	3,042[a]	2,843[b]	2,825[b]	46.5	0.001
Respiratory quotient						
Fed state	1.03	1.02	1.06	1.04	0.017	0.274
Fasting state	0.73	0.75	0.72	0.71	0.011	0.070
Energy utilization						
ME:DE	0.950[ab]	0.959[a]	0.941[b]	0.943[b]	0.0026	0.001
NE:DE	0.799[a]	0.786[ab]	0.746[b]	0.745[b]	0.0122	0.014
NE:ME	0.841[a]	0.820[ab]	0.793[ab]	0.791[b]	0.0123	0.028

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

[1]Each value represents the mean of 6 observations.

[2]P-deficient basal = a corn-soybean meal-based basal diet without inorganic phosphorus inclusion; P-adequate basal = a corn-soybean meal-based basal diet with inorganic phosphorus inclusion; HP-SFM 1 = a diet containing 70% of P-deficient basal and 30% of high-protein sunflower meal; HP-SFM 2 = a diet containing a 70% of P-adequate basal and 30% of high-protein sunflower meal.

[3]Total heat production = $(3.87 \times O_2 + 1.20 \times CO_2 - 1.43 \times$ urinary N)/DMI.

[4]Total RE = (ME intake − HP)/DMI; Retained energy as protein was calculated according to Ewan (2001) as N retention (g) × 6.25 × 5.68 (kcal/g). Whereas, retained energy as lipid was calculated as the difference between total retained energy and retained energy as protein.

[5]Fasting heat production = $(3.87 \times O_2 + 1.20 \times CO_2 - 1.43 \times$ urinary N)/DMI.

[6]Net energy = (RE + FHP)/DMI.

Table 8.5. Digestible (DE), metabolizable (ME), and net energy (NE) of high protein sunflower meal (HP-SFM) fed to growing pigs determined by the difference method using P-deficient basal and P-adequate basal diets[1]

Item	Energy content of HP-SFM		SEM	*P*-value
	P-deficient basal	P-adequate basal		
As-fed basis, kcal/kg				
DE	3,328	3,366	98.8	0.793
ME	3,057	3,037	90.3	0.881
NE	2,062	2,151	82.6	0.469
DM basis, kcal/kg				
DE	3,591	3,631	106.6	0.795
ME	3,298	3,276	97.4	0.879
NE	2,224	2,320	89.2	0.470

[1]Each value represents the mean of 6 observations.

8.5 DISCUSSION

8.5.1 Chemical Composition of Ingredients and Diets

Sunflower meal, a coproduct of sunflower seed crushing industry, has been widely used as an alternative to SBM in swine diets (Dadalt et al., 2016). The CP content of HP-SFM (48.5%) used in the current study was greater than that of de-hulled SFM reported by NRC (2012; 39.9%). In addition, the NDF and ADF contents were lower than the NRC (2012) values, which is mainly due to the differences in the method and degree of oil extraction. A new processing technique has been developed to improve the quality of de-hulling process and to allow further reductions in fiber components of SFM, by which the CP content of HP-SFM can reach approximately 46%, and fiber contents can be reduced significantly (Waititu et al., 2018a,b). The CP content was inversely associated with the crude fiber, NDF, and ADF contents, therefore indicating that the greater CP content of HP-SFM was mainly because of greater extent of dehulling (Villamide and San Juan, 1998). The nutritive values of HP-SFM fed to broiler chickens (Waititu et al., 2018a) and standardized ileal digestibility of AA in pigs (Dadalt et al., 2016) has been reported. However, no information on energy values of HP-SFM fed to pigs have been reported although DE, ME, and NE contents in regular SFM has been reported recently (Li et al., 2018c). Energy contents of a diet and an ingredient high in fiber and protein tend to be overestimated when determined with the DE and ME systems (Noblet et al., 1994), which necessitates the determination of the NE value for HP-SFM to facilitate its effective utilization.

The nutrient composition of the corn and SBM used in the current study agree with reported values (NRC, 2012; Kim et al., 2017, 2018). Also, the analyzed nutrient and energy contents of experimental diets were similar to the values calculated from the inclusion level and

analyzed composition of the ingredients. As expected, the GE, CP, NDF, and ADF contents increased as a result of HP-SFM inclusion in each basal diet.

8.5.2 Apparent Total Tract Digestibility of Dry Matter, Gross Energy, and Crude Protein and Nitrogen Utilization of Diets

In the current study, a lower ATTD of DM and GE was observed in the diets containing HP-SFM compared to the two basal diets, which is most likely attributable to the addition of HP-SFM resulting in increased dietary fiber contents as reported in previous studies (Kim and Nyachoti; 2017; Kim et al., 2018; Li et al., 2018c). However, the extent of reduction in the ATTD of DM as a result of SFM inclusion to the basal diet (percentage change; 4.3%) was much less than the value (percentage change; 11.6%) reported in the study of Li et al. (2018c) although similar level of SFM (29.5%) was included in the corn-SBM basal diet. The fiber contents in SFM likely led to this observation because the SFM used in the current study was extensively de-hulled SFM, and therefore contained less NDF and ADF contents compared to the SFM used in their study (NDF, 13.1% vs, 21.0%; ADF, 9.0% vs. 11.0%).

Phosphorus and protein deposition is closely correlated and it has been estimated that approximately 35 g of P is required to retain each kg of body protein (NRC, 2012). Standardized total tract digestible P content in the P-deficient basal diet was 0.16% which was about half of the recommended P requirement for this BW class of pigs (i.e., 0.31%), Therefore, an increase in urinary N excretion and decreases in daily N retention (g) and N retention rate (%) by pigs fed the P-deficient diet were expected although other essential nutrients (e.g., AA, Ca) were included to meet or exceed the requirements. Indeed, pigs fed the P-deficient basal diet retained significantly less amount of N compared to those fed the other diets in the current study. This

result is in agreement with the previous observation reported by Varley et al. (2011). In their study, pigs fed the diet containing 0.45% of total P had less daily N retention (g) and N retention rate (%) compared to those fed the diet containing 0.64% of total P. The lack of difference in daily N retention (g) among pigs fed the P-adequate basal diet and HP-SFM 1 and 2 diets was likely due to the high P content in HP-SFM (1.87%). To formulate the SFM 1 and 2 diets, 30% of each basal diet was replaced with the same amount of HP-SFM, which elevated the total dietary P and dietary STTD P contents, therefore met the P requirement.

8.5.3 Energy Utilization and Content of Experimental Diets

The ME:DE ratio was highest for the P-adequate basal diet and lowest for the HP-SFM 1 and 2 diets with the value for the P-deficient basal diet being intermediate in the current study. The higher CP content in the diets containing HP-SFM (on average 27.1%) than the requirement of pigs and greater N excretion by pigs fed the P-deficient basal diet compared to those fed the P-adequate basal diet may explain this observation. It has been well demonstrated that excessive AA intake more than the requirement for protein deposition is catabolized during metabolism and excreted through urine, which results in increased urinary energy loss and therefore decreased ME:DE ratio (Noblet and Perez, 1993; Le Bellego et al., 2001).

In the current study, pigs fed the diets containing HP-SFM had higher THP compared to those fed the P-deficient basal diet, which is likely attributable to an increase in dietary CP content as a consequence of the addition of HP-SFM (Noblet et al., 1994; Le Bellego et al., 2001; Noblet and van Milgen, 2013). Replacing 30% of basal diet with 30% of HP-SFM led to a decrease in dietary starch content but an increase in dietary CP content. Dietary protein has greater heat increment per unit of energy associated with metabolic utilization of energy than that

of starch because dietary protein ingestion increases protein turnover, an important activity in metabolically active organs such as liver, kidneys, and digestive tract, and energy cost related to urea synthesis for urinary excretion, resulting in less energetic efficiency (Noblet et al., 1994; Le Bellego et al., 2001; Noblet et al., 2001). The observation that pigs fed the diets containing HP-SFM retained less energy than those fed the two basal diets is also likely due to an increase in heat increment associated with high dietary protein.

The average FHP estimate of 1,299 kcal/kg DM (154 kcal/$BW^{0.6}$) observed in the current study were within the range observed in similar studies conducted with the same facility and experimental procedures (from 143 to 252 kcal/$BW^{0.6}$; Ayoade et al., 2012a; Heo et al., 2014; Velayudhan et al., 2015b; Kim and Nyachoti, 2017).

8.5.4 Energy Values of High Protein Sunflower Meal Determined Using a Phosphorus Deficient Basal diet and a Phosphorus Adequate Basal Diet

The difference method has been increasingly used to determine NE value of feed ingredients and several studies have reported no difference between determined NE using indirect calorimetry and predicted NE from prediction equations (Heo et al., 2014; Kim and Nyachoti, 2017; Li et al., 2018c). However, calculation of NE value of test ingredient determined by the difference method can be significantly biased by nutrient composition of the basal diet if nutrients composition of the basal diet is insufficient to support optimal growth of pigs (Kim et al., 2018). This is because the efficiency of dietary ME utilization depends on final utilization of dietary energy (i.e., deposition as protein vs. deposition as lipid). According to Noblet and van Milgen (2013), the energetic efficiency of dietary ME utilization is greater for lipid deposition than for protein deposition (80% vs. 60%). Therefore, dietary NE value for pigs should be

measured using balanced diets that meet nutrient requirements to support optimal growth and lean tissue gain (Noblet et al., 1993a, Noblet and van Milgen, 2013). Indeed, Li et al. (2018c) reported different NE values for corn fed to growing pigs when it was supplemented with dietary crystalline AA. Also, in our previous study (Kim et al., 2018), it was concluded that the corn-SBM basal diet that meets the nutrient requirement of pigs is a more suitable basal diet than the corn basal diet that contains insufficient AA to support growth and lean tissue gain.

In the current study, effects of dietary P content of a basal diet on energy values of HP-SFM determined by the difference method were evaluated. As hypothesized, the DE of HP-SFM determined using the P-deficient and P-adequate basal diets were not different (3,328 vs. 3,366 kcal/kg, as-fed basis), which is supported by no differences in the ATTD of DM, GE, and CP between two basal diets. Contrary to our hypothesis, however, no differences in the ME (3,057 vs. 3,037 kcal/kg, as-fed basis) and NE (2,062 vs. 2,151 kcal/kg, as-fed basis) of HP-SFM determined using the P-deficient basal diet and the P-adequate basal diet were also observed. Theoretically, significantly greater urinary N excretion observed in pigs fed the P-deficient basal diet should be extended to a decrease in ME value of an ingredient because greater urinary N excretion, therefore greater urinary energy loss would lead to underestimation of dietary ME (Noblet and van Milgen, 2013). Moreover, lower RE_P and greater RE_L observed in pigs fed the P-deficient basal diet than in those fed the P-adequate basal diet did not result in different NE value in HP-SFM despite the fact that the energetic efficiency of dietary ME utilization for protein deposition is less than for lipid deposition (Noblet and van Milgen 2013). Although there were reasonable supporting data to have different ME and NE values of HP-SFM determined by the P-deficient and P-adequate basal diets in the current study, we failed to observe the differences, which can be possibly explained by one of the inherent problems associated with

using the difference method (Kil et al., 2010). The energy values calculated with this method are generally associated with large SEM values. In case of this study, the SEM values for the ME and NE of HP-SFM were much greater than the SEM values for the ME and NE of the diets (ME; 24 vs. 90.3, NE; 46.5 vs. 82.6).

In conclusion, the DE, ME, and NE of HP-SFM determined in the current study were, on average, 3,347, 3,047, and 2,107 kcal/kg, respectively, as-fed basis. The energy values determined by using P-deficient and P-adequate diets were not different. However, measurements (e.g., urinary N excretion, RE_P, and RE_L) observed in the current study may suggest that a diet containing adequate amount of P is more suitable basal diet when the difference method is used for the calculation of NE value in a feed ingredient.

CHAPTER 9

GENERAL DISCUSSION

Feed accounts for the largest portion for total costs of swine production, and within that cost ingredients supplying dietary energy represent the greatest portion of the feed cost (Noblet and van Milgen, 2013; Velayudhan et al., 2015a). Energy is not a nutrient, however, proper amount of dietary energy should be provided to pigs for various functions such as maintaining physiological functions within the body, and for developing organs and body tissues. Moreover, dietary energy concentration has a great influence on FI because pigs consume feeds until energy requirements are fulfilled (Nyachoti et al., 2004). Therefore, dietary energy is one of the most important aspects related to swine nutrition (Chiba, 2000).

The costs of corn and SBM, the most commonly used energy and protein sources for swine diets, have risen dramatically. Cost-effective coproducts rich in dietary fiber or in protein have been used increasingly in swine diets to reduce feed cost (Woyengo et al., 2014). This could be one strategy to reduce total production costs if nutritive and energy values are accurately determined. However, one of the biggest concerns against feeding these ingredients to pigs is their high dietary fiber contents. The DE and ME systems tend to overestimate the energy content of high fiber and high protein ingredients and diets because metabolic utilization of energy during energy utilization is not taken into consideration in these systems, thereby supplying insufficient amount of available energy to pigs. Thus, it is important to use an appropriate energy evaluation system that accurately determines the amount of available energy in ingredients so as to supply adequate energy in swine diets. Among the available energy evaluation systems, the NE system is considered the most accurate system. However, NE value

of ingredients is also affected by several factors including diet composition, experimental methodology, antinutritional factors, and physiological status as are DE and ME values.

Although NE values of ingredients can be taken from feeding tables (e.g., Sauvant et al., 2004; NRC, 2012) or predicted using prediction equations for diet formulation, direct determination of NE contents in coproducts are still necessary because nutritionists and feed industry may hesitate to include these ingredients into swine diets due to the uncertainty of nutritive values of coproducts. For accelerating adoption of the NE system for swine diets, empirical data on the energy contents of specific coproducts and critical factors relevant to NE values of ingredients needs to be determined. Hence, the overall objectives of this study were to determine the effects of factors that may affect the NE values of ingredients and diets and to evaluate the NE contents of selected coproducts. The results from the studies conducted to determine the above objectives are discussed here.

In manuscript I, the energy contents of HH and processed HH products including EHH and HHP fed to growing pigs were determined using the IC method. Determined NE and predicted NE of these ingredients using prediction equation were also compared because predicted NE may differ from directly determined NE due to extremely high fiber contents in HH (NSP content, 33%). Results from this study showed that the NE of HH, EHH, and HHP determined using the IC method were 2,375, 2,320, and 2,399 kcal/kg DM, respectively, and these values were 2.9, 7.1, and 5.2% higher, respectively, compared with those obtained with prediction equations (2,308, 2,161, and 2,278 kcal/kg DM, respectively). However, no differences were observed between determined and predicted values, which suggests that prediction equations well predict the NE of ingredients containing high fiber.

In manuscript II, the energy content of CC and the effects of antinutritional factor (i.e.,

glucosinolates) originating from CC on additivity of energy in mixed diets containing increasing inclusion levels of CC were determined. Dietary glucosinolate concentration should be maintained in a range of 1.5 to 2.0 μmol/g in swine diets to avoid its negative effects on pig performance resulting from endocrine disturbance (Tripathi and Mishra, 2007). According to this safety inclusion level, it was hypothesized that NE contents (2,383 kcal/kg, as-fed basis) determined with 30% of CC would be underestimated because of the high dietary glucosinolates concentrations in the test diet and, therefore, would not show additivity of energy in the mixed diets containing 10 or 20% of CC and other multiple ingredients, which contains less dietary glucosinolates (calculated glucosinolate concentrations in the diets containing 10, 20, and 30% of CC were 2.8, 5.6, and 8.4 μmol/g, respectively). However, the results indicated that there was an additivity of energy in mixed diets containing corn, SBM, and CC up to 30%. This might have been due to the fact that enzymatic hydrolysis of the glucosinolates in CC may produce only nonvolatile isothiocyanates because of the long carbon chains in its residue. In addition to this, camelina does not contain progoitrin, which can be converted into toxic goitrin. Therefore, the adverse effect of glucosinolates in CC may be less than that in rapeseed and rapeseed coproducts (Matthäus and Zubr, 2000).

In manuscript III, the energy content of CM and effects of nutrient composition of basal diets (i.e., AA contents) and experimental methodology (difference method vs. regression method) on energy values of CM were evaluated. The efficiency of ME utilization of a diet depends on final utilization of dietary energy in pigs (i.e., deposition as protein vs. deposition as lipid). If the energy is deposited as protein, the efficiency of ME utilization of the diet will be less than when it is deposited as lipid because the efficiency of ME utilization for protein deposition is about 60% whereas that for lipid deposition is approximately 80% (Noblet and van Milgen 2013), which

may have significant effect on NE of the diet. Therefore, it has been suggested that the NE of a diet should be determined based on balanced diets that meet nutrient requirements of pigs for supporting optimal growth and lean tissue gain (Noblet et al., 1993a, Noblet and van Milgen, 2013). For this reason, a corn diet, the most commonly used basal diet for DE and ME determination, may not be appropriate for NE determination because this basal diet contains insufficient dietary AA and N to support optimal growth performance, and therefore would affect the NE of the diet. The results from this study showed that NE value of CM fed to growing pigs determined by the difference and regression methods were not different (on average, 2,099 kcal/kg, as-fed basis), which is similar to the observation with wheat bran (Jawarski et al., 2016). However, the r^2 of the regression analysis from the corn-SBM diet (adequate AA) was relatively greater than that from the corn diet (inadequate AA), which suggests that the basal diet that meets the nutrient requirements of pigs is more suitable for NE determination of feed ingredients than a basal diet that is nutritionally unbalanced.

In manuscript IV, the effect of increasing dietary NE concentrations adjusted by either dietary fat or fiber content on growth performance and energy intake of weaned pigs and the effect of physiological status (i.e., age) on NE value of diets were determined. Feed intake of growing-finishing pigs is affected by various factors such as environmental conditions, health status, genotypes, and diet composition (Nyachoti et al., 2004). Among them, dietary energy concentration is considered an important determinant of FI (Henry, 1985). As available dietary energy concentration decreases, pigs will attempt to maintain constant daily energy intake by consuming more feed, until physical FI capacity or other environmental factors restrict FI (Beaulieu et al., 2009; Quiniou and Noblet, 2012). For these reasons, the ability of young pigs (less than 20 kg), which have an immature gut system and limited physical gut capacity, to adjust

FI in response to wide range of dietary energy concentrations is known to be limited (Black et al., 1986). Results indicated that weaned pigs weighing less than 20 kg were unable to adjust FI in response to dietary NE concentration regardless of diet composition, therefore energy intake increased with increasing dietary energy concentrations. In terms of effects of age on NE values of diets, NE concentration of experimental diets (pooled within week) linearly increased with increasing age of weaned pigs from 1 to 3 week. This observation is in line with previous results reported by Le et al. (2016) who observed increased DM and GE digestibilities in weaned pigs from 1 to 3 weeks of age. Considering that GE and nutrients digestibilities are lower in weaned pigs compared with in growing pigs and the fact that NE values of feed ingredients in the feeding tables available for nursery diet formulation are predicted by the equations generated using growing pigs, careful use of NE values in the feeding tables is necessary to accurately supply dietary energy required by weaned pigs.

It has been reported that insufficient P supply led to a reduction in FI and BW gain (Baker et al., 2013), and an increase in urinary N excretion due to reduced protein deposition (Varley et al., 2011). However, dietary P content had no effect on digestibility of AA, fat, DM, and GE (Johnston et al., 2004). Based on these observations, in manuscript IV, it was hypothesized that the energy values of HP-SFM determined using the basal diets containing 2 levels of dietary P contents (i.e., P-deficient basal diet, P-adequate basal diet) would be different. The DE, ME, and NE of HP-SFM were also determined. Results indicated that the DE of HP-SFM determined using the P-deficient and P-adequate basal diets were not different (3,328 vs. 3,366 kcal/kg, as-fed basis), which is supported by no differences in the ATTD of DM, GE, and CP between two basal diets. Contrary to our hypothesis, however, there were no differences in the ME (3,057 vs. 3,037 kcal/kg, as-fed basis) and NE (2,062 vs. 2,151 kcal/kg, as-fed basis) of

HP-SFM determined using the P-deficient basal diet and the P-adequate basal diet. Theoretically, greater urinary N excretion observed in pigs fed the P-deficient basal diet should be associated with a reduction in ME value due to the fact that greater urinary N excretion, therefore greater urinary energy loss would lead to underestimation of dietary ME (Noblet and van Milgen, 2013). Moreover, lower RE_P and higher RE_L observed in pigs fed the P-deficient basal diet than in those fed the P-adequate basal diet did not result in different NE value in HP-SFM despite the fact that the energetic efficiency of dietary ME utilization for protein deposition is less than for lipid deposition (60% vs. 80%; Noblet and van Milgen 2013). The energy values calculated with the difference method often have large SEM values (Kil et al., 2010), which may explain the lack of differences between the ME and NE values of HP-SFM determined using two basal diets containing different level of dietary P. However, measurements (e.g., urinary N excretion, RE_P, and RE_L) observed in the current study may suggest that a diet containing adequate amount of P is more suitable basal diet when the difference method is used for the calculation of NE value in a feed ingredient.

By using multiple linear regression models based on DE, ME, NE, and chemical composition of test ingredients (i.e., HH, EHH, HHP, CM, and HP-SFM), following prediction equations for NE content (kcal/kg of DM) of ingredients for growing pigs from DE or ME and chemical composition has been generated.

1) NE (kcal/kg of DM) = 1,807 + 0.261 × DE + 0.32 × NDF + 0.18 × EE – 0.63 × CP – 1.20 × ADF ($P < 0.26$, $R^2 = 0.21$, RMSE = 274)

2) NE (kcal/kg of DM) = 1,169 + 0.45 × ME + 0.46 × ADF – 0.47 × CP – 0.51 × NDF – 0.28 × EE ($P < 0.09$, $R^2 = 0.29$, RMSE = 260)

3) NE (kcal/kg of DM) = 2,417 + 2.25 × EE + 1.33 × NDF + 0.15 × CP – 3.78 × ADF (*P*

< 0.24, R^2 = 0.17, RMSE = 274)

where NE, DE, and ME are in kilocalories per kilogram. DM, EE, CP, NDF, and ADF are in g/kg DM. However, all three equations had very low accuracy in terms of predicting NE contents of ingredients (i.e., low R^2) and these equations may not be applicable to predict NE contents of ingredients. High variations in NE value within the ingredient determined by the difference method and small number of replicates appear to lead to the low accuracy of equations.

In conclusion, results from the current study suggest that NE content of a feed ingredient determined by the difference method using IC would be similar to the value predicted using prediction equations. In addition, both difference and regression methods can be used for determining NE of an ingredient because NE contents determined by these methods were not different. These finding may suggest that NE content of an ingredient can be evaluated using common procedures for determination of dietary energy in feed ingredients. However, essential nutrients contents (i.e., AA and P) in a basal diet may have significant effect on energy value of an ingredient, therefore nutrient contents in a basal diet should be carefully considered to avoid bias in the calculation of NE of an ingredient when the difference method is used for determination of energy values. Antinutritional factors present in CC may have no influence on the energy value of diet and additivity energy of diets containing multiple ingredients. However, each ingredient contains different antinutritional factors that may have influences on energy values. Therefore, more investigations on antinutritional factors in different feed ingredients need to be guaranteed. Furthermore, dietary NE values increased with increasing age of weaned pigs. Hence, careful use of NE values determined using growing pigs are required to formulate piglet diets to accurately supply dietary energy required by weaned pigs.

CHAPTER 10

CONCLUSIONS AND FUTURE STUDIES

10.1. Conclusions

The following conclusions can be drawn from the present research:

1. The NE contents of HH, EHH, HHP, CC, CM, and HP-SFM determined in the current study were 2,204, 2,199, 2,190, 2,383, 2,099, and 2,107 kcal/kg, respectively, as-fed basis.
2. No differences between determined NE and predicted NE may suggest that prediction equations using chemical composition and dietary DE and ME contents published in NRC (2012) well predict the NE of ingredients high in dietary fiber.
3. The difference and regression methods do not give different NE value of an ingredient.
4. Additivity of DE, ME, and NE was observed in the mixed diets containing corn, SBM, and CC, which indicates that dietary glucosinolates originating from up to 30% of CC inclusion does not affect dietary DE, ME, and NE.
5. Goodness of fit for the linear model [i.e., greater r^2 of the regression analysis from the corn-SBM diet (balanced AA) than that from the corn diet (unbalanced AA)] suggests that a basal diet that meets the nutrient requirements of pigs is more suitable for NE determination of feed ingredients than one that is nutritionally unbalanced.
6. A diet containing adequate amount of P is a more suitable basal diet when the difference method is used for the calculation of NE value in a feed ingredient.
7. Dietary NE value increased with increasing age of weaned pigs regardless of diet composition.
8. Weaned pigs weighing less than 20 kg are unable to adjust FI in response to dietary NE concentration ranging from 9.9 to 10.7 MJ/kg which are slightly less and greater than the

NE requirement of weaned pigs.

10.2. Future Studies

1. Determine the energy value of an ingredient high in dietary soluble fiber and compare the value with predicted value.
2. Determine the effects of other antinutritional factors present in coproducts (e.g., tannin) on energy utilization in pigs.
3. Health status or disease may also affect the NE value of a diet because of increased NE_m. Therefore, this needs further investigation.
4. Validate the NE values of coproducts determined from the current study with growth performance trials where pigs will be fed with various inclusion levels of tested feed ingredients.
5. Because the number of replicates used in the current study to assess various factors affecting NE value of ingredients and diets was low, to enhance the robustness of the study, future study designs could consider using a larger number of replicates.

CHAPTER 11

LITERATURES CITED

Acosta, J. A., R. D. Boyd, and J. F. Patience. 2017. Digestion and nitrogen balance using swine diets containing increasing proportions of coproduct ingredients and formulated using the net energy system. J. Anim. Sci. 95:1243-1252.

Adeola, O. A. 2001. Digestion and balance techniques in pigs. In: A. J. Lewis and L. L. Southern, editors, Swine nutrition. 2nd ed. CRC Press, Boca Raton, FL. p. 903-916.

Agyekum, A. K., B. A. Slominski, and C. M. Nyachoti. 2012. Organ weight, intestinal morphology, and fasting whole-body oxygen consumption in growing pigs fed diets containing distillers dried grains with solubles alone or in combination with a multienzyme supplement. J. Anim. Sci. 90:3032-3040.

Agyekum, A. K., E. Kiarie, M. C. Walsh, and C. M. Nyachoti. 2016. Postprandial portal glucose and lactate fluxes, insulin production, and portal vein-drained viscera oxygen consumption in growing pigs fed a high-fiber diet supplemented with a multi-enzyme cocktail. J. Anim. Sci. 94:3760-3770.

Ahlin, K. A., M. Emmanuelson, and H. Wiktorsson. 1994. Rapeseed products from double low cultivars as feed for dairy cow: effects of long term feeding on thyroid function, fertility and animal health. Acta Vet. Scand. 35:37-53.

Almeida, F. N., J. K. Htoo, J. Thomson, and H. H. Stein. 2013. Amino acid digestibility in camelina seeds and camelina expellers fed to growing pigs. Can. J. Anim. Sci. 93:335-343.

AOAC. 1990. Official methods of analysis. 15th ed. Assoc. Offic. Anal. Chem., Arlington, VA.

AOAC. 2006. Official methods of analysis. 18th ed. Assoc. Off. Anal. Chem., Washington, DC, USA.

ARC. 1981. The nutrient requirements of pigs: Technical review. Rev. ed. Commonwealth Agricultural Bureau, Slough, England.

Ayoade, D. I., E. Kiarie, and C. M. Nyachoti. 2012a. Net energy of diets containing wheat- corn distillers dried grains with solubles as determined by indirect calorimetry, comparative slaughter, and chemical composition methods. J. Anim. Sci. 90:4373-4379.

Ayoade, D. I., E. Kiarie, T. A. Woyengo, B. A. Slominski, and C. M. Nyachoti. 2012b. Effect of a carbohydrase mixture on ileal amino acid digestibility in extruded full fat soybeans fed to finishing pigs. J. Anim. Sci. 90:3842-3847.

Aziza, A. E., N. Quezada, and G. Cherian. 2010. Feeding camelina sativa meal to meat-type chickens: effect on production performance and tissue fatty acid composition. J. Appl. Poult. Res. 19:157-168.

Bach Knudsen, K. E. and H. Jorgensen. 2001. Intestinal degradation of dietary carbohydrates-From birth to maturity. Pages 109-120 in Digestive Physiology in Pigs. Proceedings of the 8th symposium. J. E. Lindberg and B. Ogle, ed. CABI Publishing, Wallingford, UK.

Baker, S., B. Kim, and H. H. Stein. 2013. Comparison of values for standardized total tract digestibility and relative bioavailability of phosphorus in dicalcium phosphate and distillers dried grains with solubles fed to growing pigs. J. Anim. Sci. 91:203-210.

Baldwin, R. L. 1995. Energy requirements for maintenance and production. In: R. L. Baldwin, editor, Modeling ruminant digestion and metabolism. Chapman and Hall, London, UK. p. 148-188.

Baldwin, R. L., and A. C. Bywater. 1984. Nutritional energetics of animals. Annu. Rev. Nutr. 4:101-114.

Bastianelli, D., D. Sauvant, and A. Rerat. 1996. Mathematical modeling of digestion and nutrient

absorption in pigs. J. Anim. Sci. 74: 1873-1887.

Beaulieu, A. D., N. H. Williams, and J. F. Patience. 2009. Response to dietary digestible energy concentration in growing pigs fed cereal grain-based diets. J. Anim. Sci. 87:965-976.

Bell, J.M., 1993. Factors affecting the nutritional value of canola meal: a review. Can. J. Anim. Sci. 73:689-697.

Benedict, F. G., and E. H. Tompkins. 1916. Respiratory exchange, with a description of a respiration apparatus for clinical use. N. Engl. J. Med. 174:898-909.

Benson, B. N., C. C. Calvert, E. Roura, and K. C. Klasing. 1993. Dietary energy source and density modulate the expression of immunologic stress in chicks. J. Nutr. 123:1714-1723.

Birkett, S., and K. de Lange. 2001. Limitations of conventional models and a conceptual framework for a nutrient flow representation of energy utilization by animals. Br. J. Nutr. 86:647-659.

Black, J. L. 2000. Bioavailability: the energy component of a ration for monogastric animals. In: P. J. Moughan, M. W. A. Verstegen, and M. I. Visser-Reyneveld, editors, Feed evaluation: principle and practice. Wageningen Press, Wageningen, The Netherlands. p. 133-152.

Black, J. L. and C. F. M. de Lange. 1995. Introduction to the principles of nutrient partitioning for growth. In: P. J. Moughan, M. W. A. Verstegen, and M. I. Visser-Reynevel, editors, Modelling Growth in the Pig. Wageningen Press, Wageningen, The Netherlands. p. 115-122.

Black, J. L., R. G. Campbell, I. H. Williams, K. J. James, and G. T. Davies. 1986. Simulation of energy and amino acid utilisation in the pig. Res. Dev. Agric. 3:121-145.

Blaxter, K. L. 1989. Energy metabolism in animals and man. Cambridge University Press, Cambridge, UK.

Blok, M. C. 2006. Development of a new net energy formula by CVB, using the database of INRA. Int. Sym. Proc. Net energy systems for growing and fattening pigs. Vejle, Denmark. p. 40-57.

Boisen, S. 2007. New concept for practical feed evaluation systems. DJF Animal Science No. 79. Research Centre Foulum, Denmark.

Boisen, S., and M. W. A. Verstegen. 1998. Evaluation of feedstuffs and pig diets. Energy or nutrient-based evaluation systems? I. Limitations of present energy evaluation systems. Acta Agric. Scand. Sect. A Anim. Sci. 48:86-94.

Boisen, S., and M. W. A. Verstegen. 2000. Developments in the measurement of the energy content of feeds and energy utilisation in animals. In: P. J. Moughan, M. W. A. Verstegen, and M. I. Visser-Reyneveld, editors, Feed evaluation: principle and practice. Wageningen Press, Wageningen, The Netherlands. p. 57-76.

Bolarinwa, O. A., and O. Adeola. 2016. Regression and direct methods do not give different estimates of digestible and metabolizable energy values of barley, sorghum and wheat for pigs. J. Anim. Sci. 94:610-618.

Brafield, A. E., and M. J. Llewellyn. 1982. Animal energetics. Blackie, Glasgow, UK.

Brouwer, E. 1965. Report of subcommittee on constants and factors. In: Proc. 3rd EAAP Symp. Energy Metab. Troonn Publ. No. 11. Academic, London. p. 441-443.

Brown, D. 1982. The metabolic body size of the growing pig. Livest. Prod. Sci. 9:389-398.

Brown, D., T. J. Cole, M. J. Dauncey, R. W. Marrs, and P. R. Murgatroyd. 1984. Analysis of gaseous exchange in open-circuit indirect calorimetry. Med. Biol. Eng. Comp. 22:333-338.

Brown-Brandl, T. M., R. A. Eigenberg, J. A., Nienaber, and S. D. Kachman. 2001. Thermoregulatory profile of a newer genetic line of pigs. Livest. Prod. Sci. 71: 253-260.

Burrin, D. G. 2001. Nutrient requirements and metabolism. In: W. G. Pond and H. J. Mersmann , editors, Biology of the domestic pig. Cornell University Press, Ithaca, NY, USA. p. 309-389.

Callaway, J. C. 2004. Hempseed as a nutritional resource: an overview. Euphytica. 140:65-72.

Campos, P. H. R. F., E. Labussière, J. Hernández-García, S. Dubois, D. Renaudeau, and J. Noblet. 2014. Effects of ambient temperature on energy and nitrogen utilization in lipopolysaccharide-challenged growing pigs. J. Anim. Sci. 92:4909-4920.

Canadian Council on Animal Care. 2009. CCAC guidelines on: The care and use of farm animals in research, teaching and testing. Canadian Council on Animal Care, Ottawa, ON, Canada.

Canola Council of Canada. 2009. Canola Council of Canada home page. http://www.canola-council.org/meal.html Accessed Jan. 31, 2009.

Canola Council of Canada. 2015. Canola meal feed industry guide, 5th edition. Publication of Canola Council of Canada, Winnipeg, MB, Canada.

Chiba, L. I. 2000. Feeding systems for pigs. In: M. K. Theodorou, and J. France, editors, Feeding systems and feed evaluation models. CABI Publishing, Wallinford, U.K. p. 181-209.

Christensen, K., A. Chwalibog, S. Henckel, and G. Thorbek. 1988. Heat production in growing pigs calculated according to the RQ and CN methods. Comp. Biochem. Physiol. A Physiol. 91:463-468.

Chwalibog, A. 1991. Energetics of animal production. Acta Agric. Scand. 41:147-160.

Close, W. H. 1996. Modeling the growing pig: Predicting nutrient needs and responses. In: T. P. Lyons and K. A. Jacques, editors, Biotechnology in the Feed Industry. The living gut: Bridging the gap between nutrition and performance. Proc. Alltech's 12th Annual

Symposium. Nottingham University Press, Nottingham, UK. p. 289-297.

Cummings, J. H., and A. M. Stephen. 2007. Carbohydrate terminology and classification. Eur. J. Clin. Nutr. 61(Suppl. 1):S5-18.

Dadalt, J. C., D. E. Velayudhan, M. T. Neto, B. A. Slominski, and C. M. Nyachoti. 2016. Ileal amino acid digestibility in high protein sunflower meal and pea protein isolate fed to growing pigs with or without multi-carbohydrase supplementation. Anim. Feed Sci. Technol. 221:62-69.

de Goey, L. W. and R. C. Ewan. 1975. Energy values of corn and oats for young swine. J. Anim. Sci. 40:1052-1057.

de Lange, C. F. M. 2008. Efficiency of utilization of energy from protein and fiber in the pig-A case for NE systems. Pages 58-72 in Proc. Midwest Swine Nutr. Conf., Indianapolis, IN. The Ohio State Univ., Columbus.

de Lange, C. F. M., and S. H. Birkett. 2005. Characterization of useful energy content in swine and poultry feed ingredients. Can. J. Anim. Sci. 85:269-280.

de Lange, K., J. van Milgen, J. Noblet, S. Dubois, and S. Birkett. 2006. Previous feeding level influences plateau heat production following a 24 h fast in growing pigs. Br. J. Nutr. 95:1082-1087.

de Souza, T. R., A. Aumaitre, J. Mourot, and J. Peiniau. 2000. Effect of graded levels of tallow in the diet on performance, digestibility of fat, lipogenesis and body lipid deposition of the weaned piglet. Asian-Australas. J. Anim. Sci. 13:497-505.

Eastwood, L., P. R. Kish, A. D. Beaulieu, and P. Leterme. 2009. Nutritional value of flaxseed meal for swine and its effects on the fatty acid profile of the carcass. J. Anim. Sci. 87:3607-3619.

Elia, M., and G, Livesey. 1992. Energy expenditure and fuel selection in biological systems: the theory and practice of calculations based on indirect calorimetry and tracer methods. In: Simopoulus AP, ed. Metabolic control of eating, energy expenditure and the bioenergetics of obesity. World. Rev. Nutr. Diet 70:62-131.

Emmans, G. C. 1999. Energy flows. In: I. Kyriazakis, editor, A quantitative biology of the pig. CABI International, New York, NY, USA. p. 363-377.

Englyst, H. N., and J. H. Cummings. 1988. An improved method for the measurement of dietary fibre as the non-starch polysaccharides in plant foods. J. Assoc. Off. Anal. Chem. 71:808-814.

Englyst, K. N., S. Liu, and H. N. Englyst. 2007. Nutritional characterization and measurement of dietary carbohydrates. Eur. J. Clin. Nutr. 61:S19-39.

Ewan, R. C. 2001. Energy utilization in swine nutrition. In: A. J. Lewis and L. L. Southern, editors, Swine Nutrition, 2nd Ed. CRC Press, Washington, DC. p. 85-94.

Fleischer, J. E., Y. Tomita, K. Hayashi, and T. Hashizume. 1981. A modified method for determining energy of fresh faeces and urine of pig. Mem. Fac. Agric. Kogoshima Univ. 17:235-241.

Fontaine, J., U. Zimmer, P. J. Moughan, and S. M. Rutherfurd. 2007. Effect of heat damage in an autoclave on the reactive lysine contents of soy products and corn distillers dried grains with solubles. Use of the results to check on lysine damage in common qualities of these ingredients. J. Agric. Food Chem. 55: 10737-10743.

Gakhar, N., E. Goldberg, M. Jing, R. Gibson, and J. D. House. 2012. Effect of feeding hemp seed and hemp seed oil on laying hen performance and egg yolk fatty acid content: Evidence of their safety and efficacy for laying hen diets. Poult. Sci. 91:701-711.

Gibb, D. J., M. A. Shah, P. S. Mir, and T. A. McAllister. 2005. Effect of full-fat hemp seed on performance and tissue fatty acids of feedlot cattle. Can. J. Anim. Sci. 85:223-230.

Goering, H. K., and P. J. van Soest. 1970. Forage fiber analyses (apparatus, reagents, procedures and some applications). Agric. Handbook No. 379. ARS-USDA, Washington, DC.

Goldberg, E., N. Gakhar, D. Ryland, M. Aliani, R. Gibson, and J. D. House. 2012. Fatty acid profile and sensory characteristics of table eggs from laying hens fed hempseed and hempseed oil. J. Food Sci. 77:S153-S160.

Golian, A., L. D. Campbell, C. M. Nyachoti, H. Janmohammadi, and J. A. Davidson. 2007. Nutritive value of an extruded blend of canola seed and pea (Enermax^{TM}) for poultry. Can. J. Anim. Sci. 87:115-120.

Heegaard, P. M., A. Stockmarr, M. Piñeiro, R. Carpintero, F. Lampreave, F. Campbell, P. Eckersall, M. Toussaint, E. Gruys, and N. S. Sorensen. 2011. Optimal combinations of acute phase proteins for detecting infectious disease in pigs. Vet. Res. 42:50.

Henry, Y. 1985. Dietary factors involved in feed intake regulation in growing pigs: A review. Livest. Prod. Sci. 12:339-354.

Heo, J. M., D. Ayoade, and C. M. Nyachoti. 2014. Determination of the net energy content of canola meal from *Brassica napus* yellow and *Brassica juncea* yellow fed to growing pigs using indirect calorimetry. Anim. Sci. J. 85:751-756.

Humphrey, B. D., and K. C. Klasing. 2004. Modulation of nutrient metabolism and homeostasis by the immune system. World's Poult. Sci. J. 60:90-100.

Huntley, N. F., C. M. Nyachoti, and J. F. Patience. 2018. Lipopolysaccharide immune stimulation but not β-mannanase supplementation affects maintenance energy requirements in young weaned pigs. J. Anim. Sci. Biotechnol. 9:47.

Jaworski, N. W., D. W. Liu, D. F. Li, and H. H. Stein. 2016. Wheat bran reduces concentrations of digestible, metabolizable, and net energy in diets fed to pigs, but energy values in wheat bran determined by the difference procedure are not different from values estimated from a linear regression procedure. J. Anim. Sci. 94:3012-3021.

Jha, R., J. K. Htoo, M. G. Young, E. Beltranena, and R. T. Zijlstra. 2013. Effects of increasing co-product inclusion and reducing dietary protein on growth performance, carcass characteristics, and jowl fatty acid profile of growing-finishing pigs. J. Anim. Sci. 91:2178-2191.

Johnson, R. W. 2012. Fueling the immune response: What's the cost? In: J. Patience, editor, Feed efficiency in swine. Wageningen Academic Publ., Wageningen, the Netherlands. p. 211-223.

Johnston, S. L., S. B. Williams, L. L. Southern, T. D. Bidner, L. D. Bunting, J. O. Matthews, and B. M. Olcott. 2004. Effect of phytase addition and dietary calcium and phosphorous levels on plasma metabolites and ileal and total tract nutrient digestibility in pigs. J. Anim. Sci. 82:705-714.

Jørgensen, H., K. Jakobsen, and B. O. Eggum. 1993. Determination of endogenous fat and fatty acids at the terminal ileum and on faeces in growing pigs. Acta Agric. Scand. Sect. A. Anim. Sci. 43:101-106.

Jørgensen, H., X. Q. Zhao, and B. O. Eggum. 1996. The influence of dietary fibre and environmental temperature on the development of the gastrointestinal tract, digestibility, degree of fermentation in the hind-gut and energy metabolism in pigs. Br. J. Nutr. 75:365-378.

Just, A. 1982. The net energy value of balanced diets for growing pigs. Livest. Prod. Sci. 8:541-

555.

Just, A., H. Jørgensen, and J. A. Fernandez. 1983. Maintenance requirement and the net energy value of different diets for growth in pigs. Livest. Prod. Sci. 10:487-506.

Kahindi, R. K., T. A. Woyengo, P. A. Thacker, and C. M. Nyachoti. 2014. Energy and amino acid digestibility of camelina cake fed to finishing pigs. Anim. Feed Sci. Technol. 193:93-101.

Kiarie, E., and C. M. Nyachoti. 2007. Ileal digestibility of amino acids in co-extruded peas and full fat canola for growing pigs. Anim. Feed Sci. Tech. 139:40-51.

Kiarie, E., I. H. Kim, and C. M. Nyachoti. 2015. Effect of genotype on heat production and net energy value of a corn-soybean meal-based diet fed to growing pigs. Vet. Med. 60:489-498.

Kiarie, E., M. C. Walsh, L. He, D. E. Velayudhan, Y. L. Yin, and C. M. Nyachoti. 2016. Phytase improved digestible protein, phosphorous and energy contents in camelina expellers fed to growing pigs. J. Anim. Sci. 94:215-218.

Kil, D. Y., B. G. Kim, and H. H. Stein. 2013. Feed energy evaluation for growing pigs. Asian-Australas. J. Anim. Sci. 26:1205-1217.

Kil, D. Y., F. Ji, L. L. Stewart, R. B. Hinson, A. D. Beaulieu, G. L. Allee, J. F. Patience, J. E. Pettigrew, and H. H. Stein. 2011. Net energy of soybean oil and choice white grease in diets fed to growing and finishing pigs. J. Anim. Sci. 89:448-459.

Kil, D. Y., F. Ji, L. L. Stewart, R. B. Hinson, A. D. Beaulieu, G. L. Allee, J. F. Patience, J. E. Pettigrew, and H. H. Stein. 2013. Effects of dietary soybean oil on pig growth performance, retention of protein, lipids, and energy, and on the net energy of corn in diets fed to growing or finishing pigs. J. Anim. Sci. 91:3283-3290.

Kil, D. Y., T. E. Sauber, D. B. Jones, and H. H. Stein. 2010. Effect of the form of dietary fat and

the concentration of dietary neutral detergent fiber on ileal and total tract endogenous losses and apparent and true digestibility of fat by growing pigs. J. Anim. Sci. 88:2959-2967.

Kim, J. W., and C. M. Nyachoti. 2017. Net energy of hemp hulls and processed hemp hull products fed to growing pigs and the comparison of net energy determined via indirect calorimetry and calculated from prediction equations. J. Anim. Sci. 95:2649-2657.

Kim, J. W., B. Koo, and C. M. Nyachoti. 2017. Digestible, metabolizable, and net energy of camelina cake fed to growing pigs and additivity of energy in mixed diets. J. Anim. Sci. 95:4037-4044.

Kim, J. W., B. Koo, and C. M. Nyachoti. 2018. Net energy content of canola meal fed to growing pigs and effect of experimental methodology on energy values. J. Anim. Sci. 96:1441-1452.

Kleiber, M. 1975. The fire of life, R. E. Krieger, editor, 2nd Ed., New York, NY.

Knap, P. 2000. Variation in maintenance requirements of growing pigs in relation to body composition. A simulation study. PhD Diss. Wageningen Agricultural Univ., Wageningen, The Netherlands.

Knudsen Bach, E., Knud, H. N. Læ rke, and H. Jørgensen. 2013. Carbohydrates and carbohydrate utilization in swine, Sustainable Swine Nutrition. 1st ed. John Wiley & Sons, Ames, IA. p. 109-137.

Kong, C., and O. Adeola. 2014. Evaluation of amino acid and energy utilization in feedstuffs for swine and poultry diets. AsianAustralas. J. Anim. Sci. 27:917-925.

Koo, B., J. W. Kim, C. F. M. de Lange, M. M. Hossain, and C. M. Nyachoti. 2017. Effects of diet complexity and multicarbohydrase supplementation on growth performance, nutrient digestibility, blood profile, intestinal morphology, and fecal score in newly weaned pigs. J.

Anim. Sci. 95:4060-4071.

Koong, L. J., J. A. Nienaber, and H. J. Mersmann. 1983. Effects of plane of nutrition on organ size and fasting heat production in genetically obese and lean pigs. J. Nutr. 113:1626-1631.

Koong, L. J., J. A. Nienaber, J. C. Pekas, and J. T. Yen. 1982. Effects of plane of nutrition on organ size and fasting heat production in pigs. J. Nutr. 112:1638-1642.

Labussière, E., G. Maxin, S. Dubois, J. van Milgen, G. Bertrand, and J. Noblet. 2009. Effect of feed intake on heat production and protein and fat deposition in milk-fed veal calves. Animal 3:557-567.

Labussière, E., J. van Milgen, C. de Lange, and J. Noblet. 2011. Maintenance energy requirements of growing pigs and calves are influenced by feeding level. J. Nutr. 141:1855-1861.

Le Bellego, L., J. van Milgen, S. Dubois, and J. Noblet. 2001. Energy utilization of low-protein diets in growing pigs. J. Anim. Sci. 79:1259-1271.

Le Goff, G., and J. Noblet. 2001. Comparative total tract digestibility of dietary energy and nutrients in growing pigs and adult sows. J. Anim. Sci. 79:2418-2427.

Le Goff, G., L. Le Groumellec, J. van Milgen, S. Dubois, and J. Noblet. 2002. Digestibility and metabolic utilisation of dietary energy in adult sows: influence of addition and origin of dietary fibre. Br. J. Nutr. 87:325-335.

Le, M. H. A., S. Galle, Y. Yang, J. L. Landero, E. Beltranena, M. G. Gänzle, and R. T. Zijlstra. 2016. Effects of feeding fermented wheat with Lactobacillus reuteri on gut morphology, intestinal fermentation, nutrient digestibility, and growth performance in weaned pigs. J. Anim. Sci. 94:4677-4687.

Lee, G. I., K.-S. Kim, J. H. Kim, and D. Y. Kil. 2015. Growth performance of early finishing

gilts as affected by different net energy concentrations in diets. Asian-Australas. J. Anim. Sci. 28:1614-1623.

Li Y, Z. Li, H. Liu, J. Noblet, L. Liu, D. Li, F. Wang, and C. Lai. 2018c. Net energy content of rice bran, corn germ meal, corn gluten feed, peanut meal, and sunflower meal in growing pigs. Asian-Australas. J. Anim. Sci. 9:1481-1490.

Li, E., H. Liu, Y. Li, L. Liu, F. Wang, D. Li, and S. Zhang. 2018a. Determination of net energy content of dietary lipids fed to growing pigs using indirect calorimetry. J. Anim. Sci. 96:2184-2194.

Li, Q., and J. F. Patience. 2017. Factors involved in the regulation of feed and energy intake of pigs. Anim. Feed Sci. Technol. 233:22-33.

Li, Y., Z. Lyu, Z. Li, L. Liu, F. Wang, D. Li, and C. Lai. 2018b. Effects of feeding level and dietary supplementation with crystalline amino acids on digestible, metabolizable and net energy values of corn in growing pigs. Anim. Feed Sci. Technol. 240:197-205.

Li, Z. C., Y. K. Li, Z. Q. Lyu, H. Liu, J. B. Zhao, J. Noblet, F. L. Wang, C. H. Lai and D. F. Li. 2017. Net energy of corn, soybean meal, expeller-pressed rapeseed meal and solvent-extracted rapeseed meal in growing pigs. J. Anim. Sci. Biotechnol. 8:44.

Liu, D., N. W. Jaworski, G. Zhang, Z. Li, D. F. Li, and F. Wang. 2014. Effect of experimental methodology on fasting heat production and the net energy content of corn and soybean meal fed to growing pigs. Arch. Anim. Nutr. 68:281-295.

Liu, Y., N. W. Jaworski, O. J. Rojas, and H. H. Stein. 2016. Energy concentration and amino acid digestibility in high protein canola meal, conventional canola meal, and in soybean meal fed to growing pigs. Anim. Feed Sci. Technol. 212:52-62.

Lizardo, R., J. van Milgen, J. Mourot, J. Noblet, and M. Bonneau. 2002. A nutritional model of

fatty acid composition in the growing-finishing pig. Livest. Prod. Sci. 75:167-182.

Lochmiller, R., and C. Deerenberg. 2000. Trade-offs in evolutionary immunology: Just what is the cost of immunity? Oikos. 88:87-98.

Lyu, Z. Q., C. F. Huang, Y. K. Li, P. L. Li, H. Liu, Y. F. Chen, D. F. Li, and C. H. Lai. 2018. Adaptation duration for net energy determination of high fiber diets in growing pigs. Anim. Feed Sci. Technol. 241:15-26.

Matthäus, B., and J. Zubr. 2000. Variability of specific components in Camelina sativa oilseed cakes. Ind. Crop. Prod. 12: 9-18.

Mayes, P. A. 2000. Bioenergetics: The role of ATP. In: Harper's Biochemistry, R. K Murry, D. K. Granner, P. A. Mayes, and V. W. Rodwell, editors, 25th Ed., McGraw-Hill, New York, NY. p. 123-129.

McDonald, P., R. A. Edwads, J. F. D. Greenhalgh, and C. A. Morgan. 2002. Evaluation of foods: energy content of foods and the partition of food energy within the animal. In: P. McDonals, R. A. Edwads, J. F. D. Greenhalgh, and C. A. Morgan, editors, Animal Nutrition. 5th Ed., Pearson, UK. p. 263-291.

Milligan, L. P., and M. Summers. 1986. The biological basis of maintenance and its relevance to assessing responses to nutrients. Proc. Nutr. Soc. 45:185-193.

Moehn, S., J. Atakora, and R. O. Ball. 2005. Using net energy for diet formulation: Potential for the Canadian pig industry. Adv. Pork Prod. 16:119-129.

Mustafa, A. F., J. J. McKinnon, and D. A. Christensen. 1999. The nutritive value of hemp meal for ruminants. Can. J. Anim. Sci. 79:91-95.

Noblet, J. 1996. Digestive and metabolic utilization of dietary energy in pig feeds: Comparison of energy systems. In: P. C. Garnsworthy, J. Wiseman and W. Haresign, editors, Recent

advances in animal nutrition. Nottingham University Press, Nottingham, U.K. p. 207-231.

Noblet, J. 2007. Net energy evaluation of feeds and determination of net energy requirements for pigs. R. Bras. Zootec. 36:277-284.

Noblet, J., and G. Le Goff. 2001. Effect of dietary fibre on the energy value of feeds for pigs. Anim. Feed Sci. Technol. 90:35-52.

Noblet, J., and J. M. Perez. 1993. Prediction of digestibility of nutrients and energy values of pig diets from chemical analysis. J. Anim. Sci. 71:3389-3398.

Noblet, J., and J. van Milgen. 2004. Energy value of pig feeds: Effect of pig body weight and energy evaluation system. J. Anim. Sci. 82(E. Suppl.):E229-E238.

Noblet, J., and J. van Milgen. 2013. Energy and energy metabolism in swine. In: L. I. Chiba, editor, Sustainable Swine Nutrition. 1st ed. John Wiley & Sons, Ames, IA. p. 23-57.

Noblet, J., and X. S. Shi. 1994. Effect of body weight on digestive utilization of energy and nutrients of ingredients and diets in pigs. Livest. Prod. Sci. 37:323-338.

Noblet, J., and Y. Henry. 1993. Energy evaluation systems for pig diets: a review. Livest. Prod. Sci. 36:121-141.

Noblet, J., C. Karege, and S. Dubois. 1991. Influence of growth potential on energy requirements for maintenance in growing pigs. In: C. Wenk and M. Boessinger, editors, Energy metabolism of farm animals. EAAP Publication, Zurich, Switzerland. p. 107-110.

Noblet, J., C. Karege, S. Dubois, and J. van Milgen. 1999. Metabolic utilization of energy and maintenance requirements in growing pigs: Effects of sex and genotype. J. Anim. Sci. 77:1208-1216.

Noblet, J., H. Fortune, C. Dupire, and S. Dubois. 1993a. Digestible, metabolizable and net energy values of 13 feedstuffs for growing pigs: Effect of energy system. Anim. Feed Sci.

Technol. 42:131-149.

Noblet, J., H. Fortune, X. S. Shi, and S. Dubois. 1994. Prediction of net energy value of feeds for growing pigs. J. Anim. Sci. 72:344-354.

Noblet, J., J. Y. Dourmad, J. Le Dividich, and S. Dubois. 1989. Effect of ambient temperature and addition of straw or alfalfa in the diet on energy metabolism in pregnant sows. Livest. Prod. Sci. 21:309-324.

Noblet, J., L. Le Bellego, J. van Milgen, and S. Dubois. 2001. Effects of reduced dietary protein level and fat addition on heat production and nitrogen and energy balance in growing pigs. Anim. Res. 50:227-238.

Noblet, J., X. S. Shi, and S. Dubois. 1993b. Metabolic utilization of dietary energy and nutrients for maintenance energy requirements in pigs: Basis for a net energy system. Br. J. Nutr. 70:407-419.

NRC. 1998. Nutrient requirements of swine. 10th ed. Natl. Acad. Press, Washington, DC, USA.

NRC. 2012. Nutrient requirements of swine. 11th Rev. ed. Natl. Acad. Press, Washington, DC, USA.

Nyachoti, C. M., C. F. M. de Lange, B. McBride, S. Leeson, and H. Schulze. 2000. Dietary influence on organ size and in vitro oxygen consumption by visceral organs of growing pigs. Livest. Prod. Sci. 65:229-237.

Nyachoti, C. M., R. T. Zijlstra, C. F. M. de Lange, and J. F. Patience. 2004. Voluntary feed intake in growing-finishing pigs: A review of the main determining factors and potential approaches for accurate predictions. Can. J. Anim. Sci. 84:549-566.

Oresanya, T. F., A. D. Beaulieu, and J. F. Patience. 2008. Investigations of energy metabolism in weanling barrows: The interaction of dietary energy concentration and daily feed (energy)

intake. J. Anim. Sci. 86:348-363.

Patience, J. F. and A. D. Beaulieu. 2005. The merits, benefits, and challenges of adopting the net energy system in a North American context. In: Proc. 66th Minnesota Nutr. Conf. Tech. Symp.: Future of corn in animal feed. September 20-21. St. Paul. MN. p. 134-149.

Patience, J. F., M. C. Rossoni-Serão, and N. A. Gutiérrez. 2015. A review of feed efficiency in swine: biology and application. J. Anim. Sci. Biotechnol. 6:33.

Pedersen, T. F., Y. Liu, and H. H. Stein. 2016. Effects of diet energy concentration and an exogenous carbohydrase on growth performance of weanling pigs fed diets containing canola meal produced from high protein or conventional canola seeds. J. Anim. Sci. 94:5206-5218.

Pekel, A. Y., P. H. Patterson, R. M. Hulet, N. Acar, T. L. Cravener, D. B. Dowler, and J. M. Hunter. 2009. Dietary camelina meal versus flaxseed with and without supplemental copper for broiler chickens: live performance and processing yield. Poult. Sci. 88:2392-2398.

Pond, W. G., D. C. Church, and K. R. Pond. 1995. Basic animal nutrition and feeding. In: Basic Animal Nutrition and Feeding. Wiley, New York, USA. p. 149-166.

Presto, M. H., K. Lyberg, and J. E. Lindberg. 2010. Digestibility of amino acids in organically cultivated white-flowering faba bean and cake from cold-pressed rapeseed, linseed and hemp seed in growing pigs. Arch. Anim. Nutr. 65:21-33.

Quiniou, N., and J. Noblet. 2012. Effect of the dietary net energy concentration on feed intake and performance of growing-finishing pigs housed individually. J. Anim. Sci. 90:4362-4372.

Quiniou, N., S. Dubois, and J. Noblet. 1995. Effect of dietary crude protein level on protein and

energy balances in growing pigs: comparison of two measurement methods. Livest. Prod. Sci. 41:51-61.

Ramonet, Y., J. van Milgen, J. Y. Dourmad, S. Dubois, M. C. Meunier- Salaün, and J. Noblet. 2000. The effect of dietary fibre on energy utilisation and partitioning of heat production over pregnancy in sows. Br. J. Nutr. 84:85-94.

Reeds, P. J., and F. Jahoor. 2001. The amino acid requirements of disease. Clinical nutrition. 20(Suppl. 1):15-22.

Reynolds, C. K. 2000. Measurement of energy metabolism. In: M. K. Theodorou, and J. France , editors, Feeding systems and feed evaluation models. CAB International, Oxon, U.K. p. 87-107.

Rijnen, M. M. J. A. 2003. Energetic utilization of dietary fiber in pigs. Ph.D. Thesis, University of Wageningen, Wageningen, the Netherlands.

Rijnen, M. M. J. A., J. Doorenbos, J. J. Mallo, and L. A. den Hartog. 2004. The application of the net energy system for swine. In: Proc. Western Nutr. Conf., Saskatoon, Canada. p. 151-168.

Rijnen, M. M. J. A., M. W. A. Verstegen, M. J. W. Heetkamp, J. Haaksma, and J. W. Schrama. 2003. Effects of dietary fermentable carbohydrates on behavior and heat production in group-housed sows. J. Anim. Sci. 81:182-190.

Robles, A., and R. C. Ewan. 1982. Utilization of energy of rice and rice bran by young pigs. J. Anim. Sci. 55:572-577.

Russo, R., and R. Reggiani. 2015. Evaluation of protein concentration, amino acid profile and antinutritional compounds in hempseed meal from dioecious and monoecious varieties. Am. J. Plant Sci. 6:14-22.

Sauvant, D., J. M. Perez, and G. Tran. 2004. Tables of composition and nutritional value of feed

materials: Pig, poultry, sheep, goats, rabbits, horses, and fish. Wageningen Academic Publishers, Wageningen, the Netherlands and INRA ed. Paris, France.

Schiemann, R., K. Nehring, L. Hoffmann, W. Jentsch, and A. Chudy. 1972. Energetische Futterbevertung und Energienormen. VEB Deutscher Landwirtschaftsverlag, Berlin.

Schrama, J. W., M. W. Bosch, M. W. A. Verstegen, A. H. P. M. Vorselaars, J. Haaksma, and M. J. W. Heetkamp. 1998. The energetic value of nonstarch polysaccharides in relation to physical activity in group-housed, growing pigs. J. Anim. Sci. 76:3016-3023.

Schuster, A., and W. Friedt. 1998. Glucosinolate content and composition as parameters of quality of camelina seed. Ind. Crop Prod. 7:297-302.

Shi, X. S., and J. Noblet. 1993. Contribution of the hindgut to digestion of diets in growing pigs and adult sows: effect of diet composition. Livest. Prod. Sci. 34:237-252.

Slominski, B. A., and L. D. Campbell. 1987. Gas chromatographic determination of indole glucosinolates–A re-examination. J. Sci. Food Agric. 40:131-143.

Slominski, B. A., and L. D. Campbell. 1990. Non-starch polysaccharides of canola meal: Quantification, digestibility in poultry and potential benefit of dietary enzyme supplementation. J. Sci. Food Agric. 53:175-184.

Slominski, B. A., W. Jia, A. Rogiewicz, C. M. Nyachoti, and D. Hickling. 2012. Low-fiber canola. Part 1. Chemical and nutritive composition of the meal. J. Agric. Food Chem. 60:12225-12230.

Stewart, L. L., D. Y. Kil, R. B. Hinson, A. D. Beaulieu, G. L. Allee, J. F. Patience, J. E. Pettigrew, and H. H. Stein. 2013. Effects of dietary soybean hulls and wheat middlings on body composition, nutrient and energy retention, and the net energy of diets and ingredients fed to growing and finishing pigs. J. Anim. Sci. 91:2756-2765.

Tess, M. W. 1981. Simulated effects of genetic change upon life-cycle production efficiency in swine and the effect of body composition upon energy utilization in the growing Pig. Ph.D. Thi. Univ. of Nebraska, Lincoln, NE, USA.

Tess, M. W., G. E. Dickerson, J. A. Nienaber, and C. L. Ferrell. 1984. The effects of body composition on fasting heat production in pigs. J. Anim. Sci. 58:99-110.

Tess, M. W., G. E. Dickerson, J. A. Nienaber, and C. L. Ferrell. 1986. Growth, development and body composition in three genetic stocks of swine. J. Anim. Sci. 62:968-979.

Thonney, M. L., R. W. Touchberry, R. D. Goodrich, and J. C. Meiske. 1976. Intraspecies relationship between fasting heat production and body weight: A reevaluation of W.75. J. Anim. Sci. 43:692-704.

Tripathi, M. K., and A. S. Mishra. 2007. Glucosinolates in animal nutrition: A review. Anim. Feed Sci. Technol. 132:1-27.

van der Meer, Y. 2017. Nutrition of pigs kept under low and high sanitary conditions. Ph.D. Thesis, University of Wageningen, Wageningen, the Netherlands.

van Milgen, J., A. Valancogne, S. Dubois, J. Y. Dourmad, B. Sève, and J. Noblet. 2008. InraPorc: A model and decision support tool for the nutrition of growing pigs. Anim. Feed Sci. Technol. 143:387-405.

van Milgen, J., and J. Noblet. 2000. Modelling energy expenditure in pigs. In: J. P. McNamara, J. France, and D. E. Beever, editors, Modeling Nutrient Utilization in Farm Animals.. CAB International, Oxon, U. K. p. 103-114

van Milgen, J., and J. Noblet. 2003. Partitioning of energy intake to heat, protein, and fat in growing pigs. J. Anim. Sci. 81:E86-E93.

van Milgen, J., J.-F. Bernier, Y. Le Cozler, S. Dubois, and J. Noblet. 1998. Major determinants

of fasting heat production and energetic cost of activity in growing pigs of different body weight and breed/castration combination. Br. J. Nutr. 79:509-517.

Varley, P. F., J. J. Callan, and J. V. O'Doherty. 2011. Effect of dietary phosphorus and calcium level and phytase addition on performance, bone parameters, apparent nutrient digestibility, mineral and nitrogen utilization of weaner pigs and the subsequent effect on finisher pig bone parameters. Anim. Feed Sci. Technol. 165:201-209.

Velayudhan, D. E., and C. M. Nyachoti. 2016. Net energy system of feed formulation with or without enzyme supplementation in growing-finishing pigs fed barley-based diets with alternative feed ingredients. Can. J. Anim. Sci. 96:180-186.

Velayudhan, D. E., and C. M. Nyachoti. 2017. Effect of increasing dietary canola meal inclusion on lactation performance, milk composition and nutrient digestibility of lactating sows. J. Anim. Sci. 2017.95:3129-3135.

Velayudhan, D. E., I. H. Kim, and C. M. Nyachoti. 2015a. Characterization of dietary energy in swine feed and feed ingredients: A review of recent research results. Asian-Australas. J. Anim. Sci. 28:1-13.

Velayudhan, D. E., J. M. Heo, and C. M. Nyachoti. 2015b. Net energy content of dry extruded-expelled soybean meal fed with or without enzyme supplementation to growing pigs as determined by indirect calorimetry. J. Anim. Sci. 93:3402-3409.

Velayudhan, D. E., K. Schuh, T. A. Woyengo, J. S. Sands, and C. M. Nyachoti. 2017. Effect of expeller extracted canola meal on growth performance, organ weights, and blood parameters of growing pigs. J. Anim. Sci. 95:302-307.

Villamide, M. J., and L. D. San Juan. 1998. Effect of chemical composition of sunflower seed meal on its true metabolizable energy and amino acid digestibility. Poult. Sci. 77:1884-

1892.

Waititu, S. M., J. W. Kim, N. Sanjayan, P. Leterme, and C. M. Nyachoti. 2018a. Metabolizable energy and standardized ileal digestible amino acid contents of a high protein sunflower meal fed to broiler chicks. Can. J. Anim. Sci. 98:517-524.

Waititu, S. M., N. Sanjayan, M. M. Hossain, P. Leterme, and C. M. Nyachoti. 2018b. Improvement of the nutritional value of high-protein sunflower meal for broiler chickens using multi-enzyme mixtures. Poult. Sci. 97:1245-1252.

Woyengo, T. A., E. Beltranena, and R. T. Zijlstra. 2014. Nonruminant nutrition symposium: Controlling feed cost by including alternative ingredients into pig diets: A review. J. Anim. Sci. 92:1293-1305.

Woyengo, T. A., E. Kiarie, and C. M. Nyachoti. 2010. Energy and amino acid utilization in expeller-extracted canola meal fed to growing pigs. J. Anim. Sci. 88:1433-1441.

Woyengo, T. A., R. Patterson, B. A. Slominski, E. Beltranena, and R. T. Zijlstra. 2016. Nutritive value of cold-pressed camelina cake with or without supplementation of multi-enzyme in broiler chickens. Poult. Sci. 95:2314-2321.

Young, B. A., B. Kerrigan, and R. J. Christopherson. 1975. A versatile respiratory pattern analyzer for studies of energy metabolism of livestock. Can. J. Anim. Sci. 55:17-22.

Zhang, G. F., D. W. Liu, F. L. Wang, and D. F. Li. 2014. Estimation of the net energy requirements for maintenance in growing and finishing pigs. J. Anim. Sci. 92:2987-2995.