Nutritional Strategies to Control Feed Costs for Nursery Pigs

without Compromising Gut Health and Growth Performance

by

Bonjin Koo

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University of Manitoba

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ABSTRACT

One of the major challenges for the pig industry is to control nursery feed costs to maximize profits. The objective of this research was to evaluate potential economic benefits of nutritional strategies for nursery feed costs. Experiment 1 was conducted to investigate the influence of dietary wheat bran (WB) inclusion on nutrient digestibility and microbial metabolites in weaned pigs. The volatile fatty acid (VFA) concentrations and pH in the gut was beneficially modified, but WB inclusion decreased nutrient and energy digestibility and also reduced digestible energy. In Experiment 2, the effects of diet complexity and multicarbohydrase (MC) supplementation on weaned pigs were studied. No interactive effects of diet complexity and MC supplementation on growth performance and intestinal morphology were observed. Feeding weaned pigs a simplified diet improved gain-to-feed ratio (G:F) throughout the 4-week experimental period without compromising the final body weight, compared to a conventional diet. Greater reductions in total feed costs and the feed cost per kg of body weight gain for 4-week of nursery production was achieved by feeding a simple diet compared to the conventional diet. However, weaned pigs fed the conventional diet showed higher intestinal villus height and villus height:crypt depth ratio. The MC supplementation improved G:F, improved intestinal morphology and nutrient digestibility, and reduced fecal score. In Experiment 3, feeding a nursery diet containing *Lactobacillus*-fermented wheat increased nutrient and energy digestibility but did not improve lactate and VFA concentrations in the gut. Taken together, these results show that a simple diet and a fermented wheat diet would be beneficial, directly reducing feed cost and/or increasing nutrient and energy digestibility, respectively, in weaned pigs. The addition of MC in nursery diets would also provide potential economic benefits, improving feed efficiency, nutrient digestibility, and gut health.

DEDICATION

This thesis is dedicated to my pillars of support: my parents, Jawon Koo and Jongmi Kim, my

older brother, Bonseok, and my late sister, Bonhee, who remains forever in my heart

and

To my wife, Seonyeong Lee, and my daughter, Arin.

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FOREWORD

This thesis was prepared following a manuscript format and is composed of separate journal manuscripts. Manuscript I and II were accepted by the Livestock Science and Journal of Animal Science, respectively. Manuscript III is under preparation. All manuscripts were written according to the guidelines of the Journal of Animal Science for manuscript preparation.

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LIST OF ABBREVIATIONS

AA	Amino acids
ADFI	Average daily feed intake
ADG	Average daily gain
AID	Apparent ileal digestibility
ANF	Anti-nutritional factors
ATTD	Apparent total tract digestibility
BW	Body weight
Ca	Calcium
CD	Crypt depth
CG	Compensatory growth
CM	Canola meal
CP	Crude protein
DDGS	Distiller dried grains with solubles
DE	Digestible energy
DM	Dry matter
EAA	Essential amino acids
EE	Ether extracts
ETEC	Enterotoxigenic *Escherichia coli*
FSBM	Fermented soybean meal
GE	Gross energy
G:F	Gain to feed ratio
GIT	Gastrointestinal tract
Homo L.	Homofermentative *Lactobacillus*
Hetero L.	Heterofermentative *Lactobacillus*
LP	Low protein
ME	Metabolizable energy
N	Nitrogen

NC	Negative control
NE	Net energy
NDF	Neutral detergent fiber
NSP	Non-starch polysaccharides
OM	Organic matter
P	Phosphorus
PC	Positive control
PWD	Post-weaning diarrhea
RNA	Ribonucleic acids
SBM	Soybean meal
SCFA	Short chain fatty acids
SDAP	Spray-dried animal plasma
SDPP	Spray-dried porcine plasma
SID	Standardized ileal digestible or standardized ileal digestibility
STTD	Standardized total tract digestible or standardized total tract digestibility
VFA	Volatile fatty acid(s)
VH	Villus height
WB	Wheat bran

1.0 GENERAL INTRODUCTION

Weaning imposes environmental, nutritional, and psychological stress on piglets (Williams, 2003). The piglets are forced to suddenly withdraw from sow's milk and must adapt to a new solid, plant-based diet that includes various anti-nutritional factors (**ANF**). In addition, piglets are separated from their sow and littermates, and they are forced to mingle with others, during which a new hierarchy is established through fighting. In general, piglets are moved from farrowing to the nursery where they must adapt to a new environment that includes a feeder, waterer, and microbes. Because of these stresses, piglets experience marked changes in gastrointestinal physiology, microbiology, and immunology (Hampson and Kidder, 1986; Pluske et al., 1997), where additional nutrients and energy may be required to cope with these changes (Jayaraman et al., 2015). In general, the post-weaning changes also cause transient anorexia, leading to growth retardation.

Therefore, a weaner diet is formulated to be highly palatable and digestible; it also supports immunological functions. A typical weaner diet contains substantial levels of animal protein sources (e.g., sprayed dried plasma, fish meal, blood meal, and bone meal) and dairy products (e.g., dried whey, skim milk powder, and lactose). The former ingredients are nutritious and have balanced amino acids (**AA**) as well as being highly palatable for weaned pigs. Also, a weaner diet is characterized by high amounts of dairy products that provide enough lactose, the main component of sow's milk, which is known to be critical for post-weaning adaptation and subsequent growth (Mahan et al., 2004). Apart from these main ingredients, non-nutrients (feed additives), such as zinc oxide, acidifier, flavour, and antibiotics, are commonly incorporated to stimulate gut health and digestive capacity (Heo et al., 2013). However, all these ingredients are

relatively expensive because of high demand by humans as well as additional costs for processing (i.e., spray drying and rolling). Thus, the inclusion of these ingredients renders the weaner diet more complex and costly.

In conventional pork production, weaned pigs are fed three or four diets in sequence, with an average diet cost of $578 per ton, which is twofold the price compared to grower or finisher diets (Manitoba Agriculture, 2016). It should be noted that the cost was estimated when the diet is mixed at the farm. Indeed, an average for a commercial nursery diet is around $700 per ton, and a typical phase I or phase II nursery diet costs around $900 (Skinner, 2012). In hog production, this equates to feed costs during nursery phases accounting for 9% of total feeds and costs for nursery diets representing 16% of total feed costs (based on a 300-sow farrow-to-finish operation; Manitoba Agriculture, 2016).

Therefore, it is of interest to reduce the costs of a nursery diet to maximize the profit margin in swine production without compromising the weaned pigs' growth and gut health. To achieve a reduction in feed costs, it is imperative to simplify or replace conventional ingredients in nursery diets with alternative feed ingredients. However, a variety of ANF present in plant-based ingredients reduce their nutrients utilization for weaned pigs (Wenk et al., 2001; Woyengo et al., 2013) and immunologically activate the weaned pigs (Li et al., 1990; Hao et al., 2010). Thus, many efforts have been made to eliminate or reduce ANF. Of the varied solutions suggested, fermenting feedstuff and the addition of carbohydrase to the diet have been regarded as economic and practical ways to increase nutritive values of used ingredients. Microbial activities during fermentation can hydrolyze anti-nutritional factors in feedstuffs that keeps weaned pigs from fully utilizing nutrients and energy (Pal Vig and Walia, 2001; Wang et al., 2007). Furthermore, some hydrolysis products can stimulate gut health by acidifying the gastrointestinal tract (**GIT**; Pieper et al., 2012) or

suppressing pathogen proliferation (van Winsen et al., 2001). Also, there is evidence that the addition of feed enzymes to a nursery diet increase the utilization of nutrients and energy for weaned pigs (He et al., 2010; Omogbenigun et al., 2004, Diebold et al., 2004), which suggests possible opportunities to incorporate higher levels of low-quality ingredients (e.g., plant-based ingredients) into nursery diets. Furthermore, research summarized in the excellent review by Kiarie et al. (2013) indicated that the addition of feed enzymes can promote pig's gut health by producing oligosaccharides from non-starch polysaccharides (**NSP**) and reducing anti-nutritional factors and undigested substrates. Some by-products of cereal grains rich in insoluble fiber (e.g., wheat bran [**WB**] and oat bran) are not only economically competitive, but also good sources of gut health stimulant (Kim et al., 2012). The production of volatile fatty acids (**VFA**) in the gut has been known to improve enterocytes proliferation (Wenk et al., 2001) and to have bactericidal activities (Prohászka et al., 1990). Also, insoluble fiber serves as an alternative adhesion matrix to pathogenic bacteria, such as *Escherichia coli* (Molist et al., 2009), thereby reducing *E. coli* populations (Hanczakowska et al., 2008; Molist et al., 2010) in the gut and the incidences of post-weaning diarrhea (**PWD**; Hanczakowska et al., 2008; Hermes et al., 2009).

However, whether these nutritional strategies could have economic benefits by improving nutrient utilization, gut health, or growth performance remains to be determined. Therefore, the main objective of this research was the following: To evaluate whether using promising nutritional strategies could cut feed costs while improving or maintaining nutrient utilization, gut health, and growth performance.

To achieve the main objective, the following specific objectives were formulated:

i) To evaluate the influence of dietary wheat bran inclusion on nutrient digestibility and microbial metabolites in weaned pigs.

ii)   To investigate the effects of diet complexity and multicarbohydrases on growth performance, nutrient digestibility, intestinal morphology, and blood profile.

iii)   To examine nutrient digestibility and microbial metabolites in pigs fed diets containing fermented wheat.

2.0 LITERATURE REVIEW

2.1 INTRODUCTION

At weaning, pigs face environmental, nutritional, and psychological stressors, causing post-weaning anorexia (Lallès et al., 2004). These stressors further lead to changes in the GIT (Pluske et al., 1997; Lallès et al., 2004; de Lange et al., 2010; Heo et al., 2013), reducing gut barrier (Wijtten et al., 2011; Kim et al., 2012) and digestive functions (Hampson and Kidder, 1986; Pluske et al., 1997). This makes weaned pigs vulnerable to enteric diseases (e.g., PWD; Kim et al., 2012; Heo et al., 2013) and makes them more likely to poorly utilize nutrients (van Heugten et al., 2003).

To cope with these post-weaning challenges, the conventional weaner pig diet is formulated with high levels of digestible, palatable, and functional ingredients, such as animal protein sources (e.g., blood meal, fish meal, and spray-dried animal plasma), dairy products (dried whey, skim milk powder, and lactose), and varied feed additives. These ingredients have been considered necessary components of the diet that help prevent pigs from growth retardation after weaning and that promote gut health (Mahan et al., 2004; Yi et al., 2005; Heo et al., 2013). However, the inclusion of these ingredients, along with cereal grains, into the nursery diet increases diet complexity and the cost of feed. As a result, the cost of the nursery diet is the highest in swine production. Thus, it is important to reduce feed costs for nursery pigs to maximize overall swine productivity while maintaining growth performance and improving or maintaining gut health.

Various nutritional approaches for replacing conventional ingredients with other alternatives or improving gut health have been tested. However, information on the feeding strategies to control the costs of the nursery diet has not been reviewed. This review, therefore, is focused on current knowledge pertaining to (i) the conventional weaner diet and its composition

and (ii) various feeding strategies to control the cost of the weaner diet without compromising growth performance and gut health.

2.2 CONVENTIONAL NURSERY DIET

The conventional nursery pig diet should meet the following qualifications:

1. Digestibility and energy content;

2. Palatability;

3. Functionality; and

4. Cost.

Nursery diets should be highly nutritious compared to other swine diets because of the relatively lower amounts of feed intake needed for rapid growth rate of weaners. According to NRC (2012), weaned pigs (body weight [**BW**] 5–7kg) require 1.5% of standardized ileal digestible (**SID**) lysine, 0.45% of standardized total tract digestible (**STTD**) P, and 0.85% of total calcium, which is more than twice the requirements of finishing pigs. Furthermore, the NRC (2012) suggests 3,400 kcal/kg of metabolizable energy (**ME**) for pigs with BW 5–7kg while 3,300 kcal/kg of ME for those with 25kg or more. Pigs experience a period of anorexia after weaning (Lallès et al., 2004), which is known to be a major reason for the reduction in gut functionality, integrity, and, ultimately, growth rate (Pluske et al., 1997; van Beers-Schreurs et al., 1998). Thus, a weaner diet should be palatable to stimulate appetite, thereby providing the weaned pigs with continuous energy. Factors affecting diet palatability and feed intake are reviewed by Dong and

Pluske (2007) and Nyachoti et al. (2004). Typical nursery diets generally include feed additives such as antibiotics, organic acids, yeast culture, and exogenous feed enzyme, which can promote gut health and modify microbiota (de Lange et al., 2010; Heo et al., 2013). Also, animal blood protein, such as spray-dried animal plasma (**SDAP**) and blood meal, are commonly included in the weaner diet because they are known to enhance gut barrier functions, reduce the PWD (Peace et al., 2011), and improve initial growth performance after weaning (van Dijk et al., 2001), which will be further discussed below. However, to balance feed costs, cereal grains such as corn, wheat, oat, and barley, along with legumes or their by-products, represent most of the composition in nursery diets.

2.2.1 DAIRY PRODUCTS (DRIED WHEY, DRIED SKIM MILK, LACTOSE)

Dairy products such as dried whey and skim milk powder are good sources of protein and carbohydrates (mainly lactose). Dried skim milk contains 37% crude protein (**CP**) and 48% lactose and has 3,730 kcal/kg of ME, whereas dried whey contains 73% lactose and 12% CP and has 3,415 kcal/kg of ME (NRC, 2012). Also, their CP and amino acids (AA) are highly digestible, showing above 90% of SID CP and most AA (NRC, 2012). Lactose is a critical factor, influencing the growth performance of weaned pigs (Mahan et al., 2004). During lactation, pigs gain most of their energy from lactose, a major component of sow's milk (Klobasa et al., 1987), and thus, their lactase activity in the brush-border membrane is relatively high at weaning (Manners and Stevens, 1972). Also, lactose can serve as a specific substrate for *Lactobacillus* spp. and can promote *Lactobacillus* and *Bifidobacterium* proliferation (Pollmann et al., 1980; Pierce et al., 2006; Pierce et al., 2007), which may suppress the proliferation of pathogenic bacteria that impede optimal growth performance after weaning (Lessard and Brisson, 1987). Also, several studies showed that the

inclusion of dairy products in nursery diets improves the pigs' initial growth rate by increasing dry matter (**DM**) intake and nutrient digestibility (Mahan and Newton, 1993; Tokach et al., 1995). However, these ingredients are expensive components in nursery diets because of their technical processing and their growing demand in other industries and markets.

2.2.2 BLOOD MEAL AND SPRAY-DRIED ANIMAL PLASMA

There are a variety of blood-origin products available for swine feeds, including blood meal, dried blood cells, and dried blood plasma, each prepared with different drying methods. In general, blood meal and SDAP are more commonly included in a weaner pig diet because of their highly digestible nutrients and bioactive compounds. These ingredients are good sources of AA. The SID of essential amino acids (**EAA**) in blood meal and SDAP is 5% lower than that in dried skim milk (Cho et al., 1997). However, because the respective CP content in blood meal and SDAP are 74.75% and 90.42%, respectively, total SID AA is much higher than dried skim milk, showing to have three to four times higher SID Lys content, respectively (Cho et al., 1997). The results on the benefits of these blood-origin ingredients on growth performance after weaning has shown to be consistent (Bosi et al., 2004; Nofrarías et al., 2006; Zhao et al., 2007; Hernández et al., 2010). Based on 15 published studies where SDAP was fed to weaned pigs, van Dijk et al. (2001) concluded that up to 6% of dietary SDAP inclusion increases average daily gain (**ADG**), average daily feed intake (**ADFI**), and gain-to-feed ratio (**G:F**) in the first 2 wks after weaning. There is also evidence that SDAP improves intestinal morphology (Nofrarías et al., 2006; Zhao et al., 2007) and reduces incidences of diarrhea (Peace et al., 2011). Also, Bosi et al. (2004) reported that spray-dried porcine plasma (**SDPP**) contributes to maintaining mucosal integrity and reduces inflammatory responses in the gut, thereby reducing infections from pathogenic bacteria in weaned

pigs challenged with enterotoxigenic *E. coli* (**ETEC**). Similarly, in a study by Torrallardona et al. (2003), where weaned pigs were challenged with *E. coli* K99, 7% SDAP inclusion in a nursery diet increased *Lactobacilli* populations in ileal and cecal digesta. As a result, pigs fed a SDAP containing diet showed the reduction in various subsets of leukocyte in ileocolic lymph nodes, suggesting less activation of immune system (Nofrarías et al., 2006). The mechanism of action in dietary blood-origin products is still unclear, but a high feed intake due to high palatability (Ermer et al., 1994) seems to be the major reason for its benefits regarding growth performance. In addition, some bioactive compounds present in blood, such as immunoglobulins, insulin-like growth factor-I, and glycoprotein, may be associated with improved gut health (van Dijk et al., 2001), but further studies are warranted. Use of dietary SDAP in a weaner diet has received numerous reviews by Torrallardona et al. (2002), van Dijk et al. (2001), and Campbell et al. (2010). However, after a tragic outbreak of the porcine epidemic diarrhea in 2014 in North America, the use of SDPP was drastically lowered because of the concerns that SDPP may contain viral deoxyribonucleic acids or ribonucleic acids (**RNA**) that can be transmitted to the pigs fed this diet. However, several studies have indicated that the virus present in SDPP is not infectious to naive pigs (Shen et al., 2011; Opriessnig et al., 2014). However, this will not be discussed in this review.

2.2.3 FISH MEAL

Because earlier studies have shown that fish meal improves the growth performance of weaned pigs (Evans, 1952; Pike, 1979), fish meal has traditionally been included in the weaner diet. Fish meal generally has an excellent AA profile, with a high content of vitamins and minerals (Mason and Weidner, 1964). Combined data on fish meal from NRC (2012) reported that it contains 63% CP, 85% of which is SID. Total SID Lys is 3.92%, lower than blood plasma but

higher than dried skim milk (NRC, 2012). Stoner et al. (1990) reported that fish meal replacing soybean meal (SBM) increased ADFI, which ultimately improved ADG in weaned pigs. Also, Pike (1979) suggested that high levels of Se in fish meal might contribute to the improved growth of weaned pigs. Furthermore, Kim and Easter (2001) reported that SDPP in the weaner diet can be replaced with Mackerel fish meal dried at 70 °C. This substitute would not compromise the growth performance of weaned pigs. However, nutrient contents are relatively inconsistent depending on the species of fish, processing temperature, and amount of oil residue, which results in different growth responses (Kim and Easter, 2001).

2.2.4 ADDITIVES

Antibiotics were the most widely used feed additives in nursery diets. However, as concerns about food safety grows, the use of antibiotics is being prohibited around the world. The common purpose of feed additives (e.g., organic acids, zinc oxide, flavours, and probiotics) is to minimize growth retardation after weaning by positively modifying microbiota and improving gut health and feed intake. Feed additives have received great attention; even a little amount of addition provides benefits at a relatively reasonable price. Various promising alternatives to antibiotics has been reviewed by de Lange et al. (2010), Heo et al. (2013), and Thacker (2013), and they will not be discussed in this review.

2.3 STRATEGIES TO REDUCE THE DIETARY COSTS

2.3.1 ALTERNATIVE FEED INGREDIENTS

Apart from animal-protein sources, other ingredients such as corn, wheat, SBM, and supplemental oil are also becoming a considerable factor in the rising costs of nursery diets because of their soaring prices, largely in part because of increasing demand by the food and ethanol industry. Thus, it is necessary to find alternative feed ingredients that can replace conventional ingredients and increase diet formulation flexibility. The effects of including alternative feedstuffs in nursery diets on performance and economic viability of pig production were summarized in Table 1.1. The feed costs were estimated at the sum of each averaged price of ingredients over 5 years (2012–2016). In this review, feed alternatives will be categorized into cereal grains, cereals coproducts, pulse, oilseed, and oilseed coproduct.

Cereal grains

Corn and wheat are the traditional energy sources in swine diets. However, as the price of these cereal grains have risen, there has been a need for alternatives that can produce as much energy. Barley has been widely used as an alternative feed ingredient in some regions of North America and Northern Europe (Fairbairn et al., 1999). Nutrient composition in barley varies depending on the climatic and agronomic conditions (Pettersson and Lindberg, 1997). Generally, feed barley can be classified into two types: hulled and hulless barley. As the name implicates, these two are distinguished by the presence of hull of the barley kernel. Hull, mostly composed of an indigestible carbohydrate matrix, reduces nutrients and energy density in the barley, as well as lowers its digestibility for pigs (Kennelly and Aherne, 1980). Hulless barley contains 3,179 kcal of DE whereas hulled barley contains 3,073 kcal/kg of DE (NRC, 2012). Also, hulless barley showed a higher apparent total tract digestibility (**ATTD**) and apparent ileal digestibility (**AID**) of organic matter (**OM**) and energy comparted to hulled barley (Pettersson and Lindberg, 1997).

However, lower SID AA are shown in hulless barley compared to hulled barley (NRC, 2012). This is because of rich ANF in hulless barely, such as soluble β-glucan, which is known to increase digesta viscosity (Bhatty et al., 1986). The SID of Lys in barley was estimated at 69.5 ± 2.11 based on 26 studies (Spindler et al., 2014). This is 2.5% lower than in corn, but barley contains higher SID Lys (0.31%) than corn (0.18%) because of higher Lys concentration in barley.

Triticale is a man-made cereal crop; it is a hybrid of rye and wheat or durum and is now used as a feedstuff and for ethanol production. Triticale has two genotypes: a winter or spring growth habit (Goyal et al., 2011). Triticale contains 13.60% of CP, 64.31% of starch, and 3,228 kcal/kg of ME (NRC, 2012), which is similar to corn and wheat. Also, AID of CP and Lys in triticale are 78% and 76%, respectively, which is based on three studies (Pedersen and Boisen, 2002). Furthermore, Hale and Utley (1985) reported that weaned pigs fed a diet containing 66% triticale did not see changes in ADG and ADFI but did improve G:F compared to those fed a diet containing wheat.

Sorghum is cultivated in warm climates over the world. It is a good source of energy, containing 70.05% of starch and 3,532 kcal/kg of ME (NRC, 2012). However, the anti-nutritional effects of tannin should be considered when sorghum is fed to pigs as an energy source. Pan et al. (2016) reported a decrease of more than 200 kcal/kg of ME in when tannin content was increased by 1%. However, Lizardo et al. (1995) reported that sorghum as a replacement for 56% of the corn in a nursery diet did not change growth performance. More recently, Jordan et al. (2015) found that feeding a diet composed of 51%, 67%, and 72% of sorghum did not change the growth performance of weaned pigs for 21-d compared to a corn diet.

*Distiller dried grains with solubles (**DDGS**)*

Because of the growing production of bioethanol produced from grains, the price of grains has increased, and a substantial amount of its coproduct, DDGS, is produced in the feed industry (Rosentrater, 2006). Therefore, much research has been conducted to use DDGS in swine diets in North America. DDGS is an alternative source of energy, CP and P. Based on data from 11 studies, the concentration of digestible energy (**DE**) and ME in corn DDGS are 4,140 kcal/kg of DM and 3,897 kcal/kg of DM, respectively, which are similar to corn (Pdersen et al., 2007). Because most starch in grains is converted into ethanol in the fermentation process, other nutrients become highly concentrated (Stein and Shurson, 2009). For example, corn DDGS contains 0.60–0.70% of P, and its ATTD is 59%, which is a much higher value than corn (Pedersen et al., 2007). Also, DDGS is a good source of CP and AA because of their high concentrations. The CP concentrations in corn, sorghum, and wheat DDGS are approximately two, three, and four times higher, respectively, than their respective parental grains (Stein et al., 2006, Urriola et al., 2013; Widyaratne et al., 2009). However, it should be noted that DDGS contains a relatively low and variable concentration of Lys, as well as low SID of Lys because of damage sustained from heat during the process of ethanol production (Cromwell et al., 1993). Also, DDGS contains highly concentrated NSP, showing 36% insoluble and 6% soluble dietary fiber (Stein and Shurson, 2009); thus, DDGS should be carefully included in a nursery diet. Furthermore, because it has been demonstrated that a DDGS-based finisher diet deteriorates pork quality by reducing pork firmness, which is because of the unsaturated fatty acids in DDGS (Xu et al., 2010), it should also be elucidated whether DDGS inclusion in a nursery diet affects final pork quality. Much research has been conducted to investigate the effect of DDGS inclusion in a nursery diet and how it may affect growth performance in weaned pigs. Ten studies on corn DDGS were reviewed by Stein and Shurson (2009); a 10% inclusion of DDGS from 3-d post-weaning or 22.5–30% DDGS from 3-wk post-

Table 2.1 Effect of including alternative feedstuffs in nursery diets on performance and economic viability of pig production[1]

Item	Inclusion level (%)	Substituted ingredient	Initial BW	Days[2]	Δ in performance			Δ in feed cost[3]		Reference
					ADG, g/day	ADFI, g/day	G:F	$/ton	¢/kg gain	
Cereal										
Sorghum	67	Corn	10.6	21	0.0	-4.5	<0.01	36.8	5.4	Jordan et al. (2015)
Sorghum	56	Corn	7.1	21	8.0	-10.0	-0.02	24.1	1.6	Lizardo et al. (1995)
Triticale (spring)	66	Wheat	7.6	28	-3.5	-28.5	-0.02	126.3	2.4	Beltranena et al. (2008)
Triticale (winter)	66	Wheat	7.6	28	4.5	-29.5	-0.03	19.3	2.1	Beltranena et al. (2008)
Triticale	37	Corn, SBM	9.6	28	-30.0	-50.9	0.01	-5.6	-0.6	Hale and Utley, (1985)
Triticale	76	Corn, SBM	9.6	28	-30.0	28.3	0.05	-11.0	2.7	Hale and Utley, (1985)
DDGS										
Corn DDGS	10	Corn, SBM	9.9	22	-31.8	-27.2	0.02	-1.1	1.5	Linneen et al. (2006)
Corn DDGS	20	Corn, SBM	7.7	35	9.0	33.0	0.02	6.5	-1.4	Whitney and Shurson, (2004)
Corn DDGS	15	Corn, SBM	11.0	28	-15.0	-2.0	0.02	-7.5	1.1	Jones et al. (2010)
Corn DDGS	30	Corn, SBM	11.0	28	-9.0	-1.0	0.01	-12.8	0.1	Jones et al. (2010)
Corn DDGS	30	Corn, SBM	10.0	21	-14.0	-30.0	-0.01	-16.4	-3.0	Zhu et al. (2010)
Corn DDGS	30	Corn, SBM	16.0	14	-36.0	-39.0	0.01	-25.2	-3.1	Zhu et al. (2010)
Sorghum DDGS	15	Corn, SBM	11.0	28	6.5	34.5	0.02	NA	NA	Jones et al. (2010)
Sorghum DDGS	30	Corn, SBM	11.0	28	-16.0	12.0	0.03	NA	NA	Jones et al. (2010)
Wheat DDGS	10	Wheat, SBM	6.7	28	0.0	-8.0	-0.01	NA	NA	Avelar et al. (2010)
Wheat DDGS	15	Wheat, SBM	6.7	28	-13.0	-28.0	-0.01	NA	NA	Avelar et al. (2010)
Pulse										
Field pea	6	Corn, SBM	7.8	21	13.0	0.0	-0.02	1.4	-1.6	Stein et al. (2004)
Field pea	12	Corn, SBM	7.8	21	10.0	40.0	0.02	2.4	2.6	Stein et al. (2004)
Field pea	18	Corn, SBM	7.8	21	-16.0	-20.0	<0.01	4.3	1.2	Stein et al. (2004)
Field pea	12	Corn, SBM	6.0	35	-32.0	-50.0	<0.01	3.3	-0.2	Friesen et al. (2006)
Field pea	24	Corn, SBM	8.9	28	-10.0	13.0	0.02	-0.9	2.0	Stein et al. (2010)
Field pea	30	Corn, SBM	4.5	14	-25.0	47.0	0.11	-25.5	10.2	Owusu-Asiedu et al. (2009)
Faba bean	12	Wheat, SBM	9.1	21	-7.0	-38.0	-0.02	8.7	-0.7	Beltranena et al. (2009)

[1]Average price of feed ingredients for 5 years (Jan. 2012–Dec. 2016) was calculated to estimate feed cost (Corn, $185; SBM, $483; Barley, $170; Wheat, $161; Oat, $193; Triticale, $190; Flaxseed, $471; Canola seed, $478; Sunflower seed, $485; Soybean, $295; Field pea, $295; Faba bean $337; Blood meal, $1,080; Canola meal, $253; Fishmeal, $1,930; Dried whey, $973; Skim milk powder, $3,203; Lactose, $836; Corn DDGS, $260; Wheat millrun, $179; Vegetable oil, $1,022; Monocalcium phosphate, $1,011; Lysine, $1,860; Methionine, $7,370; Salt, $310; Threonine, $4,230; Limestone, $190; Vitamin-mineral premix, $4,000; Grains and Oilseeds market price, Manitoba agriculture).

[2]Experimental period.

[3]I, Phase I; II, Phase II.

Table 2.1 (continued)

Item	Inclusion level (%)	Substituted ingredient	Initial BW	days	Δ in performance ADG, g/day	ADFI, g/day	G:F	Δ in feed cost[2] $/ton		¢/kg gain		Reference
Oilseed												
Flaxseed	12	SBM, oil	6.1	28	-6.7	-18.0	-0.01	I	27.9	I	-0.1	Kiarrie et al. 2007
								II	7.6	II	0.1	
Sunflower	10	Corn, SBM, oil	5.9	35	-9.1	-13.6	<0.01		-10.7		-1.6	Fitzner et al. 1988
Sunflower	15	Corn, SBM, oil	5.9	35	-9.1	-18.1	<0.01		1.5		0.0	Fitzner et al. 1988
Sunflower	20	Corn, SBM, oil	5.9	35	-59.0	-95.3	0.01		13.6		3.9	Fitzner et al. 1988
Soybean	16	SBM, oil	NA	23	-36.0	-34.0	0.02		-17.2		-0.6	Valencia et al. 2008
Canola	8	SBM, oil	7.8	28	-20.0	33.0	0.06		-34.7		0.8	Shaw et al. 1990
Canola	15	SBM, oil	7.8	28	4.0	-27.0	-0.04		-11.9		-4.7	Shaw et al. 1990
Canola	30	SBM, oil	7.8	28	-59.0	-156.0	-0.10		24.6		-5.0	Shaw et al. 1990
Oilseed coproduct												
Canola meal	5	SBM	8.1	28	9.0	0.0	-0.01		-4.7		-1.8	Landero et al. 2011
Canola meal	10	SBM	8.1	28	-8.0	-23.0	-0.01		-8.8		-2.3	Landero et al. 2011
Canola meal	15	SBM	8.1	28	21.0	27.0	0.00		-12.3		-2.2	Landero et al. 2011
Canola meal	20	SBM	8.1	28	5.0	-23.0	-0.03		-16.4		-5.0	Landero et al. 2011
Canola meal	10	SBM	6.3	28	-24.0	-31.0	<0.01		8.9		1.5	Seneviratne et al. 2011
Canola meal	5	SBM	7.6	28	-12.0	5.0	0.02		-1.3		1.9	Sanjayan et al. 2014
Canola meal	10	SBM	7.6	28	-8.0	-1.0	0.01		-2.7		0.6	Sanjayan et al. 2014
Canola meal	15	SBM	7.6	28	2.0	9.0	<0.01		-0.8		0.3	Sanjayan et al. 2014
Canola meal	5	SBM	7.6	28	20.0	37.0	<0.01		-4.8		-0.5	Sanjayan et al. 2014
Canola meal	10	SBM	7.6	28	16.0	36.0	0.01		-7.0		-0.4	Sanjayan et al. 2014
Canola meal	15	SBM	7.6	28	1.0	13.0	0.01		-4.5		0.2	Sanjayan et al. 2014
Canola meal	6	Wheat, SBM	8.1	35	-27.0	-110.0	-0.06	I	-4.8	I	2.3	Landero et al. 2013
								II	-5.7	II	-1.7	
Canola meal	12	Wheat, SBM	8.1	35	-34.0	-41.0	0.01	I	-10.4	I	3.1	Landero et al. 2013
								II	-9.8	II	-1.6	
Canola meal	18	Wheat, SBM	8.1	35	-40.0	-43.0	0.02	I	-15.9	I	0.8	Landero et al. 2013
								II	-16.0	II	-0.9	
Canola meal	24	Wheat, SBM	8.1	35	-57.0	-84.0	<0.01	I	-21.2	I	2.4	Landero et al. 2013
								II	-22.2	II	-53.7	
Flaxseed meal	9	Wheat, SBM	NA	18	-44.0	-38.0	0.05		NA		NA	Jansman et al. 2007

weaning did not affect ADG of weaned pigs. Also, several studies reported improved G:F when DDGS was added to the nursery diet (Gaines et al., 2006; Barbosa et al., 2009). Furthermore, several studies (Whitney et al., 2006) indicated that residual yeast cells and yeast cell components in DDGS possibly promote gut health by reducing the prevalence and severity of intestinal lesions and improving villus height (**VH**) and VH: crypth depth (**CD**) ratio. On the other hand, recent studies reported that increasing wheat DDGS up to 20% adversely affected growth performance, reducing ADG and G:F (Avelar et al., 2010; Wang et al., 2016). Also, based on the average price of feed ingredients over 5 years (2012–2016), it seems hard to expect that replacing corn and SBM with corn DDGS can reduce nursery diet costs (See Table 2.1); this is because it replaces much of corn that has been cheaper than DDGS with relatively little replacement of SBM, which is the expensive part of nursery diets. Therefore, the inclusion of corn DDGS could be an economical feeding strategy when the price of corn and SBM are much higher than DDGS.

Pulse

Pulses are the dry and edible seeds of the legume plant that are not used for oil extraction (Patterson et al., 2009). In North America, the most common pulses grown for feedstuffs are peas, faba bean, lentils, and chick peas. Pulses are characterized by a relatively high CP content instead of low-fat content compared to other legumes (Patterson et al., 2009). However, the level of ANF in pulses, such as protease inhibitor, tannins, phytate, and saponin (Khattab and Arntfield, 2009), should be considered when included in nursery diets.

Field peas (*Pisum sativum*) vary and include yellow peas, green peas, and marrowfat peas. In general, yellow peas and green peas have been widely used for swine diet, and their outer colour does not affect nutrient composition. Field peas contain 22% of CP, which is less than SBM, but

they have similar SID of Lys (NRC, 2012). Also, they have less starch (43 %) but a similar net energy (**NE**; 2,419 kcal/kg) value to that of wheat (NRC, 2012). Many studies have shown that field peas can be successfully fed to weaned pigs, partially replacing SBM in nursery diets. In a study by Stein et al. (2004), weaned pigs were fed a conventional weaner diet for a 2-wk period and then fed a field pea diet (0%, 6%, 12%, or 18%) replacing corn and SBM for a 3-wk period. As a result, there were no differences in ADG, ADFI, or G:F among treatments. However, in another study (Stein et al., 2010), it was reported that ADG of weaned pigs was linearly reduced in response to increasing levels of field peas during the 28 days of the experiment. Furthermore, pigs weaned at 16-d-old and fed a diet containing 33% field peas showed lower G:F during the 2-wk period after weaning. Based on the results from these studies, it seems that a diet made up of 15–20% field peas can be successfully used, replacing corn and SBM without compromising growth performance. However, it should be avoided during the initial 2-wk post-weaning for maximum performance to be reached.

Faba beans (*Vicia faba*) could also be a good protein source in swine diets; they contain a relatively higher CP (27%) but less starch (39%) than field peas. However, a high concentration of tannin as ANF in faba beans is the main reason for limiting their inclusion in a swine diet (Oomah et al., 2011). Because energy digestibility is inversely related to the tannin content (Pan et al., 2016), faba beans also have a relatively low ME and NE (3,060 and 2,143 kcal/kg, respectively) compared to field peas (NRC, 2012). Fortunately, zero-tannin or tannin-free faba beans have been developed, containing about 1% tannin, compared to the 8–9% tannin in traditional faba beans (Oomah et al., 2011). In a study by Beltranena et al. (2009), after pigs weaned at 19-d-old were fed commercial diets for a 14-d period, they were fed a diet containing increasing levels of zero-tannin faba beans used for substituting SBM (0%, 10%, 20%, 30%, or 40%). The

authors found that increasing the dietary level of faba beans did not affect ADG, ADFI, or G:F, as well as ATTD of gross energy (**GE**) during the 21-d experimental period.

Oilseeds

Oilseed crops are grown primarily for oil extraction. The oil contents of cereal grains are only 1–3%; however, the oil contents in oilseeds range from approximately 20% for full-fat soybeans and 34% for flax seeds to over 40% for sunflower oil seeds and canola seeds (NRC, 2012). Thus, soybeans, sunflower oil seeds, and canola seeds have 2,874, 3,561, and 4,059 kcal/kg of NE, respectively (NRC, 2012). Apart from the high concentration of oil in such seeds, the oilseeds are also rich in CP, with 18% in sunflower seeds, 23% in flaxseeds, 22% in canola seeds, and 32% in soybeans. Therefore, even though the prices of oilseeds are relatively expensive compared to other cereal grains, oilseeds can substitute for SBM and supplemental oil, which could reduce the feed cost when their prices are competitive. However, oilseeds generally contain more ANF than their meals because heat-liable ANF in oilseeds such as trypsin inhibitors can be eliminated by heat during the extraction process (Khattab and Arntfield, 2009). Therefore, oilseeds may have lower SID of AA (NRC, 2012). In a study where weaned pigs were fed a diet containing 12% flaxseed, their growth performance was maintained for the 4-wk period of experiments except for wk 3 compared to those fed a wheat-SBM-based diet (Kiarie et al., 2007). Also, Fitzner et al. (1988) reported that feeding a diet containing 15% of finely ground sunflower seeds to substitute for corn, SBM, and supplemental oil did not affect ADG for 14-d after weaning. Similarly, based on results from a study by Shaw et al. (1990), up to 15% of canola seeds can be included in nursery diets for pigs weaned at 4-wk-old without negatively affecting growth performance for 4-wk post-weaning. However, in the case of soybeans replacing barley and soybean oil, 16% inclusion in

nursery diets for weaned pigs reduced ADG and G:F in pigs weaned at 23-d-old during the initial

10-d post-weaning (Valencia et al., 2008). Based on these results, it seems straightforward that

exceeding certain levels of oilseeds in nursery diets deteriorates the growth performance of weaned

pigs. However, the inclusion of oilseeds might enable economic formulation because corn,

supplemental oil, and SBM can be substituted for (See Table 2.1.).

Oilseed meals

Because of the high CP contents in oilseed meals that remain after oil extraction, they have

been widely used as a protein source in swine diets. The common oilseed meals in the feed industry

include SBM, cottonseed meal, flaxseed (linseed) meal, peanut meal, sunflower meal, and canola

meal (**CM**). Its steady production, its relatively balanced AA, and its cheap price compared to

animal protein sources has resulted in SBM being used as a major protein source in swine diets.

However, its drastic increase in price resulted in exploring other alternative oilseed meals to

replace SBM. Among the various oilseed meals, CM has received great attention in the last decade

as an alternative protein source. One of the greatest advantages of CM is that it contains relatively

low levels of ANF. Compared to rapeseed meal, CM contains lower levels of glucosinolates (< 30

µmol/g), which is known as a major ANF, limiting inclusion of rapeseed meal in swine diets

(Canola Council of Canada, 2015). According to results based on a 3-year survey, CM contains

36.7% of CP, 3.3% of ether extracts (**EE**), and 4.2 µmol/g of glucosinolates. On the other hand,

the SID of limiting AA (Lys, Trp, and Thr) in CM is lower than SBM (Trindade Neto et al., 2012,

Sanjayan et al., 2014). Therefore, digestible AA should be considered when SBM is replaced with

CM. Also, because of high fiber contents and a complex carbohydrate matrix with poor

digestibility, the energy content in CM is relatively low, showing respective DE, ME, and NE in

CM to be 3,154, 2,903, and 1,821 kcal/kg (NRC, 2012). In trials with weaned pigs, a strong preference for SBM was found (Landero et al., 2012). In this regard, Landero et al. (2013) reported that the growth performance of weaned pigs was reduced in a dose-response manner (0%, 6%, 12%, 18%, or 24%). In contrast, Sanjayan et al. (2014) found no linear effect of inclusion of increasing levels of CM (5%, 10%, or 15%) in weaned pigs. It should be noted that economic benefits can be achieved by feeding a diet containing CM in partial or total substitution for SBM. Over the last 5 years, the price of CM has been estimated at $253, which is 48% lower than SBM.

2.3.2 LACTOSE ALTERNATIVES

As discussed above, the importance of lactose from milk products for weaned pigs to reach maximum growth performance has been well documented. Lactose in a weaner diet is a source of carbohydrate (energy) for weaned pigs. Weaned pigs' ability to digest complex starch, long chain sugars with branches from grains is not fully developed (Hampson and Kidder, 1986), whereas lactose is a simple disaccharide, a combination of glucose and galactose, and lactase activity in brush border is relatively high around weaning age (Hampson and Kidder, 1986). Also, lactose serves as a sweetener in a weaner diet. Indeed, weaned pigs have a strong preference for lactose, and so inclusion of lactose can help maintain energy intake, which is the major challenge after weaning (Pluske et al., 1996). However, many attempts have been made to replace lactose with simple sugars in response to increasing price of lactose from dairy products. Various carbohydrate sources as a replacement of lactose have been examined (See Table 2.2), including glucose, sucrose, fructose, xylose, maltodextrin, starch, and hydrolyzed syrup.

Sucrose, or table sugar, has been intensively researched as an alternative to lactose. For weaned pigs to utilize sucrose as an energy source, sucrose must be split into glucose and fructose

by the action of sucrase. At birth, pigs have very low levels or the absence of the enzyme (Aherne et al., 1969). However, activity increases with age, and pigs around 2-wk old are capable of fully digesting and absorbing sucrose (Becker et al., 1954; James et al., 1987). In a study by Jin et al. (1998), 20% of sucrose totally substituted lactose in a weaner diet containing SDAP, fish meal, and blood meal, and the diet was fed to pigs weaned at 21-d old. Reduced G:F was observed in pigs fed a 20% sucrose diet compared to those fed a 20% lactose diet during the initial 7-d after weaning. However, no differences in ADG, ADFI, or G:F were observed among the pigs fed a 20% sucrose diet and 20% lactose diet for the 21-d period of the experiment. This is supported by a study where there were no changes in growth performance for the initial 10-d and following 20-d after weaning in weaned pigs fed a diet replacing all the lactose with molasses or sucrose (Mavromichalis et al., 2001). Lee et al. (2000) also reported that 20% lactose in a weaner diet containing SDPP, fish meal, and dried porcine soluble can be replaced with equivalent sucrose, showing no difference in growth performance of pigs weaned at 21-d old during the 21-d post-weaning.

Glucose is a simple sugar and a form that pigs can rapidly absorb into their bodies through sodium-dependent transporters on intestinal epithelium (Wijtten et al., 2011). Dextrins are short chains of D-glucose (dextrose) molecules linked by a α-(1, 4) or α-(1, 6) glycosidic bond and produced by enzymatic hydrolysis of starch or glycogen. Adding glucose as an energy source to a weaner diet may be a good strategy to provide energy because it does not require any enzymatic actions to digest. However, there is a discrepancy in the results from feeding glucose instead of lactose. Mahan and Newton (1993) indicated that during 5-wk experimental period, a 12% dextrose diet did not change the growth performance of pigs weaned at 23-d old compared to the equivalent lactose diet. Also, a study by Lee et al. (2000) showed that pigs weaned at 21-d old

Table 2.2 Effect on growth performance of alternative carbohydrates in partial or total substitution for lactose in a diet fed to weaned pigs

Alternative carbohydrate	Inclusion level, %	Substitution rate, %	Weaning age, d	Test Period[4], d		Response to substitution			Reference
						ADG	ADFI	G:F	
Sucrose	20	100	21		21	None	None	None	Jin et al. (1998)
Sucrose	5	50	19		30	None	None	None	Mavromichalis et al. (2001)
Sucrose	5	50	19		30	None	None	None	Mavromichalis et al. (2001)
Sucrose	20	100	21		30	None	None	Reduced	Mavromichalis et al. (2001)
Sucrose	20	100	21		21	None	None	None	Lee et al. (2000)
Molasses	10	50	19		30	None	None	None	Mavromichalis et al. (2001)
Molasses	10	50	21		30	None	None	None	Mavromichalis et al. (2001)
Dextrose	12	100	23		14	None	None	None	Mahan and Newton, (1993)
Dextrin	20	100	21		21	None	None	None	Lee et al. (2000)
Dextrin	20	100	21		21	None	Reduced	None	Lee et al. (2000)
Glucose	20	100	21		21	Reduced	Reduced	Reduced	Jin et al. (1998)
Glucose	20	100	21		21	None	None	None	Lee et al. (2000)
Starch	12	100	23		14	Reduced	Reduced	Reduced	Mahan and Newton, (1993)
Starch	20	100	21		21	Reduced	Reduced	Reduced	Jin et al. (1998)
Starch	20	100	21		7	Reduced	Reduced	Reduced	Lee et al. (2000)
	12.5 or 25	100		I	7	None	None	None	
Mixture[1,3]	10 or 20	100	23	II	14	None	None	None	Naranjo et al. (2010)
	6 or 12	100		III	7	None	None	None	
	6 or 12	100	26	I	7	None	Increased	None	
Mixture[1,3]	3 or 6	100	26	II	14	Increased	Increased	None	Naranjo et al. (2010)
	3 or 6	100	26	III	7	Reduced	None	None	
Mixture[2]	13	50	14	I	14	None	None	None	Kim and Allee et al. (2001)
	7.5	50		II	14	None	None	Reduced	
Mixture[2]	26	100	17	I	14	None	None	None	Kim and Allee et al. (2001)
	15	100		II	14	None	None	None	

[1]40% lactose, 30% sucrose, 10% glucose.
[2]10% Lactose, 40% sucrose, 1.5% glucose, 38.5% oligosaccharides and starch.
[3]Combiend data from two inclusion levels were statistically analyzed.
[4]I, Phase I; II, Phase II; III, Phase III.

could utilize glucose or dextrin as efficiently as lactose when the pigs were fed a diet containing SDPP, fish meal, and dried porcine soluble. In contrast, Jin et al. (1998) reported that adding glucose to a weaner diet instead of lactose or sucrose led to a reduction in ADG, ADFI, and G:F throughout the 21-d experimental period.

The poor performance found in pigs fed a glucose-containing diet may be attributable to reduced feed intake because of the low palatability of glucose or dextrin (Moskowitz, 1970). This is supported by a second experiment by Lee et al. (2000), where pigs were fed increasing levels of dextrin showed a linear decrease in ADFI during the 3-wk experiment. It appears that no reductions in ADFI in the two studies (Mahan and Newton, 1993; Lee et al., 2000) were because of the high inclusion of skim milk powder (45% or 28%) in the weaner diet, which may have led to maximized palatability.

However, based on studies where weaned pigs were fed a diet containing starch as a replacement for lactose, the enzyme production did not seem sufficient enough to digest and utilize as an energy source for growth. Adding hydrolyzed starch rather than sucrose or glucose appears to not be an efficient energy source. Weaned pigs fed hydrolyzed starch diet compared to lactose or glucose or a sucrose diet showed reduced ADG, ADFI, and G:F throughout the 21-d experiment (Jin et al., 1998). Similarly, a significant reduction in ADG and ADFI were found in weaned pigs fed a diet of starch as a replacement for lactose for the initial 7 (Lee et al., 2000) and 14 (Mahan and Newton, 1993) days after weaning. Thus, taken together, it can be concluded that pigs weaned at 19-d or 21-d old utilize supplemental sucrose or glucose as efficiently as lactose, suggesting that lactose sources in a weaner diet might be replaced with other simple sugars to reduce feed costs.

2.3.3 FERMENTATION TECHNIQUE

Fermentation is an ancient but scientifically sound technique for the production of healthy foods for people and animals, improving the nutritional value of both food and feed. The fermentation technique can be applied to the livestock industry either by fermenting raw feed materials or liquid feed. Thus, there is a growing interest in fermented feed and how it can be incorporated into a nursery diet to improve nutritional value and possibly replace synthesized antibiotics. However, the results of fermentation vary tremendously, depending on feed ingredients, the strains of microbes, moisture content, the presence or absence of oxygen, and the duration of fermentation. In this review, the effects of a fermented diet or ingredients for weaned pigs on performance, nutrient utilization, and gut health will be discussed.

Elimination of anti-nutritional factors

The ANF in SBM not only limits the acceptable amount of its inclusion into a weaner diet, but also inhibits the growth of weaned pigs (Li et al., 1990) which are susceptible to ANF (Pluske et al., 1997). Many studies indicated that microbes are capable of effectively degrading the ANF. Feng et al. (2007a) reported that the trypsin inhibitor content in soybean meal was eliminated from the initial level of 2.63 mg/g after fermentation with *Bacillus subtilis*. A similar result was found in a study that indicated a decrease in the level of trypsin inhibitor from 3.12 mg/g to 0.54 mg/g during fermentation with *Lactobacillus plantarum* (Wang et al., 2007). Similarly, the utilization of rapeseed meal is limited because of the presence of glucosinolates, which can reduce feed intake and induce iodine deficiency and hypertrophy of the liver, kidney, and thyroid (Tripathi and Mishra, 2007). Pal Vig and Walia (2001) found that inoculation of rapeseed meal with *Rhizopu oligosporus* reduced the initial glucosinolates content of 63.4 mg/g by 11, 13.7, 25.1, 33, and 43.1 mg/g after 1, 2, 5, 8, and 10-d of fermentation, respectively. Shi et al. (2015) found that solid-state

fermentation with *Aspergillus niger* led to a 76.89% decrease in glucosinolates of rapeseed cake. Cottonseed meal contains high levels of gossypol, which is a natural polyphenol pigment that has adverse effects on piglet growth, reproduction, gut health, and internal organs (Burlatschenko, 2003). Zhang et al. (2007) reported that gossypol content was reduced from 549.1 mg/kg to 145.5, 29.8, 63.1, 93.9, 178.4, and 81.5 mg/kg after solid-state fermentation with either *Candida capsuligena*, *Candida tropicalis*, *Saccharomyces cerevisae*, *Aspergillus terricola*, *Aspergillus oryzae,* or *Aspergillus niger*, respectively. The ability of microbes to detoxify ANF may be attributed to their enzymes, which can hydrolyze the ANF and utilize the products as metabolic substrates. Indeed, Pal Vig and Walia (2001) suggested that the reduction of glucosinolates and their by-products after fermentation is because microbial enzymes utilize the glucose and sulphur moieties of these compounds and their by-products in rapeseed meal during fermentation. Zhang et al. (2007) speculated that an observed decrease in gossypol level after fermentation might have been caused by the binding of free gossypol to microbial proteins (or AA) and by microbial enzymes secreted by microbes.

A variety of microbes have the capability to degrade different NSP in feed. For example, many studies have found that cellulases, such as endo *1.4*-β-glucanse, exo *1.4*-β-glucanse, β-glucosidase, and xylanase, can be produced by numerous types of filamentous fungi, particularly by the *Trichoderma*, *Aspergillus*, *Fusarium*, and *Penicillium* groups in the solid-state fermentation process (Ishaque and kluepfel, 1980; Semêdo et al., 2000; Silva et al., 2005). Studies have reported on a variety of fiber-degrading microbes that were isolated from different organisms. However, there appears to be limited literature on the use of fermented ingredients for nursery pig diets to take advantage of the microbes to decrease the level of NSP.

Nutrient digestibility of fermented feedstuff

Despite a large volume of literature on the nutritional value of fermented feed, there are relatively few studies on the digestibility of such feedstuffs in weaned pigs (see Table 2.3). Most digestibility studies have been conducted to investigate the efficacy of fermented soybean meal (**FSBM**), which contains less ANF content and can reduce inflammatory reactions in pigs. In contrast to the general consensus regarding the improved nutritional values of FSBM, there is much disagreement as to its digestibility. The effects of FSBM on digestibility have varied from study to study, despite the same inoculants being used for fermentation. For instance, Feng et al. (2007a) found increases in ATTD of DM, CP, and GE by 7.7%, 16.6%, and 13.3%, respectively, in weaned pigs fed a 26.5% FSBM diet. The authors assumed that the improvements were because of the elimination of TI and the degradation of large-size proteins in SBM from microbial activity during fermentation. This observation aligns with data presented by Kim et al. (2007). The ATTD of DM, GE, CP, EE, and ash significantly improved when 9% of SBM was replaced with FSBM that had been inoculated with *A. oryzae*. However, there is a discrepancy concerning improved digestibility. Results reported by Cervantes-Pahm and Stein (2010) indicated there was no difference in the AID and SID of DM, CP, and AA between unfermented SBM and FSBM that was fed as a sole source of protein to weaned pigs. Also, Yang et al. (2007) supported the result that the ATTD of nutrients in FSBM with *A. oryzae* did not significantly differ from that in SBM, except for P digestibility. It is not clear why the results for FSBM seem to be inconsistent among these experiments. It might be because of the different characteristics of FSBM. The fermentation of SBM in the experiment conducted by Cervantes-Pahm and Stein (2010) did not successfully degrade ANFs such as glycinin and β-conglycinin; no difference in the contents between SBM and

FSBM were found. Also, individual differences in the levels of tolerance to antigens that negatively affect nutrient digestibility may be attributable to this discrepancy. There can be synergistic effects of inoculants on nutritional value when it comes to digestibility. Yang et al. (2007) found that a weaner diet in which 8% of SBM was replaced by an equal amount of FSBM inoculated by both *A. oryzae* and *Bacillus subtilis* resulted in significantly improved ATTD of nutrients. On the other hand, the nutrient digestibility of FSBM inoculated solely by *A. oryzae* did not show any changes compared to the SBM diet. Also, the results presented by Chen et al. (2010) indicated that SBM fermented with a combination of two inoculants (*Aspergillus* and *Lactobacillus*) had approximately ten times more trichloroacetic acid soluble protein, which is an indicator of digestible proteins, 30% improved available Lys, and 7% greater in vitro CP digestibility, which might partially have contributed to the rise in nutrient digestibility. This is possibly because each bacterial species has a different ability to degrade or hydrolyze particular substrates.

Growth performance in weaned pigs fed fermented feed

Most experiments on fermented feed and its effects on growth performance have dealt with SBM. Kim et al. (2007) reported that replacing some of the unfermented SBM in a 15% SBM diet for weaned pigs by 3%, 6%, and 9% of FSBM could improve growth performance; the researchers observed an increase in ADG, ADFI, and G:F during the 35-d post-weaning period. This is partially in agreement with the results reported by Yang et al. (2007). The authors found that the inclusion of FSBM, replacing 8% of commercial unfermented SBM, significantly increased ADG and ADFI during the same period. However, G:F did not change. The authors purported that the lower ADFI compared to FSBM was partially because of the poor palatability of SBM, which led to lower ADG. Another possible reason for better performance was the

elimination of ANF that hinder growth performance (Kim et al., 2007). Additionally, Kim et al. (2010) reported that when the diet was supplemented with Lys, Thr, Trp, Met, and lactose, FSBM can partially replace the use of dried skim milk and SDAP without having adverse effects on the growth of weaned pigs. Jones et al. (2010) showed that FSBM can replace 5% of fish meal without any reduction in the growth performance of nursery pigs.

It appears that ingredients with improved nutritional value through fermentation can successfully replace other animal protein sources that have formerly been regarded as necessary options for high-nutrient weaner diets. However, there seems to have been little research to date on the differing effects of various fermented feeds on growth performance. Such research might lead to developing strategies to replace costly feed ingredients with different fermented ingredients and other types of microbes. Also, further studies are needed to investigate how feeding weaned piglets fermented feed can affect their finishing periods and carcass traits in the long run.

Effects of fermented feed on the gut health of weaned pigs

As with other studies on digestibility and growth performance, most research has focused on FSBM and gut morphology. This is because intestinal mucosal integrity and the function of the digestive tract can be impaired by ANF of SBM, such as with TI, lectins, and soybean globulins (Dunsford et al., 1989), which are known to be degraded during the fermentation process. The result reported by Feng et al. (2007b) represented a significantly higher VH in the duodenum, jejunum, and ileum of weaned pigs when fed 24% FSBM inoculated by *B. subtilis*. Interestingly, reduced CD in the duodenum was observed, indicating a higher VH:CD. Similarly, the electron microscopic pictures of villi in the intestines of weaned pigs fed FSBM present a smooth and tapering shape, which is in contrast to those of piglets fed unfermented SBM (Kim et al., 2007;

Table 2.3 Nutrient digestibility in a diet containing fermented feedstuffs fed to weaned pigs

Substrate[1]	Inclusion level, %	Inoculant[2]	Digestibility[3]	Δ in nutrients digestibility							Source
				DM	GE	EE	CP	Total AA	Ca	P	
SBM	27	*A. oryzae*	ATTD	+6.0*	+10.0*	-	+12.0*	-	-	-	Feng et al. (2007a)
SBM	30	*A. oryzae*	AID	+1.1	-	-	+0.1	+2.1	-	-	Cervantes-Pahm and Stein (2010)
SBM	3			+1.7*	+1.4*	+3.6*	+3.6*	-	-0.5	+4.7*	
	6	*A. oryzae*	ATTD	+1.6*	+1.9*	+5.3*	+4.9*	-	+0.9	+2.1	Kim et al. (2007)
	9			+2.2*	+2.3*	+4.3*	+7.1*	-	+1.6*	+1.0	
SBM	8	*A. oryzae* *B. subtilis*	ATTD SID for AA	+2.8*	+3.4*	+11.6*	+6.8*	+2.7	+9.0*	+3.9	Yang et al. (2007)
	8	*A. oryzae*		+0.5	-0.2	-1.7	+1.5	+3.4	+2.2	+7.6*	
SBM	22	*A. oryzae*		-		-	+2.0	+4.4	-	-	
	22	*A. oryzae* *L. casei*	AID	-	-	-	+4.1*	+4.4	-	-	Chen et al. (2010)
SBM	6	*A. oryzae*	ATTD AID for AA	+2.2*	+2.2*	+8.3*	+6.8*	+4.7*	+7.0*	+7.3*	Yun et al. (2005)
SBM	5			+3.0			+1.7	-1.5			
	10	*A. oryzae*	ATTD	+2.3	-	-	+1.3	+2.3	-	-	Cho et al. (2007)
	15			+3.0			+2.5	+3.4			
Wheat	96			+1.4	-	+12.0*	+1.8*	-	-	+22.0*	
Barley	96	*L. plantarum*	ATTD	+1.8	-	+10.0	-1.0	-	-	+14.4	Pieper et al. (2011)[5]
Triticale	96			+1.4*	-	+24.5*	+2.0	-	-	+22.7*	
RSC	-	*A. niger*	*In vitro*	-	-	-	-	+3.9*	-	-	Shi et al. (2015)[4]
CSM	-	*C. tropicalis*	*In vitro*	-	-	-	+2.9*	+2.8*	-	-	Zhang et al. (2007)[4]
Barley	100			+0.1	-	-4.3	+0.3	-7.1*	-	-	
Wheat		*L. plantarum*	ATTD SID for AA	+1.9	-	+27.1*	+1.0	+10.4*		-	Hackl et al. (2010)
Triticale				+0.7	-	+22.9*	+2.9	-	-	-	
Rye				-0.2	-	+7.0*	+0.4	-	-	-	
Mixture[5]	25	*L. fermentum* *S. cerevisae*	ATTD	-	-0.29	-	+0.8	-	-2.7	-1.9	Hu et al. (2008)

[a]SBM, soybean meal; RSC, rapeseed cake; CSM, cotton seed meal.
[b]*A*, *Aspergillus*; *L*, *Lactobacillus*; *S*, *Saccharomyces*; *B*, *Bacillus*; *C*, *Candida*.
[c]ATTD, apparent total tract digestibility; AID, apparent ileal digestibility; SID, standardized ileal digestibility.
[d]SID of Lys instead of total AA.
[e]Organic matter instead of DM.
*Statistic difference was observed.

Feng et al., 2007b). The authors suggested that fermentation with *B. subtilis* can effectively hydrolyze the acidic and basic subunits of soybean glycinin, thereby improving intestinal morphology

Microbial modification of the gastrointestinal tract (GIT)

It has been known since the days of antiquity that fermented food products benefit human gut health. Similarly, it has been reported that feeding fermented liquid compound diets to weaned pigs alters the gastrointestinal environment in a more desirable direction compared to feeding the pigs a normal diet (Russell et al., 1996). Generally, fermented feed, especially fermented liquid feed, is characterized as containing high concentrations of lactic acid, VFA, and abundant lactobacilli and low pH, which positively affect the bacterial ecology of the GIT (van Winsen et al., 2001). Indeed, Canibe et al. (2003) found that weaned pigs fed fermented liquid feed had significantly higher lactic acid bacteria in the stomach and small intestine compared to those fed a dry diet. Also, the number of *Enterobacteriaceae* decreased by more than 15.8%, 25.5%, 15.3%, and 24.2% in the stomach, distal small intestine, cecum, and mid-colon, respectively. It is believed that lactate and VFA can successfully reduce the numbers of *Enterobacteriaceae*, including *Salmonella* spp. (van Winsen et al., 2001; Prohászka et al., 1990). The level of total short chain fatty acids (**SCFA**) in fermented liquid feed has been found to be significantly higher than that in dry feed, having been measured at 38.4 mmol/kg in fermented liquid feed as opposed to only 5.8 mmol/kg in dry feed. In addition, the low pH of liquid feed has beneficial effects on the GIT of weaned pigs; these effects have been discussed in a previous literature review (Ravindran and Kornegay, 1993). According to the review, stomach pH levels and bacterial populations in GIT are evidently interconnected; van Winsen et al. (2001) found a significant positive correlation between

pH and the number of bacteria in the family *Enterobacteriaceae* in the stomach contents of pigs fed dry feed.

One study was on the benefits of feeding lactic acid fermented feed and showed fermented barley, wheat, triticale, and rye have abundant SCFA and low pH values (Pieper et al., 2011). Several studies have been conducted to investigate the effects of fermented soybean on PWD. Kiers et al. (2003) found that the severity of diarrhea was significantly less in piglets fed a diet of 20% *Rhizopus*-fermented soybean rather than toasted soybeans. In line with this finding, Kim et al. (2010) found that piglets fed a diet in which 6% unfermented SBM content was replaced with the same amount of FSBM had significantly lower diarrhea scores during the first 2-wk post-weaning period; to measure diarrhea, the authors used a scale of 1 (watery) to 5 (normal), showing averages of 4.16 with the FSBM diet compared to 3.66 with the unfermented SBM. The underlying reason is unclear. Reduced undigested substrates that can be potentially used by pathogenic bacteria in the gut might partially explain the benefit. Although the mode of action in FSBM is different from that in liquid, it appears obvious that fermentation-processed feeds have benefits on the gut health of weaned pigs.

2.3.4 EXOGENOUS FEED ENZYMES

Exogenous feed enzymes have been extensively researched for the last decade to explore their benefits in livestock and poultry feeds. Currently, their mass production is available at a reasonable price. Broadly, feed enzymes are classified into phytase and carbohydrase. In this review, the potential cost benefits of adding carbohydrase or phytase to nursery diets will be discussed.

Phytase

For swine diets, phosphorus (**P**) in plant-based ingredients is primarily present in the form of phytate (myo-inositol hexakisdihydrogen phosphate), which is composed of an inositol ring with 6 phosphate groups and 12 protons (Woyengo and Nyachoti, 2013). The activity of endogenous phytase secreted in the small intestinal mucosa of pigs cannot efficiently dephosphorylate phytate-P (Selle and Ravindran, 2008). For example, the total P in corn accounts for 0.58%, in which 80% is phytate P; thus, the STTD of P is around 44% (NRC, 2012). In addition, the requirements for STTD P are the greatest during the nursery phase (NRC, 2012). Therefore, a swine diet should incorporate higher levels of total P content, generally supplementing inorganic P, including mono- or dicalcium phosphate or animal-source ingredients (known to have a higher P availability) to meet the P requirements. This indicates that P in a swine diet is a considerable component in determining dietary costs. Also, phytate has a strong capability to chelate multivalent metal ions such as Zn, Ca, Fe, and protein residue (Selle and Ravindran, 2008). As reviewed by Woyengo and Nyachoti (2012), because of these physiological properties, phytate in swine diets creates antinutritional effects by decreasing minerals, CP, and energy digestibility and by increasing the endogenous losses of minerals and nitrogen (**N**).

Exogenous phytase is produced the most in the feed enzyme industry. The phytase liberates 6 P moieties and inositol in the digestive tract by hydrolyzing phytate (Woyengo and Nyachoti, 2012). Many studies have been conducted to find opportunities to reduce the P content in nursery diets by adding exogenous phytase and thereby increasing P utilization. In a study by Omogbenigun et al. (2003), weaned pigs were fed a conventional complex diet containing corn, lactose, SBM, and SDPP with low (negative control; 0.76% of total P) or high P (positive control; 1.33% of total P). Lower P content in a diet led to poorer growth performance while the addition

of phytase (500 FTU/kg) in a negative control diet enhanced ADG and G:F, showing similar growth performance with the positive control; this is in agreement with many other studies conducted on weaned pigs (Radcliffe et al., 1998; Valencia et al., 2002; Shelton et al., 2005; Williams et al., 2005; Braña et al., 2006; Kies et al., 2006; Sands and Kay, 2007; Olukosi et al., 2007; Zeng et al., 2011; Jolliff and Mahan, 2012; Zeng et al., 2013). This is mainly because exogenous phytase improves P and Ca utilization, showing improved retention rate (Revy et al., 2004; Sands and Kay, 2007; Létourneau-Montminy et al, 2010; Zeng et al., 2013) or digestibility (Valencia et al., 2002; Kies et al., 2006; Olukosi et al., 2007; Létourneau-Montminy et al., 2010; Zeng et al., 2011; Bento et al., 2012; Jolliff and Mahan, 2012; Zeng et al., 2013) in nursery pigs. This advantage is further extended to a reduction in environmental pollution because the amount of P in manure decreases. Furthermore, many studies have reported that phytase supplementation in a nursery diet enhances other mineral utilization, including Mg, Fe, Cu, and Zn (Revy et al., 2004; Kies et al., 2006). In this regard, Shelton et al. (2005) showed that the addition of phytase can successfully substitute for a trace-mineral premix in nursery diets without compromising growth performance.

Despite relatively consistent results on the effects of phytase on mineral utilization and growth performance, there is a controversy on whether phytase affects AA digestibility in nursery diets. Interestingly, Liao et al. (2005) formulated four different nursery diets (corn-SBM-based diet, wheat-SBM-based diet, wheat-SBM-CM-based diet, and barley-peas-CM-based diet) that can be commonly formulated in Canada, each with a similar available P content. Each diet was fed to weaned pigs fitted with a T-cannula without or with either 500 or 1,000 FTU/kg of phytase to estimate AID of AA. The results indicated that the beneficial effect of phytase on AID of AA was

observed only in the wheat-SBM-CM-based diet, suggesting the efficacy of microbial phytase in terms of AA digestibility in weanling pigs depends on diet composition.

Non-starch polysaccharides-degrading enzyme (carbohydrase)

The term NSP includes a wide variety of polysaccharides molecules, except for α-glucan (starch). The major NSP found in feed ingredients can be simply classified as cell wall NSP (cellulose, arabinoxylans, arabinogalactans, xyloglucans, mixed-linked β-glucan, and rhamnogalacturans glalactans) and non-cell wall NSP (fructans, mannas, pectins, and galactomannas; Montagne et al., 2003). Their structure and physiochemical properties have been well described by Choct (1997). The NSP in feed ingredients cannot be hydrolyzed by porcine endogenous enzymes and have been regarded as ANF because of their negative effects on digestion, increasing DM flow, intestinal viscosity, endogenous nutrient losses, and nutrient encapsulation, as reviewed by Souffrant (2001) and Wenk (2001). Plant-based ingredients are generally rich in NSP, which is a limiting factor for inclusion in a swine diet, particularly in a nursery diet. Thus, there has been a growing interest in the use of NSP-degrading enzymes to improve the nutrient availability in the ingredients and decrease ANF. In practice, NSP-degrading enzymes mainly target NSP present in the plant-source feed ingredients described above. Also, α-amylases are sometimes included as a carbohydrase for the digestion of excessive amounts of starch in diets. Sometimes, the combination of enzymes, including various NSP-degrading enzymes, α-amylase and protease, are used to maximize the beneficial effects of each specific hydrolyzing ability.

The addition of exogenous carbohydrase in a swine diet provides several benefits in the GIT for increasing the pigs' nutrient utilization. To be specific, carbohydrase has the ability to partially hydrolyze NSP, decrease digesta viscosity, and rupture cell walls consisting of NSP,

releasing the nutrients inside (Bedford and Schulze, 1998). Furthermore, carbohydrate-protein complexes, such as glycoproteins and proteoglycans, may be hydrolyzed by exogenous carbohydrase, enhancing protein or AA digestibility (Meng et al., 2005; Ayoade et al., 2012). These benefits may further improve G:F in nursery pigs (Pettey et al., 2002; Kim et al., 2003; Omogbenigun et al., 2004; Fan et al., 2009; Owusu-Asiedu et al., 2012; Zhang et al., 2014) and enable the formation of a nursery diet with lower energy. However, no information is available on whether supplementation of carbohydrase in a low-nutrient or energy-density diet can lead to comparable performance in weaned pigs with a requirements-met diet. Apparently, the supplementation of carbohydrase in a swine diet seems to improve nutrient and energy digestibility. However, there are difficulties when comparing the results from study to study because of different enzymes used, activities, substrates (diet composition), and individual variances (age, genotype, etc.). The effects of carbohydrase on nutrient digestibility and growth performance has been reviewed by Adeola and Cowieson (2011) and Bedford and Schulze (1998). Individual studies will not be discussed in this review.

Exogenous enzyme supplementation has recently received greater attention because of growing evidence that pigs' gut health is possibly promoted by altering GIT microbial ecology and providing potential prebiotic effects, as reviewed by Kiarie et al. (2013). Increased intestinal viscosity is known to be associated with the proliferation of pathogenic bacteria (McDonald et al., 2001); thus, the ability of carbohydrase to degrade soluble NSP would result in a favorable gut ecology, increasing the proportion of lactic acid (Kiarie et al., 2007) and VFA concentrations (Hübener et al., 2002) and reducing ammonia production (Kiarie et al., 2007). Furthermore, exogenous feed enzymes promote the digestion of feedstuff, reducing undigested nutrients that could be potential microbial substrates in the gut, thereby modifying microbial ecology. However,

no studies are currently available on its benefits on weaned pigs. Also, it was postulated that hydrolyzed products could potentially provide a prebiotic effect. Kiarie et al. (2008) incubated different feedstuffs with carbohydrase and found various sugars were produced, including mannose, which is a known immune stimulant. When these enzyme hydrolysis products were infused into small intestinal segments of pigs infected with ETEC, it was observed that fluid absorption through the segments was greater than the control segments (Kiarie et al., 2010), suggesting the possibility that products hydrolyzed by carbohydrase can maintain fluid balance and control ETEC-induced diarrhea in weaned pigs.

2.3.5 SIMPLE DIET AND COMPENSATORY GROWTH

Compensatory growth (**CG**) is defined as a physiological process resulting in the accelerated growth of animals after their growth retardation compared to age-matched control animals (Hornick et al., 2000). In pork production, CG could be beneficially introduced in a stage of nursery production to reduce feed costs by lowering the quality of nursery diets by simplifying diet composition or phase-feeding. This may also have positive effects on gut health, reducing excessive protein contents in a diet, which will be discussed in the 2.3.6. For extensive reviews on the mechanism of compensatory growth understood so far, refer to Martínez-Ramírez and de Lange (2008) and Hornick et al. (2000). In this review, growth catch-up following growth retardation caused by low-quality diet during the nursery phase will be discussed.

Many attempts have been made to simplify diet complexity during the nursery stage without compromising carcass yield and quality when pigs reach market weight. However, it has been documented that a simplified diet (low-quality diet; corn-SBM-based diet) reduces the growth performance of weaned pigs (Dritz et al., 1996; Mavromichalis et al., 2001; Wolter et al.,

2003; Mahan et al., 2004; Sulabo et al., 2010; Skinner et al., 2014). This is where the concept of CG can be employed. Many researchers have hypothesized that lagged growth in nursery pigs resulting from a simple low-quality nursery diet can be compensated during the re-alimentation period with improvements in feed efficiency. In 1973, Zimmerman and Khajarern reported that growth compensatory was observed in pigs following feeding low-protein (**LP**) nursery diets (9% or 12%) or restricted amounts (75% of ad-libitum group) during the nursery phase compared to pigs fed ad-libitum with 16% CP; the results showed no difference in ADG during the overall experimental period (until 93kg of BW). Similarly, several studies (Campbell and Biden, 1978; Dritz et al., 1996; Whang et al., 2000; Wolter et al., 2003; Martínez-Ramírez et al., 2009; Chaosap et al., 2011; Skinner et al., 2014;) have also shown an increased growth rate following the reduced growth rate during the nursery phase in pigs fed a low-quality diet (corn-SBM-based simple diet) or low-protein diet compared to those fed a high-quality diet (conventional complex diets containing dairy products and animal-protein source) or high-protein diet. Also, nursery diet complexity (simple vs. complex) did not alter carcass characteristics (Campbell and Biden, 1978; Dritz et al., 1996; Martínez-Ramírez et al., 2009; Skinner et al., 2014), carcass contents (Skinner et al., 2014), longissimus muscle quality (Dritz et al., 1996), loin meat quality (Skinner et al., 2014), or ham composition (Campbell and Biden, 1978). Furthermore, Kristensen et al. (2004) found that gilts fed a restricted amount (69% of ad-libitum) during the initial 80-d after weaning had higher longissimus muscle tenderness scores than those fed ad-libitum during the overall experimental period (d 28 to d 140). Whang et al. (2000) reported that pigs fed a low-quality diet during the starter period had higher protein but lower fat content at d 152 than those fed a high-quality diet, suggesting a leaner pork yield through compensatory growth. This is because fat deposition is affected more than protein deposition during the restricted period (Hornick et al., 2000). However,

the reduced initial growth rate during the nursery period was not always compensated for in the following growing–finishing period (Pond et al., 1980; Hines et al., 1993; Chiba, 1995), and this negatively changed carcass characteristics (Wolter and Ellis, 2001) compared to a high growth rate. This disagreement may result from variations in genotype (initial body composition), weaning weight, age, and sex, as well as degree, duration, type, beginning point of energy, and nutrient-intake restriction (Martínez-Ramírez and de Lange, 2008). Thus, further studies are needed to more precisely control CG and adapt CG to reduce the feed amount or nutritional quality nutritional value, which may provide great economic benefits.

2.3.6 LOW-PROTEIN DIET FORTIFIED WITH CRYSTALLINE AMINO ACIDS

According to NRC (2012), the average requirements for Lys in weaned pigs (BW 5-7 kg) is 1.50%, which is the greatest among the other stages of production. This is because of a high growth rate (e.g., protein deposition) with a relatively low feed intake (approximately 400g DM/d) during the first wk post-weaning. Therefore, in commercial swine production, the protein content of a diet is the greatest during the nursery period. Weaner diets are sometimes formulated to contain between 20–24% CP (Heo, 2010). This is mainly because of the excessive inclusion of protein sources to meet the requirements for limiting amino acids. Furthermore, higher levels of AA than the recommended requirements are included in nursery diets to avoid any AA deficiencies in weaned pigs under various health statuses (Jayaraman et al., 2015), which is ultimately done to maximize potential growth. However, protein in a complete diet accounts for the greatest proportion in total feed costs after carbohydrate (energy) sources. Also, there is evidence that a high-protein diet is associated with PWD (Heo et al., 2013), which is one of major factors in reducing swine productivity. Therefore, reducing the protein content fortified with crystalline AA

in weaned-pig diets could be a beneficial feeding strategy to reduce dietary costs and improve gut health. Furthermore, Kerr and Easter (1995) reported that a 1%-unit reduction in dietary protein level could lower 8% N excretion in manure, suggesting a strategy for sustainable livestock production. However, this review is focused on the effects of LP diet on gut health, PWD, and growth performance.

Effect on gut health and function, and immune response

A relatively low production of HCl and high gastric pH are often observed in pigs after weaning; this combination reduces enzyme activity such as pepsin (Kim et al., 2005). Also, intestinal absorption function is impaired after weaning (Pluske et al., 2007), and reduced enzyme activities in the brush-border surface of enterocytes are found in weaned pigs (Heo et al., 2013). Furthermore, pancreatic enzyme activity, such as chymotrypsin and trypsin, also dramatically decrease after weaning (Kim et al., 2005). Because of incomplete digestion and higher gastric pH, pathogenic bacteria are more prone to proliferate (Wijtten et al., 2011). Furthermore, proteolytic bacteria hydrolyze undigested proteinaceous materials in the gut, primarily in the large intestine and deamination or decarboxylation of AA occurs by them, during which toxic substances indoles, ammonia, biogenic amines, phenols, and branched chain fatty acids are produced (Rist et al., 2013). These toxic compounds together with the proliferation of pathogenic bacteria are associated with PWD (Heo et al., 2013).

Many studies have reached an agreement that decreasing dietary CP reduces microbial metabolites. In a study by Nyachoti et al. (2006) where pigs were weaned at 18-d old were fed diets with decreasing dietary CP (23% 21%, 19%, or 17%) while maintaining a balance of EAA, linear reduction in ammonia N and individual VFA concentrations were observed in the small

intestine. This aligns with findings showing that feeding weaned pigs an LP diet resulted in reduced ammonia N concentration in cecum (Htoo et al., 2007; Wang et al., 2011), colon (Bikker et al., 2006), or feces (Heo et al., 2008, 2009, 2010). Also, numerous studies have shown that individual VFA (Pierce et al., 2006; Htoo et al., 2007; Zhang et al., 2013), total VFA (Htoo et al., 2007; Hermes et al., 2009; Heo et al., 2009; Pieper et al., 2012), or total SCFA concentration (Hermes et al., 2009) were reduced. Furthermore, reductions in concentrations of individual amine in the colon (Pieper et al., 2012) and in the ileum (Htoo et al., 2007) were observed when dietary CP was reduced by 5% and 4%, respectively. Low ammonia N and amine productions in the gut have beneficial effects on growth and the differentiation potential of intestinal epithelial cells (Heo et al., 2013) and may preserve energy for detoxification by urea cycle in the liver or for maintenance of gut mucosa (Gaskin, 2001). However, only several studies have reported that an LP diet led to a reduction in microbial population, such as the *Clostridium leptum* group (Pieper et al., 2012), total anaerobes (Wang et al., 2011) in the colon, and *Escherichia coli* (Wang et al., 2011) in cecum *E. coli* in feces (Pierce et al., 2006), along with an increase in *Lactobacilli* in the feces (Pierce et al., 2006) of weaned pigs fed the LP diet. In contrast, most studies have shown no effect of LP on microbial populations (Bikker et al., 2006; Nyachoti et al., 2006; Jeaurond et al., 2008; Yue and Qiao, 2007; Heo et al., 2009; Hermes et al., 2009). Also, Zhang et al. (2013) reported no difference in diversity and similarity of microbial communities in the colon of weaned pigs fed either normal diet or LP diet. Similarly, some studies have shown that an LP diet reduced diarrhea incidences (Heo et al., 2008, 2009, 2010) or improved fecal scores (Lordelo et al., 2008; Yue and Qiao, 2008; Heo et al., 2009, 2010; Wang et al., 2011); others did not change diarrhea incidences (Hermes et al., 2009) or fecal scores (Le Bellego and Noblet, 2002; Nyachoti et al., 2006; Htoo et al., 2007;

Hermes et al., 2009). Different diet composition among the experiments and a relatively clean experimental environment may have contributed to this disagreement.

Caution should be exercised when an LP diet is formulated for weaned pigs regarding gut morphology and development. Yue and Qiao (2008) indicated that VH of duodenum and jejunum and disaccharides activities such as lactase and sucrase were linearly decreased with the reduction of dietary CP levels (23%, 21%, 18%, and 17%), even though the LP diets were fortified with supplemental AA, including Lys, Thr, Trp, Met, Ile, Val, His, and Phe. Similarly, when LP diets were fortified with common limiting AA (Lys, Met, Thr, and Trp) containing 17% CP, Zhang et al. (2013) showed there was a reduced VH at all sties of small intestine, AA-related transporter mRNA expression (Na^+-neutral AA exchanger 2), and protein expression (related to $b^{0,+}$ AA transporter) compared to the control diet (21% CP). However, when the LP diet was further fortified with branched chain amino acids (Leu, Ile, and Val) together with the common limiting AA, all parameters were recovered compared to the control diet. These results indicate that branched chain AA (particularly Leu) content should be considered to maintain gut health and function, as their importance for gut health has been stressed by Yin et al. (2010). However, based on the results from a study by Peng et al. (2016), where decreasing dietary CP levels from 20% to 14% fortified with all EAA showed impaired intestinal morphology, suggesting other non-essential AA such as Arg or Glu are imperative. Wu (2009) stated the importance of non-essential AA on gut development. Therefore, gut development seems to be carefully considered when dietary CP is further decreased to below 17%.

Effect on growth performance

In general, pigs do not have specific requirements for CP. However, ideal patterns of AA in swine diet are critical for their optimum growth because protein synthesis proceeds only to an extent when limiting AA is available. That is, if a diet is not balanced in terms of AA, a shortage of one or more EAA will cause growth reduction. In conventional diet formulation on the corn-SBM-basis, Lys is the most common limiting AA, followed by Trp and Thr. In practice, nursery diets are characterized with a high CP content to meet these limiting AA, hence providing excessive amounts of other AA. Thus, there has been growing interest to incorporate synthetic AA into swine diet to meet the limiting AA without excessive inclusion of protein source ingredients. There are varying results regarding the effect of LP fortified with synthetic AA on growth performance (Table 2.3). In general, based on previous results, synthetic industrial AA, such as Lys, Met, Thr, and Trp, may reduce dietary CP levels to 19% without compromising growth performance. However, an LP diet below 19% CP appears to require additional AA supplementation, such as Ile and Val, which are branched chain AAs. The importance of a branched chain AA was stressed in a study by Lordelo et al. (2008), where weaned pigs were fed 20% CP, Lys-Met-Thr-Trp-fortified 17% LP diet supplemented with or without Ile and/or Val. The results indicated that supplemental Val and Ile are needed to reduce dietary CP from 20% to 17% in a corn-wheat-SBM-based weaner diet. Also, Nychoti et al. (2006) reported linear reduction in pigs fed a corn-wheat-SBM-based diet with decreasing dietary CP fortified with Lys, Met, Thr, Trp, and Ile, suggesting Val might be the limiting AA. This has been supported by several studies showing additional supplementation of Ile and Val, together with 4 major limiting AA reduced dietary CP to 20% (Htoo et al., 2007), 18% (Heo et al., 2008, 2009; Le Bellgo and Noblet, 2002), and 16% (Hermes et al., 2009) without compromising growth performance. Peng et al. (2016) reported that growth was not compromised in weaned pigs fed a corn-SBM-based-LP diet (15.3%

CP) fortified with EAA, except Arg. However, they found an extremely low protein diet (13.9% CP) did not lead to optimal growth, even though the diets met all standardized digestible EAA requirements by supplementing with synthesized AA. Similarly, Gloaguen et al. (2014) indicated that a corn-wheat-barley-SBM-based LP diet (14.0% CP) fortified with synthetic Lys, Met, Thr, Trp, Val, His, Ile, Lue, and Phe, which met SID EAA requirements (NRC, 2012), led to poorer performance in weaned pigs compared to a 19.7% CP diet. This indicates that non-essential AA and total N along with EAA might play an imperative role in the growth and levels of non-essential AA because intact protein might be limited for growth when the CP in a corn-SBM-based diet is decreased to 14%.

Indeed, in another experiment (Gloaguen et al., 2014), a wheat-barley-based LP diet (14%) without any protein-source-cereals (e.g., SBM) was supplemented with all EAA plus Glu, Gly, and Pro to meet the requirements for standardized digestible EAA and to provide the same level of SID Pro:Lys and (Gly+Ser):Lys with the 15.6% CP:Lys levels. Feeding this diet to weaned pigs did not lead to poorer growth performance compared to a wheat-barley-corn-SBM-based 17.6% CP diet. Therefore, all things considered, dietary CP levels in nursery diets can be successfully reduced if all EAA, as well as non-essential AA content, are carefully considered. In practice, however, commercially available synthetic AA includes Lys, Met, Thr, and Trp, but their prices are relatively exorbitant ($1,500, $3,400, $1,600, and $6,300/ton, respectively; Jan 28, 2017 access, Agricultural Electronic Bulletin Board, University of Missouri). Therefore, additional studies are needed to estimate the benefits of LP on gut health of weaned pigs and, thereby, on its productivity in a commercial environment. Also, how to balance the benefits and additional costs for synthetic AA should be estimated to find the optimum dietary CP level for weaned pigs.

2.3.7 FERMENTABLE CARBOHYDRATE AS A SOURCE OF DIETARY FIBER FOR GUT HEALTH

The NSP in feed ingredients has been regarded as ANF for pigs, as described above. Ironically, the inclusion of specific NSP sources into a weaner diet has received much attention because of its ability to improve the gut health of weaned pigs. Generally, the most active degradation of NSP in the GIT occurs in the large intestine because of microbial enzyme, during which SCFA and gasses (CO_2, H_2, and CH_4) are produced (Wenk, 2001). These SCFA are absorbed from the lumen into the body at a rate dependent on luminal pH and the concentration of SCFA (Montagne et al., 2003). The SCFA absorbed are utilized as energy sources, which represent 15–24% of net energy of the maintenance needed for growing and finishing pigs (Montagne et al., 2003). Also, these SCFA, particularly butyrate, play substantial roles in intestinal integrity by stimulating epithelial cell proliferation and the resorption of water and sodium in the large intestine (Williams et al., 2001). However, it does not appear that all NSP provide beneficial effects on gut health in weaned pigs. It was reported that there is a positive relationship between soluble NSP and incidence of PWD in weaned pigs (McDonald et al., 2001). This is supported by results from a study where increasing levels of soluble NSP linearly increased the viable counts of β-hemolytic ETEC (Hopwood et al., 2004). This is mainly because soluble NSP generally increases intestinal viscosity, resulting in a decrease of digesta transit time and nutrient digestibility and an increase of endogenous N flow in the GIT (Choct, 1997), thereby providing a more favorable environment for the proliferation of pathogens.

On the other hand, the inclusion of insoluble fibers has been recognized as a feeding strategy to reduce the incidence of PWD and improve gut health. Early in 1968, Smith and Hallas

reported that compared to a barley meal (rich in soluble β-glucan) diet, barley hull (mostly composed of insoluble fibers) prevented ETEC-challenged weaned pigs from PWD. Also, the inclusion of 20% of the oat hull in an extruded rice-dried whey-fish meal diet decreased PWD incidence by 9% P in weaned pigs (Kim et al., 2008), which is in agreement with the results by Mateo et al. (2006). Also, Hanczakowska et al. (2008) found that 2.0% of cellulose can be beneficially incorporated into a barley-wheat-corn-SBM-based nursery diet by reducing the population of *E.coli* and *Clostridium* in cecum and PWD. Recently, a series of studies by Molist et al. (2009, 2010, 2011) showed the potential gut health benefits when WB as an insoluble fiber was included. It was reported that 4% (Molist et al., 2011) or 8% of WB (Molist et al., 2009) in a corn-barley-whey-based diet increased butyric acid concentration in fecal sample and colon digesta, respectively. In another study (Molist et al., 2010), it was reported that 4% of coarsely milled WB decreased *E. coli* K88 adhesion to ileal mucosa and fecal score in weaned pigs challenged with *E.coli* K88[+]. As such, insoluble NSP appears to serve as an alternative adhesion matrix to pathogenic bacteria such as *E. coli* because of its similarity with the intestinal receptors (Molist et al., 2011). Also, the authors compared the blocking capacity against *E. coli* K88 against insoluble fiber sources, including WB, rice hulls, soybean hulls, pea hulls, and oat hulls *in vitro*, and they found that WB extracts showed the greatest ability to bind F4-fibriated *E. coli* K88.

Post-weaning diarrhea poses tremendous economic losses in pork production. Thus, supplementing insoluble NSP can improve nursery productivity. Also, insoluble fiber sources such as WB or other hulls are by-products produced from grain processing, and thus, the prices are relatively reasonable, which gives broader opportunities to incorporate them into a weaner diet. However, excessive insoluble fibers may reduce nutrient and energy digestibility, so the optimum amount of NSP should be found, a topic discussed by Kim et al. (2012).

2.3.8 SUMMARY OF THE LITERATURE REVIEW

Nursery diets are much more expensive than other swine diets because of their high levels of animal-protein sources and dairy products. Therefore, an improvement in swine productivity can be achieved by reducing feed costs for nursery diets. Many feeding strategies have been made to replace conventional ingredients with alternative ingredients and/or functional feed additives while maintaining or improving growth performance and gut health. Numerous studies have indicated that traditional energy or protein sources (e.g., corn, wheat, supplemental oils, and SBM) in nursery diets can be partially or totally replaced with other cereal grains, pulses, oilseeds, DDGS, or oilseed meals without compromising the growth performance of weaned pigs. Also, dairy products as a source of lactose for pigs weaned later than 18-d old can be successfully substituted with simple sugars without having negative effects on growth performance. The feeding of a simple diet (low-quality) during the nursery phases followed by compensatory growth would lead to huge savings on nursey diets. Nutrients and energy utilization for weaned pigs can be enhanced by using fermentation techniques or exogenous feed enzymes that could improve feed efficiency and save on feed costs. Also, improving the gut health of weaned pigs by feeding them a diet that includes insoluble fibers or fermented feeds and that has been formulated with a low CP content or supplemented with exogenous feed enzyme could lead to potential cost savings.

However, it is difficult to calculate the accurate amount of cost savings with these feeding strategies because of the fluctuating market prices of feed ingredients, nutritional quality of alternative ingredients, environmental or sanitation conditions in farms, and the pigs' genetics. Also, the potential benefits from improved gut health is difficult to economically estimate. Further

studies are needed to investigate if synergic cost savings can be approached by combining the feeding strategies discussed above.

3.0 MANUSCRIPT I

Effect of dietary wheat bran inclusion on nutrient and energy digestibility and microbial metabolites in weaned pigs

B. Koo, **M.M. Hossain, C.M. Nyachoti**

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

3.1 ABSTRACT

This study was conducted to assess effect of including 4% of wheat bran (WB) in weaner diet on nutrient and energy digestibility and on microbial metabolites. Six weaned piglets (7.3 ± 0.1 kg of initial body weight) were surgically fitted with a T-cannula at the distal ileum. Pigs were fed 1 of 2 test diets consisting of: 1) control (a barley-wheat-corn-soybean meal-based diet), and 2) WB diet (4% of coarsely milled WB; 1,091 µm), which were assigned according to a 2×2 Latin square design repeated 3 times. At the end of the second period, all pigs were fed a corn starch-based diet containing 50 g casein/kg to estimate basal endogenous amino acids (AA) losses. Piglets fed the WB diet had lower coefficient of apparent total tract digestibility of dry matter (DM), organic matter (OM), crude protein (CP) and gross energy than those fed the control diet ($P < 0.05$). Also, inclusion of WB tended to reduce coefficient of apparent ileal digestibility of OM and DM ($P \leq 0.10$). Furthermore, the WB diet showed less digestible energy compared to the control diet ($P < 0.05$). There were no differences or trends in the coefficient of standardized ileal digestibility (CSID) of CP and AA between the 2 diets. Feeding the WB diet increased ($P < 0.05$) fecal acetate, butyrate, and total volatile fatty acids (VFA) concentrations in feces and tended to increase ($P \leq 0.10$) acetate, propionate, valerate, and total VFA in intestinal digesta compared with the control diet. In conclusion, results indicated that dietary inclusion of WB decreased nutrients and energy digestibility, but increased VFA production in the ileal digesta and feces.

Key words: Nutrient and energy digestibility, Standardized ileal digestibility, Volatile fatty acids, Weaned pigs, Wheat bran

3.2 INTRODUCTION

Weaned pigs generally show low nutrients and energy utilization because of their immature gut function and integrity (Lallès et al., 2004) as well as low diversity of intestinal microbiota (Konstantinov et al., 2004). Post-weaning challenges such as high incidence of diarrhea, gut disorder, and low enzymes activities further reduces the utilization of nutrients (Pluske et al., 1997). Wheat bran (WB), as a source of insoluble fiber, has received increasing attention because of its potential benefits on gut health in weaned pigs. Previous studies have shown that the inclusion of dietary WB in weaner diet beneficially decreased *Escherichia coli* K88$^+$ population in the ileal mucosa (Molist et al., 2010), thereby reducing the incidence of diarrhea (Molist et al., 2009). Also, the production of volatile fatty acids (VFA) by bacterial fermentation from fiber sources such as WB has been known to improve gut morphology and function (Williams et al., 2001). However, no studies have explored whether the benefits from dietary WB affect nutrients and energy digestibility in weaned pigs. Therefore, the objective of this study was to determine the influence of dietary WB on nutrients and energy digestibility and microbial metabolites.

3.3 MATERIALS AND METHODS

All experimental procedures were 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 (CCAC, 2009). Six barrows [(Yorkshire × Landrace) × Duroc; 7.3 ± 0.1 kg of initial body weight] were fitted with a T-cannula at the distal ileum and given a 14-d postsurgical recovery period before the start of the experiment. Pigs were individually housed in plastic-covered, woven metal floored pens (1.47 × 1.14 m). Room temperature was maintained at 29 ± 1ºC during wk 1 and then reduced by 1.5ºC/wk thereafter. Each pen was equipped with a

stainless-steel feeder and a nipple drinker, which allowed pigs to have unlimited access to water at all times.

The experimental diets (Table 3.1) included a corn-soybean meal-wheat-barley-based diet without WB (control) and a WB diet containing 4% of WB at the expense of barley, corn, wheat, and soybean meal. A casein-corn starch-based diet (50 g casein/kg; LP diet) was used to estimate basal endogenous losses of crude protein (CP) and amino acids (AA) for determining coefficient of standardized ileal digestibility (CSID) of CP and AA. The WB used in this study was manually sieved to obtain a 1,091-μm particle size of the mean value, whereas corn, barley, and wheat were ground in a hammer mill to pass through a 3,175-μm sieve. Titanium dioxide (0.3%) was included in the diets as an indigestible marker. Control and WB diets were formulated to meet or exceed the NRC (2012) requirements and had identical or similar standardized ileal digestible AA.

The 2 test diets (with or without the inclusion of 4% of WB) were assigned according to a 2 × 2 Latin square design replicated 3 times to give 6 observations per diet. At the end of the second period, all pigs were fed the LP diet. Each period lasted 9 d and started with a 5-d adaptation period followed by two 2-d collection periods for fecal (d 6 and 7) and digesta (d 8 and 9). Daily feed allowance was set at 4% of body weight at the start of each period and offered in 2 equal portions at 0800 and 1600 h as a dry mash. Fresh fecal samples and ileal digesta for digestibility assessment were collected and immediately frozen (-20ºC). Subsamples of digesta without formic acid and feces were used for pH determination using a pH meter (AB15 plus; Fisher Scientific, Toronto, ON, Canada); the rest were snap-frozen in liquid nitrogen and transferred immediately into a -80ºC freezer until required for VFA analysis as described by Agykeum et al. (2016).

Digesta samples were lyophilized and finely ground prior to chemical analysis. Samples of diets, digesta and feces were analyzed for dry matter (DM), gross energy (GE), ether extracts (EE),

CP, titanium, calcium, phosphorus, neutral detergent fiber (NDF), acid detergent fiber (ADF), ash and AA. Dry matter was determined according to official method 934.01 of the Association of Official Analytical Chemists (AOAC, 2006). Nitrogen content was determined using the combustion analyzer (model CNC-2000; Leco Corporation, method 984.13A-D); N was used to calculate the CP concentration (N × 6.25). The GE was determined using an adiabatic bomb calorimeter (Parr Instrument Co., Moline, IL), which had been calibrated using benzoic acid as a standard. Samples for titanium analysis were ashed and digested according to the method proposed by Lomer et al. (2000) and read on an inductively coupled plasma spectrometer (Vista-MPX; Varian Canada Inc., Mississauga, ON, Canada). The content of EE was determined using hexane extraction (method 920.39A) of AOAC (2006). The Ca (method 968.08) and P (method 946.06) concentrations were analyzed according to the AOAC (2006) and read on a Varian inductively coupled plasma mass spectrometer (Varian Inc., Palo Alto, CA). The ADF and NDF contents were analyzed according to the method of Goering and van Soest (1979) using the Ankom 200 Fiber Analyzer (Ankom Technology, Fairport, NY). Ash content was determined according to official method 942.05 of the Association of Official Analytical Chemists (AOAC, 2006). Organic matter (OM) was calculated as DM minus ash. Amino acid contents were determined according to the AOAC (1990); Code No. 982.30. Briefly, 100 mg sample was hydrolyzed with 6M HCl at 110°C for 24 h, followed by neutralization with 4 mL of 25% (wt/vol) NaOH and cooled to room temperature. The mixture was then equalized to 50 mL volume with sodium citrate buffer (pH 2.2)

Table 3.1 Composition and chemical analysis of experimental diets (%, as-fed)[1]

Item	Experimental Diets		
	Control	WB	LP
Ingredient			
Barley	100.0	93.0	—
Corn	300.0	280.0	—
Wheat	200.0	190.0	—
Wheat bran[2]	—	40.0	—
Soybean meal (460 g CP/kg)	236.0	224.0	—
Fish meal (620 g CP/kg)	50.0	50.0	—
Casein	—	—	50.0
Dried whey	35.0	35.0	—
Dextrose	—	—	220.0
Vegetable oil	37.0	45.2	—
Cornstarch	—	—	627.0
Cellulose	—	—	50.0
Limestone	11.1	11.5	6.0
Monocalcium phosphate	10.0	10.0	30.0
Iodized salt	2.5	2.5	4.0
Vitamin-mineral premix[3]	10.0	10.0	10.0
$_L$-Lys · HCl	3.1	3.3	—
$_{DL}$-Met	1.3	1.4	—
$_L$-Thr	1.0	1.1	—
Titanium dioxide	3.0	3.0	3.0
Energy and nutrient composition[4]			
DM	890.3	892.5	900.0
Metabolizable energy (MJ/kg)	14.02	14.02	14.20
CP (N × 6.25)	216.0	204.9	49.7
NDF	99.6	112.1	34.1
ADF	38.5	40.6	25.9
NSP			
Water-insoluble	83.1	102.1	—
Water-soluble	5.9	9.5	—
Total	89.0	111.6	—
P	7.8	7.9	7.7
Ca	10.3	10.5	6.4
Ether extract	45.7	62.8	—

[1]WB = 4% of wheat bran diet; LP = low protein diet (5% of casein); DM = dry matter; CP = crude protein; N = nitrogen; NDF = neutral detergent fiber; ADF = acid detergent fiber; NSP = non-starch polysaccharides; P = phosphorus; Ca = calcium.
[2]The mean particle size of wheat bran was 1091 μm; 891.2 g of DM, 161.7 g of CP, 42.3 g of NDF, 12.6 g of ADF, 300.2 g of water-insoluble NSP, 36.6 g of water-soluble NSP, 11.5 g of P, 0.9 g of Ca, and 22.8 g of ether extract per kilogram.

[3]Supplied per kilogram of diet: vitamins A, 8,250 IU; vitamin D_3, 825 IU; vitamin E, 40 IU; vitamin K, 4 mg; thiamine, 1 mg; riboflavin, 5 mg; niacin, 35 mg; pantothenic acid, 15 mg; vitamin B_{12}, 25 µg; folic acid, 2 mg; Cu, 15 mg (copper sulfate); I, 0.21 mg (potassium iodine), Fe, 100 mg (ferrous sulfate); Mn, 20 mg (manganese oxide); Se, 0.15 mg (sodium selenite); and Zn, 100 mg (zinc oxide).

[4]The metabolizable energy was calculated, whereas all other values were analyzed.

and analyzed using an AA analyzer (Sykam GmbH, Fürstenfeldbruck, Germany). Methionine and Cysteine were oxidized with performic acid prior to hydrolysis. Tryptophan was not determined. Total NSP was analyzed by using GLC for component neutral sugars (Varian CP3380 gas chromatography, Varian Inc., Palo Alto, CA), colorimetry for uronic acids (Biochrom Ultrospec 50, Biochrom Ltd., Cambridge, UK), and the procedure described by Englyst and Cummings (1988) with some modifications (Slominski and Campbell, 1990). Water-soluble NSP content was determined according to the method described by Slominski et al. (1993). The water-insoluble NSP content was calculated as the difference between total NSP and water-soluble NSP content. Volatile fatty acid concentrations were determined using gas chromatography (Varian Chromatography System, model Star 3400; Varian Medical Systems, Palo Alto, CA) with a capillary column (30 m by 0.5 mm; Restek Corp., Belefonte, PA) according to the method described by Erwin et al. (1961). Briefly, 1 mL of 25% metaphosphoric acid was mixed with 5 mL of digesta fluid in a 15-mL centrifuge tube, and the mixture was frozen overnight. Samples were then thawed, neutralized with 0.4 mL of 25% NaOH, and vortexed. Thereafter, 0.64 mL of 0.3 M oxalic acid was added to the samples, which were then vortexed again. The samples were then centrifuged for 20 min at 3,000 × g at 4ºC, and 2 mL supernatant was transferred into the gas chromatography vial for VFA analysis.

Apparent total tract digestibility (ATTD) was calculated using the following equation:

$$ATTD\ (\%) = 100 - \{[(N_{feces}/N_{diet}) \times (Ti_{diet}/Ti_{feces})] \times 100\}$$

where N_{feces}, nutrient concentration in the feces (g/kg); N_{diet}, nutrient concentration in the diet (g/kg); Ti_{diet}, TiO_2 concentration in the diet (g/kg); Ti_{feces}, TiO_2 concentration in the feces (g/kg) Apparent ileal digestibility (AID) was calculated using the following equation:

$$AID\ (\%) = 100 - \{[(N_{ileal}/N_{diet}) \times (Ti_{diet}/Ti_{ileal})] \times 100\}$$

where N_{ileal}, nutrient concentration in the ileal digesta (g/kg); N_{diet}, nutrient concentration in the diet (g/kg); Ti_{diet}, TiO_2 concentration in the diet (g/kg); Ti_{ileal}, TiO_2 concentration in the ileal digesta (g/kg)

Standardized ileal digestibility (SID) of AA was calculated using the following equation:

$$SID\ (\%) = AID + [(EAL/AA_d) \times 100]$$

EAL, basal endogenous loss of AA at the distal ileum (g/kg of DMI); AA_d, dietary content of the AA (mg/kg DM).

Basal endogenous loss of AA was measured at the distal ileum after feeding a low-casein diet and calculated according to the following equation:

$$EAL\ (mg/kg\ of\ DMI) = AA_{ileal} \times (Ti_d/Ti_{ileal})$$

where AA_{ileal}, concentration of that AA in the ileal digesta (mg/kg DM), and Ti_d and Ti_{ileal} are the TiO_2 concentrations in the diet and the ileal digesta, respectively, (mg/kg DM).

Data were analyzed by analysis of variance (ANOVA) using the PROC MIXED (SAS Institute, Inc., Cary, NC, USA). Experimental diet was fixed effect and, pig and period were random effects. Differences were considered to be significant at $P < 0.05$, and trends ($P < 0.10$) were also presented.

3.4 RESULTS

The inclusion of 4% of WB in the weaner diet did not affect the CAID of dietary components (CP, EE, NDF, Ca, and P) and energy (Table 3.2). However, the piglets fed the WB diet tended to have lower CAID of the DM and OM ($P \leq 0.10$) than those fed the control diet. On the other hand, a decrease in the CATTD of DM, OM, CP and GE ($P < 0.05$) was observed in

weanling pigs fed the WB diet. Also, the WB diet had lower digestible energy (DE) than the control diet. The 4% inclusion of WB did not affect the CAID and the CSID of N and AA, except for arginine, glutamine and serine (Table 3.3) whose CSID values tended to be reduced ($P \leq 0.10$) because of the WB inclusion. The inclusion of WB increased acetate, butyrate, and total VFA concentrations in feces compared to the control diet ($P < 0.05$; Table 3.4). However, in ileal digesta, dietary WB inclusion only tended to increase acetate, propionate, valerate, and total VFA concentrations compared to control ($P \leq 0.10$). Ileal digesta pH was not affected by the inclusion of WB, whereas the WB diet tended to reduce fecal pH ($P \leq 0.10$).

3.5 DISCUSSION

The pH and concentrations of VFA in the gastrointestinal tract are used as indicators of intestinal gut health (Nyachoti et al., 2006). The VFA are known to play essential trophic roles in intestine epithelial proliferation, showing the positive correlation between villus height and luminal level of VFA, particularly butyrate (Galfi and Bokori, 1990). In the present study, the dietary WB increased fecal production of acetate, butyrate, and total VFA concentration and it also tended to increase ileal acetate, propionate, valerate, and total VFA concentration. This is consistent with previous findings that formation of butyrate can be further stimulated by the starch and bran from wheat or oats compared to pectin-rich fractions and xylans (Jensen and Jørgensen, 1994). In addition, high levels of insoluble NSP in cereal grains has been shown to stimulate the proliferation of acetate-producing microbes in vitro (Bindelle et al., 2010). Similarly, Molist et al. (2011) reported that the 4% of WB diet increased acetate, propionate, butyrate, and total

Table 3.2 Apparent ileal digestibility and apparent total tract digestibility of dietary components and energy in weanling pigs fed diets containing 4% wheat bran[1]

Item	Dietary component	Control	WB	SEM	P-value
Ileal	DM	66.4	59.4	1.5	0.068
	OM	70.6	64.5	1.7	0.070
	EE	90.1	84.0	3.9	0.335
	NDF	28.0	18.5	3.2	0.185
	GE	64.2	58.2	1.8	0.110
	Ca	64.6	53.8	7.5	0.368
	P	52.4	42.0	4.5	0.199
Total tract	DM	87.0	83.3	0.4	0.004
	OM	89.8	86.5	0.3	0.003
	CP (N×6.25)	93.3	87.9	1.0	0.020
	EE	63.5	66.5	3.0	0.523
	NDF	56.7	52.9	1.5	0.155
	GE	88.7	85.4	0.4	0.003
	Ca	65.8	61.1	4.2	0.471
	P	59.8	52.6	2.6	0.118
DE, MJ/kg of DM		15.3	14.9	0.64	0.013

[1]SEM, standard error of the mean; WB, 4% of wheat bran diet; $n = 6$.
[2]DM = dry matter; OM = organic matter; GE = gross energy; EE = ether extract; NDF = neutral detergent fiber; Ca = calcium; P = phosphorus.
[3]Digestible energy.

Table 3.3 Apparent ileal digestibility (AID) and standardized ileal digestibility (SID) of amino acids and crude protein in weanling pigs fed diets containing 4% wheat bran[1]

Item	AID				SID			
	Control	WB	SEM	P-value	Control	WB	SEM	P-value
CP (N×6.25)	70.7	63.9	2.9	0.166	80.8	74.2	5.9	0.174
Indispensable AA								
Arg	83.8	81.4	1.5	0.323	89.0	87.4	0.7	0.075
His	39.0	36.5	3.0	0.530	64.9	60.0	4.6	0.280
Ile	79.1	77.5	1.9	0.640	80.3	80.8	1.2	0.759
Leu	80.2	76.6	2.1	0.300	84.4	82.3	3.2	0.403
Lys	80.8	79.6	1.7	0.523	85.3	83.6	1.1	0.368
Met	88.6	86.8	1.2	0.332	91.8	89.8	1.2	0.316
Phe	80.8	78.9	2.5	0.385	81.3	79.3	1.7	0.378
Thr	70.6	69.2	1.9	0.430	79.6	77.3	1.3	0.257
Val					82.1	81.3	1.1	0.597
Dispensable AA								
Ala	72.9	71.3	1.5	0.528	79.5	77.3	2.6	0.400
Asp	71.7	70.0	1.6	0.531	72.7	70.8	2.5	0.503
Cys	66.0	60.4	2.8	0.214	78.8	73.5	3.5	0.225
Glu	83.6	79.7	1.9	0.083	88.9	84.8	1.0	0.073
Gly	64.3	61.3	2.2	0.305	79.7	75.6	1.5	0.195
Pro	68.8	68.8	6.5	0.409	76.2	84.3	7.5	0.414
Ser	71.3	71.0	0.4	0.611	84.0	82.5	0.7	0.092
Tyr	80.3	78.1	2.7	0.346	84.6	82.2	1.8	0.312

[1]SEM, standard error of the mean; WB, 4% of wheat bran diet; n =6.

Table 3.4 Volatile fatty acids (VFA) concentrations and pH values in ileal digesta and feces in weanling pigs fed a diet containing 4% wheat bran[1]

Item	Control	WB	SEM	P-value
Digesta (mmol/L)				
Acetic acid	10.64	13.01	0.686	0.070
Propionic acid	3.49	4.36	0.275	0.087
Isobutyric acid	0.52	0.69	0.060	0.121
Butyric acid	1.85	2.53	0.328	0.216
Isovaleric acid	0.77	1.06	0.105	0.126
Valeric acid	0.52	0.83	0.098	0.088
Total VFA	17.78	22.48	1.497	0.091
Fecal (mmol/L)				
Acetic acid	43.83	77.43	7.225	0.030
Propionic acid	4.87	5.77	0.771	0.458
Isobutyric acid	0.22	0.61	0.238	0.312
Butyric acid	0.63	2.24	0.272	0.014
Isovaleric acid	1.47	2.82	0.642	0.212
Valeric acid	0.3	1.73	0.778	0.265
Total VFA	51.34	90.58	9.183	0.039
pH				
Digesta	7.26	7.2	0.112	0.619
Feces	6.61	6.33	0.063	0.060

[1]SEM, standard error of the mean; WB, 4% of wheat bran diet.

concentrations of VFA in the feces of weanling piglets. Because undigested nutrients are fermented by microbes in the large intestine producing their metabolites, it seems straightforward that the fibrous diet can modify the pH of feces (Canh et al., 1998). Also, the greater production of VFA in the hindgut of pigs fed WB diet may be a contributing factor of the decrease in pH value in feces in the current study. However, 4% of WB inclusion into weaner diets reduced nutrients and energy digestibility. This is mainly because WB was included at the expense of barley, corn, wheat and soybean meal, thereby increasing total NSP from 89.05 to 111.58 g/kg. Insoluble NSP, a main component of WB, has been well documented to produce antinutritional effects, lowering nutrients and energy digestibility (Wenk, 2001). Therefore, based on our results, microbial metabolites beneficially modified by WB does not improve nutrients digestibility. Rather, increased level of NSP in weaner diet reduced nutrients and energy digestibility, and DE of WB diet. Thus, when WB is included in weaner diet to enhance gut health, nutrients and energy availability for weaned pigs should be taken into account. Also, our results might explain an underlying reason for no improvement of growth performance in weaned pigs fed a WB diet ad libitum although benefits on gut health were shown (Molist et al., 2009, 2010, 2011).

4.0 MANUSCRIPT II

Effects of diet complexity and multicarbohydrase supplementation on growth performance, nutrient digestibility, blood profile, intestinal morphology, and fecal score in newly-weaned pigs

B. Koo,* J. W. Kim,* C. F. M. de Lange,† M. M. Hossain,* and C. M. Nyachoti*

*Department of Animal Science, University of Manitoba, Winnipeg, MB, Canada R3T 2N2; and †Department of Animal and Poultry Science, University of Guelph, Guelph, ON, Canada N1G 2W1

4.1 ABSTRACT

To study the effects of diet complexity and multicarbohydrase (MC) supplementation, 144 piglets (6.70 ± 0.81 kg of BW) weaned at 21 ± 2 d of age (1:1 male to female ratio) were assigned to 1 of 6 dietary treatments in a 3 × 2 factorial arrangement based on diet complexity (complex1, complex2, and simple) and MC addition (0 vs. 0.1% of MC). Diets were provided in a 2-phase feeding program with phase I (d 0 to 14) and phase II diets (d 15 to 28). Complex1 was formulated to mimic a conventional weaner diet with blood plasma, fish meal, dried whey, and skim milk powder, whereas complex2 partially or totally replaced these ingredients with various plant-based ingredients. The simple diet comprised primarily of corn, wheat, and soybean meal. No interactions were found between diet complexity and MC supplementation ($P > 0.10$), except for apparent total tract digestibility (ATTD) of ash and globulin concentration on d 28 ($P < 0.05$). Pigs fed the complex1 diet had greater ($P < 0.01$) ADG, ADFI, and G:F than those fed the other diets during the first wk post-weaning. However, no differences in ADG or ADFI were observed for pigs fed the complex1 and simple diets throughout the 28-d experimental period ($P > 0.10$), whereas the complex2 diet led to lower ADG and ADFI compared with the complex1 diet ($P < 0.05$). Overall, G:F was greater for pigs fed the simple diet compared to those fed the complex1 diet ($P < 0.01$). Pigs fed the simple diet showed comparable ATTD of DM, OM, GE, and ash to those fed the complex1 diet on d 14 ($P > 0.10$). In contrast, greater ATTD of DM, OM, NDF, GE, and ash was observed ($P < 0.05$) in pigs fed the complex1 diet than those fed the complex2 or the simple diet on d 28. The simple diet increased the lymphocyte proportion in serum compared to the complex1 diet ($P < 0.01$) on d 14. Pigs fed the complex1 diet had greater ($P < 0.05$) ratio of villus height to crypt depth (VH:CD) in the ileum compared with the other diets. Compex2 diet decreased ($P < 0.05$) fecal score during 3-wk of post-weaning period compared to the complex1

diet. Dietary MC supplementation increased ($P < 0.05$) G:F during the overall experimental period, VH in the ileum, VH:CD ratio in the duodenum, and ATTD of DM and GE, but it reduced fecal score ($P < 0.05$). In conclusion, feeding a simple diet showed comparable BW to complex1 diet, but it led to inferior intestinal morphology and ATTD of nutrients on d 28. Also, MC supplementation could be beneficial regardless of diet complexity.

Key words: diet complexity, digestibility, growth performance, intestinal morphology, multicarbohydrase, weaned pigs

4.2 INTRODUCTION

To prevent post-weaning challenges such as impaired gut morphology and a high incidence of diarrhea (Pluske et al., 1997), highly digestible and palatable ingredients are included in nursery pig diets. This increases the complexity of the diet composition, which in turn lead to increase feed costs. Therefore, to reduce feed costs, it would be beneficial to simplify the diet composition without compromising growth performance and gut health. Previous studies show that pigs fed a conventional complex diet have superior performance at the beginning of the post-weaning period when compared to those fed a simple corn-soybean meal diet (Mahan et al., 2004; Sulabo et al., 2010; Skinner et al., 2014). However, the positive effects of the complex diet observed during the early post-weaning period seem to dissipate during subsequent growth (Mavromichalis et al., 2001; Pettey et al., 2002; Wolter et al., 2003). Enzyme supplementation has been shown to increase G:F and nutrient digestibility (Omogbenigun et al., 2004) in nursery pigs, which may increase the flexibility of nursery feed formulation by allowing inclusion of low-quality alternative ingredients.

However, no information is available on the interaction between diet complexity and multicarbohydrase (**MC**) supplementation. Also, no studies have compared the effects of a complex diet containing various alternative ingredients to the effects of a conventional complex diet. Thus, we hypothesized that a conventional complex diet would be beneficial during the beginning of the post-weaning period, however pigs would be able to adapt to an alternative complex diet or a simple diet showing comparable performance in nursery pigs. Also, MC supplementation could help in efficiently utilizing alternative ingredients in nursery diets. The objective of this study was to investigate the effects of diet complexity and MC supplementation on growth performance, nutrient digestibility, intestinal morphology, blood profile, and fecal score in weaned pigs.

4.3 MATERIALS AND METHODS

All experimental procedures were reviewed and approved by the University of Manitoba Animal Care Committee (Protocol #, AC11018), and pigs were cared for according to the guidelines of the Canadian Council on Animal Care (CCAC, 2009).

Animals, Housing, Experimental Design, and Dietary Treatments

One hundred and forty-four piglets [(Yorkshire × Landrace) × Duroc] with an initial BW of 6.70 ± 0.81 kg and weaned at 21 ± 2 d of age (1:1 male to female ratio) were obtained from the Glenlea Swine Research Unit at the University of Manitoba and assigned to dietary treatments in a randomized complete block design. Pigs were assigned to one of six dietary treatments in a 3 × 2 factorial arrangement of treatments based on diet complexity (**complex1**, **complex2**, and **simple**) and feed enzyme addition (0 vs. 0.1% of MC [Superzyme OM, Canadian Bio-System Inc., Calgary,

Alberta, Canada]; Table 4.1), giving 8 replicates per treatment with 3 pigs per pen. Nursery diets were provided in a 2-phase feeding program with phase I (d 0 to 14) and phase II diets (d 15 to 28; Table 4.1). The complex1 diet contained typical levels of highly digestible animal protein sources (e.g., dried whey, skim milk powder, fish meal, and spray-dried animal plasma), and relatively more digestible cereal grains, whereas the complex2 diet replaced these ingredients with a variety of plant-based ingredients such as distillers dried grains with solubles, wheat middlings, canola meal, field peas and flaxseed meal, maintaining diet complexity. The simple diet was comprised primarily of corn, wheat, and soybean meal (**SBM**). Dried whey and fish meal were included in all diets during phase I. Titanium dioxide (TiO_2; Sigma Chemical Company, St. Louis, MO) was added at 0.3% of the experimental diets as an indigestible marker to determine apparent total tract digestibility (**ATTD**). The 0.1% of MC supplementation supplied 3,400 U of cellulase, 2,200 U of pectinase, 480 U of mannanase, 60 U of galactanase, 2,400 U of xylanase, 720 U of glucanase, 3,000 U of amylase, and 240 U of protease per kilogram of diet. Within each phase, calculated nutrient and ME contents were similar in all diets, and exceeded or met requirements according to NRC (2012). All experimental diets were fed in mash form. Each pen (1.2 × 1.5 m) had a plastic-covered expanded metal floor, a stainless-steel feeder, and a low-pressure nipple drinker. Pigs had free access to feed and water throughout the experiment. Room temperature was maintained at 29 ± 1°C during the first wk and then reduced by 1.5°C per wk thereafter.

Sample Collection and Slaughter Procedure

Body weight and feed disappearance were determined weekly and the two variables were used to calculate G:F. Samples were pooled by pen and frozen at -20°C until required for further analyses. On the last day of each phase, pigs were fasted overnight, and then fed at 0600. After 2

h, blood samples (10 mL) were collected from the jugular vein into vacutainer tubes with and without heparin (Becton Dickson, Rutherford, NJ) from 1 pig (median BW) in each pen, and the same pigs were sampled again on the final day of the experiment. Blood samples were placed on ice during collection, and then sent to the Veterinary Diagnostic Services at Manitoba Agriculture (Winnipeg, MB, Canada). On d 29, the same pigs (1 pig per pen) were then anesthetized by an intramuscular injection of ketamine:xylazine (20:2 mg/kg; Bimeda-MTC Animal Health Inc., Cambridge, ON, Canada) and euthanized by an intravenous injection of sodium pentobarbital (50 mg/kg of BW; Bimeda-MTC Animal Helath Inc.). Thereafter, the abdomen and the thorax were cut open by a midline incision. A sample of the duodenum was taken 30 cm away from the stomach. Jejunum and ileal samples were taken at 2 m and 30 cm before the ileal-cecal junction, respectively. Tissue samples were immediately rinsed with cold physiological saline (0.9% saline) and then stored in 10% buffered formalin to fix the villi and the crypts.

Lab Analyses

Total NSP was analyzed using GLC (component neutral sugars) using Varian CP3380 gas chromatography (Varian Inc., Palo Alto, CA) and by colorimetry (uronic acids) using a Biochrom Ultrospec 50 (Biochrom Ltd., Cambridge, UK) as described by Englyst and Cummings (1988) with some modifications (Slominski and Campbell, 1990). Serum samples for glucose, total protein, albumin, and urea nitrogen were analyzed at a Veterinary Diagnostic Services (Winnipeg, MB, Canada) using an Ortho Diagnostics Vitros 250 Chemistry System (Ortho-Clinical Diagnostics Inc., Johnson & Johnson, Rochester, NY). Total globulin was determined by subtracting albumin from total protein. The whole blood samples were analyzed for white blood cells (**WBC**), and lymphocyte counts using an automatic blood analyzer (Advia 2120, Siemens,

Erlangen, Germany). A total of WBC was counted by light microscopy at 100× magnification from stained blood smears. The relative percentages of lymphocytes were determined based on the WBC morphology. Six cross sections were obtained from each formalin-fixed sample and processed for histological examination using the standard hematoxylin and eosin method. The measurement of villus height (VH) and crypt depth (CD) was made on at least 15 well-oriented villi per segment from each pig using an Axio Scope A1 microscope (Carl Zeiss MicroImaging GmbH, Gottingen, Germany) coupled with an Infinity 2 digital camera (Lumenera Corporation, Ottawa, ON, Canada). The captured images were analyzed and measured using Infinity Analyze software (version 6.5. 4; Lumenera Corporation). The VH was measured from the villous tip to the crypt-villous junction and the CD was measured from the crypt-villous junction to the base. Subjective fecal scores were recorded daily during the experimental period by the same person and were evaluated as follows: 1=normal, 2=soft, looser than normal feces, slight diarrhea, 3=moderate diarrheic feces, and 4=liquid, severe diarrhea as described by Yi et al. (2005). Scores were recorded for each pen following observations of individual pig and signs of stool consistency in the pen.

Statistical Analyses

All data were analyzed as a randomized complete block design using the MIXED procedure of SAS (version 9.4; SAS Inst. Inc., Cary, NC) with the pen as the experimental unit. For evaluating data for intestinal morphology and blood profile, the individual pig (median BW in each pen) was used as the experimental unit. Diet complexity, the addition of MC, and their interaction were considered as main effects, with block and sex regarded as random effect. Tukey's test was used to separate and test the differences among treatment means. Means were considered to be significantly different at $P < 0.05$, whereas $0.05 < P < 0.10$ was considered to indicate a trend.

4.4 RESULTS

Growth Performance

There were no interaction effect of diet complexity and MC supplementation on growth performance ($P > 0.10$), except for a trend for ADFI during d 8 to 14 ($P < 0.10$). A difference in BW among dietary treatments was observed during the 4-wk experiment ($P < 0.05$; Table 4.2). Pigs fed the complex1 diet had greater BW ($P < 0.01$) than those fed either the complex2 diet or the simple diet on d 7 and d 14, but there were no differences in BW on d 21 and 28 between pigs fed the complex1 and the simple diet ($P > 0.10$). Similarly, pigs fed the complex1 diet had greater ($P < 0.01$) ADG, ADFI and G:F than those in the other groups during the first wk post-weaning. However, no differences in ADG or ADFI were observed between pigs fed the complex1 and simple diets during the entire experimental period ($P > 0.10$). Pigs fed the simple diet had higher G:F than those fed the complex1 diet ($P < 0.01$) during the overall experimental period. Dietary supplementation with MC did not affect ADG or ADFI ($P > 0.10$) but did improve G:F during the overall experimental period ($P < 0.05$).

Apparent Total Tract Digestibility of Nutrients and Energy, and Digestible Energy and Digestible Energy Intake

There were no interactive effects of diet complexity and MC supplementation on ATTD of nutrients and energy on d 14 and 28, except for ATTD of ash on d 28 ($P < 0.01$; Table 4.3). Differences in ATTD of DM, OM, EE, NDF, GE, and ash were observed by dietary treatments on d 14 and 28 ($P < 0.01$), but the dietary treatments had no effect on ATTD of EE during phase II ($P > 0.10$). A complex2 diet had lower ATTD of DM, OM, NDF, GE, and ash in both phase I and II

than the complex1 or simple diet ($P < 0.01$). However, no differences in DM, OM, GE, or ash were observed between the complex1 and simple diets on d 14 ($P > 0.10$). The ATTD of EE and NDF were greater ($P < 0.01$) in the simple diet compared to the complex1 diet whereas ATTD of DM, OM, NDF, and GE in the complex1 diet were higher ($P < 0.05$) than in the simple diet on d 28. The addition of MC increased ($P < 0.05$) ATTD of DM, OM and GE during phase I, and NDF during phase II ($P < 0.05$), whereas it tended to increase ATTD of NDF during phase I ($P < 0.10$), and that of DM, OM, and GE during phase II ($P < 0.10$) irrespective of diet complexity. The DE contents were greater ($P < 0.05$) for the complex1 and the simple diet than for the complex2 diet during phase I and II. However, DE intake in pigs fed the complex1 diet was greater than those fed the complex2 or the simple diet during phase I and II ($P < 0.05$), but no difference in DE intake was observed in pigs fed the complex1 diet and the simple ($P > 0.10$). The addition of MC tended to increase the DE content on d 14 irrespective of diet complexity ($P < 0.10$), whereas no effect of MC supplementation on the DE content was observed on d 28 ($P > 0.10$).

Blood Profile, Small Intestinal Morphology, and Fecal Score

There were no interaction effects of diet complexity and MC supplementation on blood profiles (Table 4.4), intestinal morphology (Table 4.5), or fecal scores (Figure 4.1) in weaned pigs ($P > 0.10$), except for total protein concentration on d 14 ($P < 0.10$) and globulin concentration ($P < 0.05$). Enzyme supplementation did not alter the blood profile ($P > 0.10$) on d 14 and 28 of the experiment, except that albumin content was greater ($P < 0.05$) in pigs fed diets supplemented with MC than those fed non-supplemented diets on d 14. On d 14, pigs fed the complex2 diet had increased total protein compared to those fed the other diets ($P < 0.05$). Feeding of the simple diet increased lymphocyte proportion in WBC on d 14, compared to the complex1 diet ($P < 0.01$).

Table 4.1 Ingredient and nutrient composition of experimental diets (%, as-fed basis)[1]

Item	Phase I (d 1-14)			Phase II (d 15-28)		
	Complex1	Complex2	Simple	Complex1	Complex2	Simple
Ingredient						
Barley	—	3.00	—	—	5.00	—
Corn	24.52	31.40	31.25	35.55	36.90	37.30
Wheat	24.00	8.00	23.00	25.00	9.00	25.00
DDGS[2]	—	3.00	—	—	5.00	—
Wheat middlings	—	3.00	—	—	5.00	—
Canola meal	—	4.00	—	—	4.00	—
Soybean meal, CP 46%	10.00	17.00	24.00	20.00	18.50	30.00
Field peas	—	4.00	—	—	4.00	—
Flaxseed meal	—	4.00	—	—	4.00	—
Oat groats	5.00	—	—	—	—	—
Spray-dried animal plasma	5.00	—	—	2.50	—	—
Fish meal	5.00	4.50	4.00	4.00	—	—
Dried whey	18.00	10.00	10.00	7.50	—	—
Skim milk powder	3.00	—	—	—	—	—
Vegetable oil	2.50	4.53	3.99	2.53	4.40	3.66
Limestone	0.85	0.84	0.92	0.91	1.14	1.15
Monocalcium phosphate	0.30	0.60	0.70	0.25	0.76	0.85
Salt	0.20	0.20	0.20	0.20	0.20	0.20
Vit-Min premix[3]	1.00	1.00	1.00	1.00	1.00	1.00
L-Lys·HCl	0.24	0.43	0.41	0.20	0.55	0.36
DL-Met	0.09	0.10	0.12	0.05	0.10	0.09
L-Thr	—	0.10	0.11	0.01	0.15	0.09
Titanium dioxide	0.30	0.30	0.30	0.30	0.30	0.30
Calculated composition						
ME, Mcal/kg	3.44	3.44	3.44	3.39	3.39	3.39
SID[4] Lys	1.35	1.36	1.36	1.24	1.24	1.24
SID Thr	0.79	0.79	0.78	0.74	0.74	0.74
SID Met	0.40	0.43	0.43	0.36	0.38	0.37
Ca	0.80	0.80	0.80	0.70	0.70	0.70
STTD[5] P	0.45	0.45	0.45	0.35	0.35	0.35
Analyzed composition						
Ether extract	4.22	7.43	5.70	4.41	6.20	5.79
CP	21.05	21.54	21.51	21.86	21.14	21.27
Total NSP, g/kg	61.18	92.66	77.18	79.13	108.42	84.49

[1]Diets were formulated without or with multicarbohydrases (Superzyme OM, Canadian Bio-System Inc., Calgary Alberta, Canada) added at 1kg/ton, which supplied 3,400 U of cellulase, 2,200 U of pectinase, 480 U of mannanase, 60 U of galactanase, 2,400 U of xylanase, 720 U of glucanase, 3,000 U of amylase, and 240 U of protease per kilogram of the diets.

[2]DDGS=distillers dried grains with solubles produced from a 1:1 mixture of wheat and corn.

[3]Supplied per kilogram of diet: vitamins A, 8,250 IU; vitamin D_3, 825 IU; vitamin E, 40 IU; vitamin K, 4mg; thiamine, 1mg; riboflavin, 5mg; niacin, 35mg; pantothenic acid, 15 mg; vitamin B_{12}, 25 µg; folic acid, 2 mg; Cu, 15 mg (copper sulfate); I, 0.21 mg as potassium iodine), Fe, 100 mg (ferrous sulfate); Mn, 20 mg (manganese oxide); Se, 0.15 mg (sodium selenite); and Zn, 100mg (zinc oxide).

[4]Standardized ileal digestible.

[5]Standardized total tract digestible P.

Table 4.2 Effects of diet complexity and multicarbohydrase (MC) on growth performance in weaned pigs

Item	Diet complexity			Multicarbohydrase[1]		SEM	P-value[2]		
	Complex1	Complex2	Simple	+	-		Diet	MC	Diet × MC
BW, kg									
d 0	6.69	6.71	6.69	6.70	6.69				
d 7	8.92^x	8.33^y	8.36^y	8.49	8.58	0.125	<0.001	0.412	0.843
d 14	11.83^x	11.07^y	11.23^y	11.31	11.45	0.193	0.001	0.372	0.411
d 21	15.68^x	14.82^y	15.26^{xy}	15.20	15.30	0.302	0.026	0.695	0.669
d 28	20.05^x	19.04^y	19.86^{xy}	19.70	19.60	0.386	0.030	0.758	0.691
ADG, g/d									
d 1 to 7	316^x	233^y	238^y	255	270	17.7	<0.001	0.344	0.883
d 8 to 14	416	391	411	402	410	15.9	0.290	0.571	0.155
d 15 to 21	549	536	575	557	550	20.0	0.151	0.685	0.976
d 22 to 28	626	602	657	642	615	26.2	0.109	0.192	0.792
d 1 to 28	477^x	441^y	470^{xy}	464	461	13.7	0.028	0.785	0.674
ADFI, g/d									
d 1 to 7	350^x	300^y	286^y	309	315	18.3	0.002	0.635	0.763
d 8 to 14	635^x	576^{xy}	572^y	581	608	24.4	0.024	0.196	0.083
d 15 to 21	912	858	908	883	903	25.3	0.059	0.329	0.852
d 22 to 28	1,111	1,025	1,064	1,047	1,087	41.0	0.128	0.240	0.697
d 1 to 28	752^x	690^y	707^{xy}	705	728	19.3	0.008	0.164	0.911
G:F, g/g									
d 1 to 7	0.91^x	0.77^y	0.83^y	0.83	0.85	0.023	<0.001	0.304	0.162
d 8 to 14	0.65	0.68	0.73	0.96	0.68	0.031	0.076	0.526	0.512
d 15 to 21	0.60	0.63	0.63	0.63	0.61	0.018	0.246	0.124	0.932
d 22 to 28	0.57	0.59	0.63	0.61	0.57	0.028	0.097	0.065	0.818
d 1 to 28	0.63^y	0.64^{xy}	0.67^x	0.66	0.63	0.013	0.038	0.018	0.647

[1] +, with MC; -, without MC.

[2]Diet, main effect of diet complexity; MC, main effect of diet complexity.

x,y,zMeans within the main effect of diet complexity and within row lacking a common superscript letter differ (P <0.05).

Table 4.3 Effects of diet complexity and multicarbohydrase (MC) on apparent total tract digestibility of nutrients and energy, and calculated digestible energy (DE) and digestible energy intake (DEI) in weaned pigs

Item	Diet complexity			Multicarbohydrase[1]		SEM	P-value[2]		
	Complex1	Complex2	Simple	+	-		Diet	MC	Diet × MC
Phase I									
DM	82.5^x	71.0^y	81.7^x	79.3	77.5	1.03	<0.001	0.049	0.867
OM	84.3^x	73.3^y	83.5^x	81.3	79.5	0.96	<0.001	0.034	0.894
EE	49.9^y	54.2^{xy}	61.4^x	57.0	53.3	2.95	0.002	0.129	0.755
NDF	49.7^y	34.4^z	58.3^x	49.8	45.1	2.98	<0.001	0.061	0.571
GE	80.9^x	69.4^y	80.3^x	77.9	75.9	1.09	<0.001	0.034	0.918
Ash	57.2^x	39.8^y	56.5^x	51.9	50.5	2.30	<0.001	0.463	0.556
DE, kcal/kg[3]	3,272^x	2,950^y	3,303^x	3,210	3,140	45.27	<0.001	0.066	0.909
DEI, kcal/d[4]	1,613^x	1,289^y	1,414^y	1,429	1,448	36.62	<0.001	0.699	0.367
Phase II									
DM	86.1^x	79.3^z	83.6^y	83.6	82.4	0.75	<0.001	0.068	0.925
OM	87.9^x	81.3^z	85.6^y	85.5	84.4	0.72	<0.001	0.064	0.950
EE	65.4	68.6	68.6	67.1	68.0	2.59	0.374	0.693	0.630
NDF	70.3^x	55.8^z	61.1^y	64.1	60.7	1.70	<0.001	0.019	0.140
GE	85.5^x	78.7^z	83.2^y	83.1	81.8	0.89	<0.001	0.090	0.866
Ash	58.7^x	47.6^y	50.4^y	52.8	51.7	1.35	<0.001	0.322	0.002
DE, kcal/kg[3]	3,489^x	3,313^y	3,453^x	3,437	3,400	57.46	<0.001	0.233	0.583
DEI, kcal/d[4]	3,530^x	3,119^y	3,405^x	3,319	3,384	99.00	<0.001	0.855	0.855

[1] +, with MC; -, without MC.
[2] Diet, main effect of diet complexity; MC, main effect of diet complexity.
[3] Digestible energy, DM basis.
[4] Digestible energy daily intake was calculated by ADFI (kg) × DE, DM basis.
[x,y,z] Means within the main effect of diet complexity and within row lacking a common superscript letter differ (P <0.05).

Table 4.4 Effects of diet complexity and multicarbohydrase (MC) on blood characteristics of weaned pigs

Item	Diet complexity			Multicarbohydrase[1]		SEM	P-value[2]		
	Complex1	Complex2	Simple	+	-		Diet	MC	Diet × MC
Glucose, mmol/L									
d 14	7.58	7.74	7.83	7.79	7.64	0.349	0.780	0.612	0.229
d 28	6.33	6.46	6.99	6.80	6.38	0.441	0.297	0.246	0.620
Total protein, g/L									
d 14	42.94^y	45.44^x	43.50^y	43.67	44.25	0.654	0.001	0.282	0.057
d 28	49.38	49.13	48.25	49.38	48.46	1.176	0.608	0.346	0.403
Albumin, g/L									
d 14	25.56^y	27.25^x	26.19^{xy}	25.79	26.88	0.644	0.041	0.047	0.458
d 28	30.75	31.38	30.44	31.04	30.67	0.896	0.572	0.611	0.700
Globulin, g/L									
d 14	17.38	18.19	17.31	17.88	17.38	0.428	0.089	0.162	0.175
d 28	18.63	17.75	17.81	18.33	17.79	0.567	0.241	0.250	0.050
Urea nitrogen, mmol/L									
d 14	3.46	3.06	3.49	3.29	3.39	0.260	0.186	0.640	0.452
d 28	4.56	4.10	4.71	4.38	4.54	0.337	0.186	0.559	0.282
White blood cells, 10^9/L									
d 14	15.44	16.49	15.17	15.36	16.03	1.077	0.441	0.450	0.757
d 28	14.29	14.56	14.75	14.08	14.99	1.371	0.944	0.418	0.827
Lymphocyte, %[3]									
d 14	44.88^y	48.88^{xy}	55.56^x	49.46	50.08	2.765	0.002	0.784	0.822
d 28	51.75	46.79	48.00	46.98	50.71	5.162	0.569	0.352	0.623

[1] +, with MC; -, without MC.
[2] Diet, main effect of diet complexity; MC, main effect of multicarbohydrase.
[3] Relative percentage to white blood cells.
^{x,y,z}Means within the main effect of diet complexity and within row lacking a common superscript letter differ ($P < 0.05$).

Table 4.5 Effects of diet complexity and multicarbohydrase (MC) on small intestinal morphology of weaned pigs

Item	Diet complexity			Multicarbohydrase[1]		SEM	P-value[2]		
	Complex1	Complex2	Simple	+	-		Diet	MC	Diet × MC
Villus height, μm									
Duodenum	542.5	510.7	528.9	548.8	505.9	32.82	0.545	0.066	0.733
Jejunum	508.0	525.1	496.1	520.6	498.8	27.49	0.572	0.338	0.656
Ileum	495.9	453.2	462.8	488.1	453.3	19.67	0.075	0.029	0.304
Crypt depth, μm									
Duodenum	296.7	298.5	322.2	297.5	314.1	21.02	0.259	0.256	0.695
Jejunum	269.3	298.8	282.3	281.4	285.6	18.78	0.305	0.784	0.705
Ileum	253.4[y]	270.6[xy]	288.7[x]	270.1	271.8	12.89	0.023	0.866	0.508
Villus height:crypt depth ratio									
Duodenum	1.84	1.74	1.65	1.87	1.62	0.097	0.090	0.001	0.294
Jejunum	1.91	1.78	1.77	1.87	1.77	0.080	0.132	0.126	0.680
Ileum	1.97[x]	1.69[y]	1.63[y]	1.84	1.70	0.092	0.001	0.054	0.948

[1] +, with MC; -, without MC.

[2]Diet, main effect of diet complexity; MC, main effect of multicarbohydrase.

[x,y,z]Means within the main effect of diet complexity and within row lacking a common superscript letter differ ($P < 0.05$).
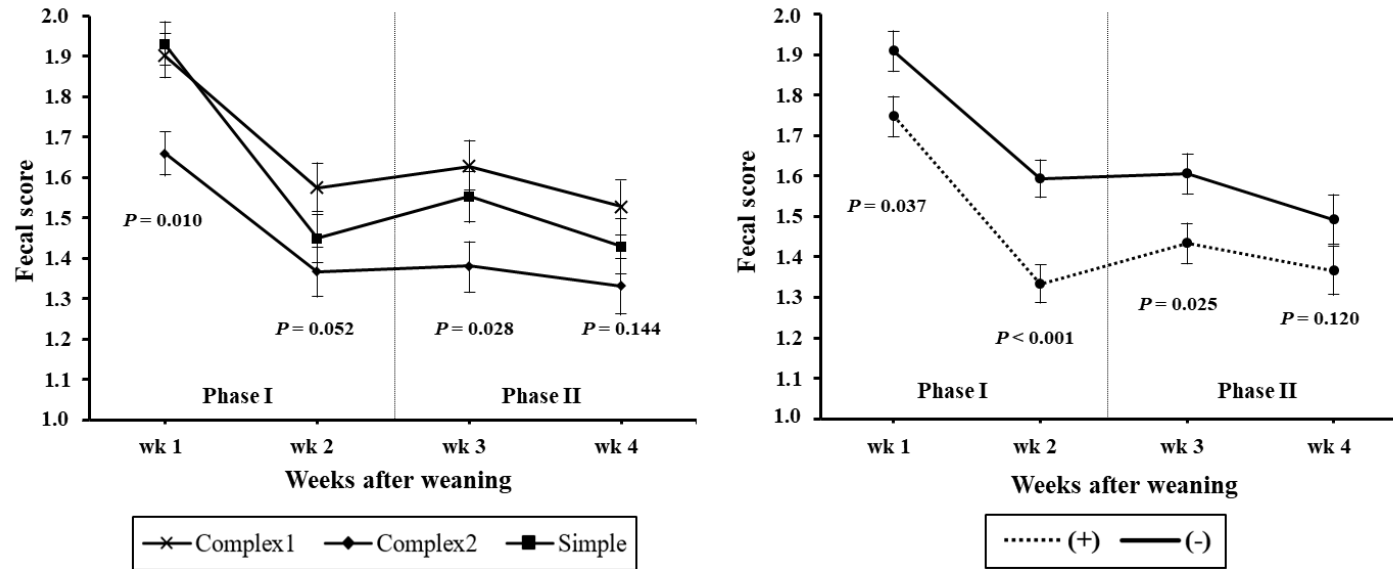
Figure 4.1 Effect of diet complexity and multicarbohydrase supplementation on fecal score in weaned pigs. Fecal score was recorded for each pen following observations of an individual pig and signs of stool consistency in the pen and evaluated as follows: 1=normal, 2=soft, looser than normal feces, 3=moderate diarrheic feces, and 4=liquid, severe diarrhea. Each value represents the mean of 8 replicates per each treatment. No interactions between diet complexity and multicarbohydrase supplementation were observed for 4-wk after weaning ($P > 0.10$). (+), with multicarbohydrase; (-), without multicarbohydrase.

There were no effects of diet complexity on blood profile on d 28 ($P > 0.10$).

The complex1 diet tended to increase ($P < 0.10$) VH in the ileum and the ratio of VH:CD in the duodenum, compared to the complex2 and the simple diet, respectively (Table 4.4). The lower CD and greater VH:CD ratio were observed ($P < 0.05$) in the ileal segment of pigs fed the complex1 diet compared to the simple diet and the other two diets, respectively. Although dietary MC supplementation did not alter the CD in the all segments ($P > 0.05$), it increased ($P < 0.05$) the VH in the ileum and the ratio of VH:CD in the duodenum. Trends for increases ($P < 0.10$) in VH and VH:CD ratio were observed in duodenal and ileal segment of pigs fed MC supplemented diets, respectively, compared to those fed non-supplemented diets.

Complex2 diet-fed pigs showed lower fecal scores for 3-wk post-weaning, compared to complex1 diet-fed pigs ($P < 0.05$). Also, dietary MC supplementation reduced fecal scores for 3 wk after weaning ($P < 0.05$).

4.5 DISCUSSION

Diet Complexity

Diet complexity refers to the dietary composition state of containing different feed ingredients and additives. Nursery diets are often characterized by high diet complexity because animal protein sources and dairy products that are not used for grower or finisher diets are included. Because of this, high complexity in nursery diets generally indicates high feed costs. Therefore, the potential to use less complex nursery diets without compromising growth performance has been extensively investigated in order to reduce the cost for nursery diets. Also, even though diet complexity is high, a great reduction in costs for nursery diets may be achieved if conventional ingredients can be replaced with various low-quality alternative ingredients while maintaining

growth performance of weaned pigs. Therefore, in the present study, the simple and complex2 diets were formulated for alternative nursery diets.

Numerous studies have shown that nursery pigs fed a simple corn-SBM-based diet have inferior ADG, ADFI, and G:F than those fed conventional complex diets (Dirtz et al., 1996; Mavromichalis et al., 2001; Wolter et al., 2003; Mahan et al., 2004; Sulabo et al., 2010; Skinner et al., 2014). These results are in line with those found in the present study, showing less ADG, ADFI and G:F in the pigs fed the simple diet in the first wk post-weaning compared to those fed a complex1 diet. Highly palatable and digestible ingredients such as skim milk powder, dried whey, blood protein, fish meal and oat groats increases feed intake and thus provides sufficient nutrients and energy to support adequate growth performance. Indeed, in the present study, pigs fed a complex1 diet showed the greatest DE intake during phase I.

Greater ADG in pigs fed the complex1 diet was observed only in the first wk after weaning in the present study. Inclusion of a certain amount of fish meal and dried whey in all phase I may help weaned pigs to adapt to the nursery diets. This is supported by several studies (Mavromichalis et al., 2001; Pettey et al., 2002; Wolter et al., 2003) showing that the negative effect of the simple diet was limited to the beginning of post-weaning when a certain amount of animal protein sources was included in the phase I simple diet. In contrast, lower ADG lasted for 4 (Mahan et al., 2004) or 5-wk (Tokach et al., 1995) when piglets were fed simple corn-SBM-based diets without any animal protein and lactose sources after weaning, compared to conventional complex diets. Thus, it appears that the composition of diet fed in the immediate post-weaning (e.g., phase I) period is critical for the nursery growth performance, as suggested by Dritz et al. (1996).

Soybean meal contains conglycinin, β-conglycinin, and antigenic compounds (Li et al., 1990). These may elicit a clinical allergic reaction in weaned pigs, which is the reason for limiting

SBM inclusion in swine diets, particularly for young pigs. Diets containing SBM have been shown to cause lymphocyte proliferation as well as differentiation of T-cells as an immune response (Li et al., 1990; Sun et al., 2008; Hao et al., 2010). This might be the reason for our finding that pigs fed the simple diet, which contained high levels of SBM showed increased serum lymphocyte proportions. As such, this antigenic reaction to SBM might explain the reduced ADFI during phase I and the high diarrhea scores of pigs fed the simple diet during phase I, which is consistent with the findings of Li et al. (1990) and Dréau et al. (1994). Due to the reduced ADFI, pigs fed a simple diet showed a 19.6% lower DE intake than those fed a complex1 diet during phase I, although no differences in ATTD of GE or DE were shown between two diets. However, as the results showed no difference in lymphocyte proportions across the dietary treatments on d 28, the hypersensitivity to antigens in SBM appears to be transient, indicating a digestive adaptation to the diet, as discussed by Li et al. (1990). As a result, there was no difference in ADFI and DE intake between pigs fed the complex1 diet and a simple diet, which might have contributed to the catch-up in BW for those on the simple diet during phase II. However, the BW of pigs fed the complex2 diet was less than that of the pigs fed the complex1 diet for the entire experimental period. This might have been because of the antinutritional effects of NSP on nutrient and energy utilization of pigs (Wenk, 2001). The complex2 diet for phase I and phase II contained 51% and 37% greater total NSP than the complex1 diet, respectively. Also, it should be noted that the negative effects are more pronounced in weaned pigs because they have a less developed digestive system and a low diversity of microbiota in the large intestine (Sergey et al., 2004; Montagne et al., 2013). There is also earlier evidence that adding fat and high levels of NSP to the nursery diet reduces lactose and dried whey utilization (Cheeke and Stangel, 1973). In the present study, weaned pigs showed the lowest ATTD of nutrients and DE with the complex2 diet during both phase I and II, which might

indicate that insufficient nutrients and energy to support optimal growth were supplied. Also, it was observed that the complex2 diet increased serum total protein, albumin, and tended to increase globulin compared to the other diets, suggesting the alteration of protein metabolism in the liver (Swamy et al., 2003), but this requires further examination.

It is of interest to measure VH, CD, and the VH:CD ratio of weaned pigs, as they offer an indication of the functional capability of enterocytes and the extent of their maturity (Pluske et al., 1997). Reasons for the increased VH and the VH:CD ratio in complex1 diet-fed pigs are not clear. However, it is possible that greater feed intake in complex1 diet-fed pigs after weaning stimulated enterocyte proliferation as demonstrated by Pluske et al. (1997), and the higher VH and VH:CD ratio may have endured until the end of experiment. Also, the hypersensitivity-inducing ANF from SBM has been known to cause VH atrophy and crypt hyperplasia in weaned pigs (Dunsford et al., 1989; Li et al., 1990; Makinde et al., 1996), which might explain the deeper crypt depth in the ileum of pigs fed the simple diet compared to those on the complex1 diet.

However, in contrast to intestinal morphology, fecal scores were higher in pigs fed the complex1 diet than those fed the complex2 diet. This could have been mainly because of overconsumption of feed, presumably outweighing the digestive and volumetric capability of weaned pigs due to its higher palatability (Bayley and Carlson, 1970; Ball and Aherne, 1982). Ekstrom et al. (1976) reported that feeding pigs a diet containing dried whey for an extended time caused osmotic diarrhea due to the limited intestinal lactase activity.

Supplementation of Multicarbohydrase

One of the advantages of adding carbohydrases to swine diets is to hydrolyze the outer cell membrane of grains, mostly composed of NSP that cannot be degraded by the digestive

enzymes of monogastric animals (Adeola and Cowieson, 2011). Thus, MC supplementation enables pigs' digestive enzymes to have access to nutrients entrapped inside the cell walls (Bedford and Schulze, 1998). In the present study, the supplementation of MC improved ATTD of nutrients, energy, and DE content during phase I and II, which might be one of the reasons for the increased G:F in pigs fed a nursery diet supplemented with MC during the overall experimental period. This is consistent with the findings of Omogbenigun et al. (2004), where the addition of 0.1% carbohydrase to the corn-SBM-based diet containing some animal protein sources improved G:F by 12.5% throughout the 35-d of the experimental period; ATTD of energy was improved by 4.2%.

An increase in intestinal viscosity has been known to shorten VH and deepen crypt depth (Montagne et al., 2003). Indeed, McDonald et al. (2001) reported that inclusion of 4% water-soluble synthetic viscous polysaccharide increased intestinal viscosity and reduced VH and VH:CD ratio of newly-weaned pigs. It was speculated that amines, NH_3, and toxins produced during the fermentation of viscous digesta by intestinal microbes cause a detrimental effect on the gut (Langhout et al., 2000). Thus, because supplementation with carbohydrase is known to reduce digesta viscosity (Choct et al., 1996; Yin et al., 2001; Wang et al., 2008), it may reduce the detrimental effect of soluble NSP on intestinal structure. Indeed, it has been reported that weaned pigs fed a corn-SBM-based diet supplemented with carbohydrase showed higher VH in the ileum or jejunum (Kim et al., 2003; Waititu et al., 2016). This is in agreement with our findings that MC-supplemented diets increased the duodenal and ileal VH thereby increasing the ratio of VH:CD. In the present study, however, no detailed analysis of change in intestinal viscosity by MC was carried out. As an increased VH:CD ratio indicates a favorable intestinal structure for nutrient digestion and absorption in newly-weaned pigs (Pluske et al., 1997), the increased ATTD of nutrients in pigs fed diets supplemented with MC may be attributable to the improved VH:CD ratio.

Low ileal absorptive capacity after weaning could be contributed to high incidences of osmotic diarrhea because undigested and unabsorbed nutrients lead to osmotic diarrhea *per se* and serve as substrates for proliferation of pathogenic bacteria that induce diarrhea (van Beers-Schreurs et al., 1992; Pluske et al., 2002). Thus, the reduced fecal score in pigs fed diets supplemented with MC in the present study might be explained by the improved intestinal histology and digestion of nutrients by the addition of MC. This is supported by Tapingkae et al. (2008), who reported that supplementation of xylanase reduced the incidence of post-weaning diarrhea in pigs fed a rice-SBM-based diet. Furthermore, it has been suggested that the addition of carbohydrase can produce short-chain oligosaccharides from cell wall NSP, which can create potential prebiotic effects, thereby decreasing diarrhea-inducing pathogenic bacteria (Kiarie et al., 2013), but a microbial assay was not carried out in the present study.

The effects of MC on the complex2 or simple diets were expected to be greater than for the complex1 diet because the former diets contained higher levels of complex carbohydrate on which supplemental MC could act. However, no interaction effects between diet complexity and MC were observed except for ATTD of ash, and globulin concentration, suggesting that the MC used had similar effects irrespective of diet complexity. This might be due to the high levels of wheat inclusion, which is known to be rich in soluble NSP (Zijlstra et al., 1999), along with other ingredients in the complex1 diet. This may have provided sufficient substrates for maximum activity of the supplemented enzymes.

4.6 CONCLUSIONS

Feeding newly-weaned pigs a complex1 diet led to superior growth in the first wk of the post-weaning period, but there was no difference in ADG throughout the 4-wk of the experimental

period between feeding a complex1 or a simple diet. Also, pigs fed the simple diet had a greater G:F throughout the 4-wk experimental period. In contrast, the complex2 diet led to reduced ADG during the entire experimental period, compared to the complex1 diet. Dietary supplementation with MC improved intestinal morphology, ATTD of nutrients, G:F and reduced fecal score irrespective of nursery diet complexity. Taken together, it is implied that economic benefits may be achieved by feeding a simple diet and supplementation of MC in the 4-wk of nursery production without compromising growth performance. However, further investigation is needed into whether nursery diet complexity affects subsequent growth under commercial management conditions and sanitation environments.

5.0 MANUSCRIPT III

Nutrient and energy digestibility, and microbial metabolites in weaned pigs fed diets containing lactobacillus-fermented wheat

5.1. ABSTRACT

The objective of this study was to investigate the effects of nursery diets containing fermented wheat, with or without an enzyme complex, on nutrient and energy digestibility as well as microbial metabolites in weaned pigs. In addition, we also compared two inoculants: homofermentative *Lactobacillus* (Homo L.) - *Lactobacillus plantarum (L. plantarum)* and heterofermentative *Lactobacillus* (Hetero L.) - *Lactobacillus buchneri* (*L. buchneri*). Wheat was fermented using either *L. plantarum* or *L. buchneri*, with or without an enzyme complex for 90 d. Control diets were formulated with unfermented wheat without (negative control) or with (positive control) multicarbohydrase (MC) blend. A casein (5%)-corn starch-based diet was used to estimate the basal endogenous AA losses. Fourteen weaned piglets (7.8 ± 0.3 kg initial BW) were surgically fitted with a T-cannula at the distal ileum and allocated as per an incomplete triplicate 7×3 Latin square design with 7 dietary treatments and 3 periods. Inclusion of fermented wheat increased ($P < 0.05$) the apparent ileal digestibility (AID) of GE and starch, and apparent total tract digestibility (ATTD) of DM, OM, GE, and P. Further, pigs fed fermented wheat diets showed greater ($P < 0.05$) AID of Met, Cys, and Tyr, and standardized ileal digestibility (SID) of Met, Ala, and Pro than those fed negative control diet. Pigs fed *L. buchneri*-fermented wheat diets had greater ($P < 0.05$) AID of DM, OM, and GE, and ATTD of Ca and P than those fed *L. plantarum*-fermented wheat. The

MC supplementation for wheat fermentation increased ($P < 0.05$) the ATTD of Ca and P; the AID of Ile, Phe, Val, and Tyr; as well as the SID of Ile, Phe, Val, and Tyr. However, there was no marked change ($P > 0.05$) in the volatile fatty acids and lactic acid concentrations and pH of the feces or the ileal digesta. In conclusion, feeding a diet comprising fermented wheat grains to weaned pigs increased the nutrient and energy digestibility.

Key words: apparent ileal digestibility, fermented wheat, lactobacillus, standardized ileal digestibility, volatile fatty acids, weaned pigs

5.2. INTRODUCTION

Use of fermented cereals such as wheat can be beneficial for weaned pigs whose digestive capacity is immature (Pluske et al., 1997). Fermentation with *Lactobacillus* increases the nutritive value of feed ingredients by increasing digestible nutrients contents (Hackl et al., 2010) and reducing antinutritional factors (ANF) such as phytic acids (Skrede et al., 2007) and non-starch polysaccharides (NSP) (Canibe and Jensen, 2003). In addition, lactic acid, a product of *Lactobacillus* fermentation, can suppress the proliferation of pathogens by lowering gut pH (Scholten et al., 1999).

Lactobacillus plantarum (homofermentative *Lactobacillus* [**Homo L.**]), generally ferments monosaccharides into lactic acid. Lactic acid is further metabolized into acetic acid and ethanol by *L. buchneri* (heterofermentative *Lactobacillus* [**Hetero L.**]; Holzer et al., 2003). Therefore, *L. plantarum* inoculation is preferred at the beginning of the fermentation process because it can rapidly lower the pH, thus inhibiting the action of spoilage bacteria (Mayne, 2010). Thereafter, fermentation stability is more effectively achieved by *L. buchneri* inoculation because the acetic acid that is produced has effective antifungal activity (Holzer et al., 2003). Moreover,

exogenous enzymes have been widely used as additives to improve the fermentation quality by hydrolyzing the complex linkages of grains and producing more substrates that *Lactobacillus* can ferment (Yitbarek and Tamir, 2014).

However, the comparative effect of these inoculants used alone and in combination with enzymes on digestibility has only been studied in ruminant animals (Filya, 2003). In addition, to the best of our knowledge, no studies have investigated the influence of fermented wheat on standardized ileal digestibility (SID) of AA and microbial metabolites in weaned pigs. Therefore, this study was performed 1) to investigate the effects of including fermented wheat in the diet of weaned pigs on their nutrient and energy digestibility as well as microbial metabolites 2) to compare the effects of Hetero L. and Homo L.-fermented wheat diets on nutrient digestibility, and 3) to examine the effects of adding an enzyme complex during fermentation.

5.3 MATERIALS AND METHODS

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

Animals, Housing, and Experimental Design

The animal description (except that 14 piglets were used in the present study) and surgical procedures were as described previous in Manuscript I. Pigs were allocated according to an incomplete triplicate 7 × 3 Latin square design with 7 dietary treatments and 3 periods.

Preparation of Fermented Wheat and Experimental Diets

The following treatments were given to the wheat (12% CP, Pasteur): 1) Control (untreated), 2) Inoculation with *L. plantarum* (DSMZ 8862 and DSMZ 8866, Biosil®, Dr. Pieper, Germany), 3) Inoculation with *L. plantarum* and enzyme complex, 4) Inoculation with *L. buchneri* (Biotal, Buchneri 40788, Lallemand Inc., Montreal, QC, Canada), and 5) Inoculation with *L. buchneri* and enzyme complex. Distilled water was added to adjust the moisture content to 27%; the inoculants were added at the rate of 6×10^5 CFU/g of fresh wheat. The enzyme complex (0.05%; Superzyme™ Plus, Calgary, AB, Canada) was mixed with wheat before inoculation, which supplied 600 U of phytase, 700 U of cellulase, 1,100 U of pectinase, 240 U of mannanase, 30 U of galactanase, 1,200 U of xylanase, 360 U of glucanase, 1,500 U of amylase, and 120 protease per kilogram of wheat. The mixture was transferred into 55-gallon plastic barrels, compacted, sealed tightly to create an anaerobic environment, and fermented for 90 d at room temperature. On d 90, fermented wheat was taken out and mixed with other ingredients for making the experimental diets (Table 5.1). The dietary composition of the positive control (**PC**) and negative control (**NC**) wheat was similar, except that the PC wheat had been supplemented with the enzyme complex at 0.05 g/kg. In addition, a casein-corn starch-based diet was used to estimate the basal endogenous losses of CP and AA. Titanium dioxide (0.3%) was included in the diets as an indigestible marker. All experimental diets containing wheat were stored at -20 ºC until fed, to inhibit microbial proliferation.

Sample Collection, Sample Preparation, and Chemical analyses

All procedures were as described previously in Manuscript I. Total starch (AOAC, 2006; method 996.11) was analyzed using a test kit (Megazyme International Ireland, Wicklow, Ireland). Water-soluble NSP content was determined according to the method described by Slominski et al.

(1993). The water-insoluble NSP content was calculated as the difference between total NSP (described in Manuscript II) and water-soluble NSP content.

Calculation and Statistical Analyses

Digestibility was calculated using equations in described in Manuscript I. The following orthogonal contrasts were used to test differences between the treatments: 1) NC vs. PC, 2) NC vs. fermented wheat, 3) Homo L. vs. Hetero L., and 4) fermentation with MC vs. fermentation without MC. Pigs fed fermented wheat diets were re-analyzed using ANOVA to test the interaction between the inoculant and the MC. Differences were considered significant at $P < 0.05$, and trends ($P < 0.10$) were also presented.

5.4 RESULTS

Chemical Composition and pH of Experimental Diets

Fermented wheat diets contained less DM, OM, starch, NSP, NDF, and GE than unfermented wheat diets (Table 5.2). In particular, the inclusion of fermented wheat to the nursery diets instead of unfermented wheat resulted in approximately 30% lower soluble NSP content. However, although the CP contents were similar across experimental diets ($22.06 \pm 0.22\%$), most AA contents in fermented wheat diets were greater than in unfermented wheat diets. Replacing unfermented wheat with fermented wheat in the weaner diets decreased the dietary pH value by 0.5. On the other hand, using two different types of inoculants (Homo L. vs. Hetero L.) or adding MC did not cause any notable changes in the chemical composition or pH of the experimental diets.

Nutrient and Energy Digestibility, and Digestible Energy

Weaned pigs fed fermented wheat diets tended to show greater ($P < 0.10$) AID of DM, OM, and EE than those fed the NC diets (Table 5.3). In addition, greater ($P < 0.05$) AID of GE and starch was observed in pigs fed the fermented wheat diets than in those fed the NC diet. Similarly, ATTD of DM, OM, GE, and P were greater ($P < 0.05$) in weaned pigs fed fermented wheat diets than in those fed the NC diets. The addition of MC tended to increase ($P < 0.10$) the AID of DM, GE, and ileal DE. Moreover, a greater ($P < 0.01$) AID of starch was found in weaned pigs fed a PC diet than those fed a NC diet. However, the addition of MC to the nursery diets did not affect ($P > 0.10$) the ATTD of nutrients and energy. Weaned pigs fed a diet containing Hetero L.-fermented wheat showed greater ($P < 0.05$) AID of DM, OM, and GE as well as greater ATTD of EE, Ca, and P than those fed a diet containing Homo L.-fermented wheat. Further, ileal DE was greater ($P < 0.05$) in Hetero L.-fermented wheat diets than in Homo L.-fermented wheat diets. The addition of MC for wheat fermentation did not affect ($P < 0.10$) the AID of nutrient and energy but did increase ($P < 0.05$) AID of EE, Ca, and P.

Feeding a fermented wheat diet tended to increase ($P < 0.10$) the AID of Arg, Leu, and Val compared to NC diets (Table 5.4). On the other hand, greater ($P < 0.05$) AID of Met, Ala, Cys, and Tyr was observed in pigs fed fermented wheat diet than in those fed the NC diets. Similarly, the inclusion of fermented wheat instead of unfermented wheat led to greater ($P < 0.05$) SID of Met, Ala, and Pro (Table 5.5). However, the type of inoculant used for fermentation did not affect ($P > 0.10$) the AID and SID of CP and AA. Nevertheless, MC supplementation as a wheat fermentation additive tended to increase ($P < 0.10$) the AID of Arg, Leu, Lys, and Asp, and the SID of Arg, Leu, Lys, Asp in a diet comprising fermented wheat. Similarly, the AID of Phe, Val, and Tyr and the SID of Ile, Phe, Val, and Tyr were greater ($P < 0.01$) by the addition of MC for wheat fermentation. In addition, pigs fed the PC diets showed greater ($P < 0.05$) AID and SID of

Cys than those fed the NC diets. Interactive effects of the inoculant and MC addition ($P < 0.05$) were observed in the AID of starch and the ATTD of Ca and P.

Volatile Fatty Acids Concentrations and pH

The addition of MC to a wheat-based diet tended to increase ($P < 0.10$) the acetate concentrations in the ileal digesta and the lactate concentration in the feces (Table 5.6). Moreover, significantly greater ($P < 0.05$) total VFA concentration was observed in the feces of weaned pigs fed the PC diet than those fed the NC diet. However, there were no effects ($P > 0.10$) of fermentation, the type of inoculants, or the addition of MC for wheat fermentation on the VFA and lactate concentrations or pH of the feces and ileal digesta.

5.6 DISCUSSION

The technique of fermentation has been historically used in the food and feed industries to increase preservability, nutritive value, and palatability and the gut health of consumers. Considering that highly digestible and palatable diets are necessary for weaned pigs that have an immature digestive system and a susceptibility to gut disorders, the inclusion of fermented ingredients into the nursery diet would be highly beneficial.

Given that wheat was the only different component across experimental diets, comparing the diet's chemical compositions may provide a valuable background to understand the changes that occurred in wheat during fermentation. Based on the data showing the reduction in the starch, NSP, and NDF content, it appears that *Lactobacillus* utilized the carbohydrates in wheat for energy.

Table 5.1 Ingredient composition of the experimental diets (%, as-fed basis)

Item	Experimental diet		
	Control	Fermented	5% Casein diet
Ingredient			
Wheat	43.75	—	
Fermented wheat[1]	—	43.75	—
Soybean meal	21.62	21.62	—
Whey permeate	11.73	11.73	—
Canola meal	9.97	9.97	—
Peas	4.99	4.99	—
Cornstarch	—	—	64.60
Sucrose	—	—	20.00
Casein	—	—	5.00
Cellulose	—	—	5.00
Vegetable oil	2.67	2.67	2.50
Limestone	1.39	1.39	1.10
Dicalcium Phosphate	1.15	1.15	0.90
L-Lysine	0.69	0.69	—
Salt	0.66	0.66	0.40
Celite	0.40	0.40	—
L-threonine	0.23	0.23	—
DL-Met	0.20	0.20	—
Vit-Min premix[2]	0.15	0.15	0.20
Choline chloride	0.11	0.11	—
Titanium dioxide	0.30	0.30	0.30

[1]Fermented with either homofermentative (*L. plantarum*) or hetero fermentative *Lactobacillus* (*L. buchneri*), with or without enzyme complex (Superzyme™ Plus, Calgary, Canada).
[2]Vitamin-mineral premix provided the following nutrients (per kg of air-dry diet): Vitamins: A, 2000 IU, D_3 200 IU, E, 40 mg, K, 2 mg, B_1, 1.5 mg, B_2, 7 mg, B_6, 2.5 mg, B_{12}, 25 µg, calcium pantothenate, 14 mg, folic acid, 1 mg, niacin, 21 mg, biotin, 70 µg. Minerals: Cu, 10 mg (as copper sulphate), iodine, 0.4 mg (as potassium iodine), iron, 120 mg (as ferrous sulphate), Mn, 10 mg (as manganous oxide), Se, 0.3 mg (as sodium selenite), Zn, 110 mg (as zinc oxide).

Table 5.2 Analysed chemical composition and pH of experimental diets, as-fed basis

Item	Control	Fermented[1] Homo -	Fermented[1] Homo +	Fermented[1] Hetero -	Fermented[1] Hetero +	5% Casein
DM, %	89.5	86.8	85.0	85.5	85.0	91.0
OM, %	82.6	80.1	78.8	79.1	78.4	
GE, Mcal/kg	3.91	3.80	3.77	3.80	3.77	3.86
EE, %	3.6	3.1	3.4	3.6	3.9	0.8
Starch, %	30.4	28.8	28.4	26.1	27.9	—
NSP, g/kg						
Total	100.1	89.6	85.3	89.1	85.4	—
Insoluble	81.1	76.1	76.1	73.6	74.0	—
Soluble	19.0	13.5	12.4	15.4	11.4	—
NDF, %	10.2	7.7	7.9	8.4	8.0	3.9
ADF, %	4.8	4.1	4.2	4.7	4.6	1.6
Ca, %	1.09	0.94	0.86	0.90	0.97	—
P, %	0.78	0.74	0.72	0.73	0.76	—
CP, %	21.9	22.0	22.4	22.1	22.0	4.6
pH	5.46	4.98	4.98	4.98	4.81	—
Indispensable AA, %						—
Arg	1.11	1.26	1.25	1.11	1.23	0.21
His	0.56	0.64	0.63	0.58	0.63	0.13
Ile	0.69	0.76	0.79	0.68	0.81	0.24
Leu	1.27	1.44	1.42	1.29	1.46	0.42
Lys	1.39	1.58	1.63	1.53	1.60	0.39
Met	0.44	0.49	0.51	0.47	0.48	0.18
Phe	0.85	0.95	0.96	0.84	1.02	0.24
Thr	0.84	0.96	0.94	0.88	0.96	0.20
Val	0.79	0.88	0.90	0.80	0.93	0.33
Dispensable AA, %						
Ala	0.76	0.89	0.89	0.82	0.86	0.15
Asp	1.64	1.88	1.83	1.71	1.91	0.41
Cys	0.30	0.34	0.37	0.33	0.33	0.06
Glu	3.98	4.44	4.37	3.96	4.45	1.05
Gly	0.79	0.91	0.89	0.82	0.89	0.09
Pro	1.19	1.33	1.26	1.18	1.34	0.50
Ser	0.94	1.06	1.04	0.97	1.08	0.27
Tyr	0.52	0.60	0.63	0.51	0.62	0.19

[1]Homo, inoculated with homofermentative *Lactobacillus* (*L. plantarum*); Hetero, inoculated with heterofermentative *Lactobacillus* (*L. buchneri*); +, with enzyme complex added for the wheat fermentation; -, without enzyme complex.

Table 5.3 Apparent ileal digestibility (AID) and apparent total tract digestibility (ATTD) in weaned pigs fed diets containing fermented wheat[1,2]

Item	NC	PC	Fermented[3]				SEM	Significance[4]			
			Homo		Hetero			C_1	C_2	C_3	C_4
			-	+	-	+					
AID, %											
DM	60.0	62.6	60.1	61.8	63.0	64.2	1.15	0.090	0.059	0.021	0.188
OM	62.7	65.0	63.0	64.2	66.0	66.0	1.14	0.118	0.059	0.017	0.402
GE	63.1	66.1	63.8	65.5	67.7	68.1	1.18	0.058	0.015	0.006	0.371
EE	82.7	89.4	83.5	88.2	91.5	90.6	2.88	0.114	0.089	0.082	0.522
Starch[5]	93.0	95.6	95.8	97.7	97.4	97.3	0.57	0.004	<0.001	0.280	0.121
ATTD, %											
DM	84.4	85.8	85.1	88.2	88.5	88.1	1.35	0.428	0.033	0.215	0.319
OM	86.7	87.8	87.5	89.9	90.3	89.8	1.20	0.452	0.036	0.244	0.407
GE	84.7	85.8	85.3	88.3	88.9	88.3	1.42	0.532	0.048	0.188	0.368
EE	75.4	69.3	64.8	76.9	80.6	83.2	1.42	0.169	0.768	0.002	0.029
NDF	50.8	50.7	48.4	55.7	59.4	54.3	4.40	0.981	0.435	0.274	0.795
Ca[5]	71.1	68.9	64.7	75.3	75.4	75.1	2.50	0.518	0.581	0.042	0.046
P[5]	54.5	54.4	52.4	65.7	63.5	65.9	2.60	0.993	0.018	0.035	0.005
DE[6], Mcal/kg of DM											
Ileal	2.47	2.59	2.42	2.47	2.53	2.56	0.04	0.070	0.603	0.029	0.375
Total tract	3.31	3.36	3.24	3.33	3.38	3.33	0.06	0.525	0.933	0.200	0.682

[1]NC = negative control; PC = positive control, which was supplemented with 0.05% of enzyme complex.
[2]Each value represents the mean of 6 replicates per each treatment.
[3]Homo, inoculated with homofermentative *Lactobacillus* (*L. plantarum*); Hetero, inoculated with heterofermentative *Lactobacillus* (*L. buchneri*); +, wheat fermentation with enzyme complex; -, wheat fermentation without enzyme complex.
[4]Contrast 1 (C_1) = NC vs. PC; contrast 2 (C_2) = NC vs. Fermented; contrast 3 (C_3) = Homo. vs. Hetero.; contrast 4 (C_4) = (+) vs. (-).
[5]Inoculant and enzyme complex interaction ($P < 0.05$).

Table 5.4 Apparent ileal digestibility (AID) of crude protein (CP) and amino acids (AA) in weaned pigs fed fermented wheat[1,2]

Item	NC	PC	Fermented[3]				SEM	Significance[4]			
			Homo		Hetero			C_1	C_2	C_3	C_4
			-	+	-	+					
CP	72.6	75.3	72.5	73.9	74.2	74.6	1.14	0.076	0.307	0.286	0.402
Indispensable AA											
Arg	81.7	82.0	83.4	85.2	82.4	84.7	1.15	0.822	0.063	0.493	0.065
His	48.4	52.2	49.0	49.2	47.7	49.9	3.95	0.453	0.886	0.922	0.753
Ile	76.2	77.7	76.6	80.9	77.2	80.8	1.60	0.484	0.118	0.885	0.013
Leu	76.2	77.2	77.7	80.5	77.5	80.3	1.54	0.610	0.084	0.864	0.061
Lys	81.5	83.3	81.1	83.8	81.7	83.4	1.17	0.231	0.385	0.946	0.055
Met	84.4	87.1	86.4	88.8	89.1	89.3	1.38	0.138	0.008	0.219	0.322
Phe	77.9	79.3	79.0	82.3	77.9	82.5	1.44	0.451	0.101	0.723	0.007
Thr	72.5	73.0	72.9	75.1	72.5	74.9	1.49	0.780	0.367	0.824	0.104
Val	72.4	73.9	73.6	77.5	72.7	77.6	1.63	0.483	0.083	0.782	0.007
Dispensable AA											
Ala	68.6	72.2	73.3	76.7	74.1	76.4	2.22	0.225	0.008	0.907	0.182
Asp	70.0	71.5	71.0	73.3	70.3	74.3	1.75	0.530	0.230	0.928	0.065
Cys	64.6	71.9	67.0	72.3	71.1	72.1	2.74	0.046	0.039	0.462	0.227
Glu	83.7	84.9	83.8	85.3	84.1	85.0	1.25	0.461	0.509	0.989	0.316
Gly	65.1	66.9	66.3	68.6	66.0	69.4	2.01	0.480	0.239	0.875	0.135
Pro	77.3	79.1	78.4	77.7	77.4	78.6	1.50	0.362	0.658	0.988	0.851
Ser	71.8	73.2	73.3	75.2	72.6	75.9	1.68	0.510	0.158	0.997	0.111
Tyr	77.2	79.1	79.8	83.9	77.2	82.1	1.59	0.376	0.039	0.155	0.005

[1]NC = negative control; PC = positive control, which was supplemented with 0.05% of enzyme complex.
[2]Each value represents the mean of 6 replicates per each treatment.
[3]Homo, inoculated with homofermentative lactobacillus (*L. plantarum*); Hetero, inoculated with heterofermentative (*L. buchneri*); +, wheat fermentation with enzyme complex; -, wheat fermentation without enzyme complex.
[4]Contrast 1 (C_1) = NC vs. PC; contrast 2 (C_2) = NC vs. Fermented; contrast 3 (C_3) = Homo. vs. Hetero.; contrast 4 (C_4) = (+) vs. (-).

Table 5.5 Standardized ileal digestibility (SID) of crude protein (CP) and amino acids (AA) in weaned pigs fed fermented wheat[1,2,3]

Item	NC	PC	Fermented[4] Homo -	Homo +	Hetero -	Hetero +	SEM	Significance[5] C_1	C_2	C_3	C_4
CP	79.5	82.2	79.2	80.4	80.8	81.1	1.14	0.072	0.457	0.283	0.479
Indispensable AA											
Arg	85.9	85.9	86.9	88.6	86.3	88.2	1.15	0.979	0.176	0.649	0.097
His	62.3	64.7	60.2	60.3	59.8	61.0	3.95	0.641	0.620	0.966	0.858
Ile	79.7	80.8	79.5	83.7	80.4	83.4	1.60	0.583	0.222	0.818	0.022
Leu	79.1	79.9	80.1	82.9	80.1	82.6	1.54	0.700	0.153	0.909	0.076
Lys	83.7	85.3	83.0	85.6	83.6	85.2	1.17	0.281	0.609	0.925	0.065
Met	86.9	89.5	88.6	90.8	91.3	91.5	1.38	0.158	0.015	0.196	0.359
Phe	80.4	81.6	81.1	84.3	80.2	84.4	1.44	0.529	0.170	0.760	0.010
Thr	78.7	78.8	78.0	80.2	77.9	79.9	1.49	0.934	0.812	0.887	0.143
Val	76.8	77.9	77.3	81.0	76.7	81.0	1.63	0.611	0.201	0.839	0.014
Dispensable AA											
Ala	74.3	77.3	77.8	81.0	78.8	80.9	2.22	0.303	0.026	0.817	0.210
Asp	73.7	74.8	74.0	76.3	73.5	77.1	1.75	0.628	0.405	0.897	0.081
Cys	71.5	78.2	72.8	77.4	76.9	77.8	2.74	0.065	0.099	0.389	0.286
Glu	86.0	87.0	85.7	87.2	86.2	86.8	1.25	0.536	0.697	0.955	0.368
Gly	79.3	80.1	77.7	80.1	78.6	80.9	2.01	0.747	0.986	0.655	0.224
Pro	107.3	107.0	103.4	103.8	105.3	103.0	1.50	0.841	0.032	0.687	0.493
Ser	78.5	79.3	78.8	80.7	78.5	81.2	1.68	0.714	0.461	0.938	0.155
Tyr	80.1	81.8	82.1	86.1	79.9	84.4	1.59	0.429	0.076	0.200	0.009

[1]NC = negative control; PC = positive control, which was supplemented with 0.05% of enzyme complex.
[2]Each value represents the mean of 6 replicates per each treatment.
[3]Values for SID were calculated by correcting apparent ileal digestibility values for basal endogenous losses (mg/kg of DMI), which were determined by feeding a 5%-casein diet used to quantify endogenous CP and AA losses: CP, 17,015; Arg, 507; His, 821; Ile, 253; Leu, 395; Lys, 332; Met, 120; Phe, 226; Thr, 565; Val, 373; Ala, 456; Asp, 644; Cys, 224; Glu, 981; Gly, 1,206; Pro, 3,854; Ser, 671; Tyr, 163.
[4]Homo, inoculated with homofermentative *Lactobacillus* (*L. plantarum*); Hetero, inoculated with heterofermentative *Lactobacillus* (*L. buchneri*); +, wheat fermentation with enzyme complex; -, wheat fermentation without enzyme complex.
[5]Contrast 1 (C_1) = NC vs. PC; contrast 2 (C_2) = NC vs. Fermented; contrast 3 (C_3) = Homo. vs. Hetero.; contrast 4 (C_4) = (+) vs. (-).

Table 5.6 Volatile fatty acids and lactate concentrations, and pH in fresh feces and digesta in weaned pigs fed containing fermented wheat[1,2]

Item	NC +	PC -	Fermented[3] Homo -	Homo +	Hetero -	Hetero +	SEM	C_1	C_2	C_3	C_4
Ileal digesta, mmol/L											
Acetate	17.72	27.86	21.01	19.20	19.99	15.13	3.91	0.053	0.782	0.490	0.368
Propionate	2.44	3.15	1.71	3.51	1.26	1.38	1.01	0.609	0.655	0.202	0.347
Butyrate	0.31	0.45	0.12	0.21	0.35	0.12	0.11	0.381	0.403	0.547	0.528
Valerate	0.19	0.26	0.21	0.17	0.31	0.27	0.07	0.432	0.449	0.134	0.498
Total VFA	21.52	35.78	20.97	25.39	19.36	18.05	4.18	0.018	0.895	0.272	0.701
Lactate	5.58	2.16	5.08	5.17	6.38	1.73	2.42	0.280	0.694	0.647	0.331
Feces, mmol/L											
Acetate	42.75	45.04	48.37	42.87	50.03	47.18	6.99	0.728	0.410	0.542	0.398
Propionate	16.90	16.45	21.56	15.35	14.94	17.51	2.26	0.877	0.850	0.297	0.396
Butyrate	8.95	7.79	12.67	7.30	8.12	7.12	2.15	0.678	0.946	0.275	0.141
Valerate	1.93	2.29	2.75	2.14	2.12	2.10	0.32	0.394	0.299	0.278	0.302
Total VFA	76.86	77.28	92.25	72.30	84.78	81.40	9.69	0.973	0.565	0.932	0.233
Lactate	3.80	10.12	5.17	3.45	9.28	3.78	2.96	0.090	0.585	0.394	0.170
pH											
Ileal	7.26	7.15	7.42	7.44	7.61	7.47	0.12	0.510	0.098	0.073	0.591
Fecal	6.79	6.74	6.72	6.86	6.74	6.69	0.10	0.695	0.736	0.453	0.610

[1]NC = negative control; PC = positive control, which was supplemented with 0.05% of enzyme complex.
[2]Each value represents the mean of 6 replicates per each treatment.
[3]Homo, inoculated with homofermentative lactobacillus (*L. plantarum*); Hetero, inoculated with heterofermentative (*L. buchneri*); +, wheat fermentation with enzyme complex; -, wheat fermentation without enzyme complex.
[4]Contrast 1 (C_1) = NC vs. PC; contrast 2 (C_2) = NC vs. Fermented; contrast 3 (C_3) = Homo. vs. Hetero.; contrast 4 (C_4) = (+) vs. (-).

In particular, the water-soluble carbohydrates in the wheat dissolved in the added water, and those carbohydrates might have been more accessible for the microbes to hydrolyze. Many other studies have found that *Lactobacillus* fermentation reduces the water-soluble carbohydrate content after fermentation (Ranjit and Kung, 2000; Yang et al., 2006). This is in agreement with our results regarding the significant reduction in soluble NSP content in fermented wheat diets. The loss of these OM during fermentation might have resulted in less GE in the fermented wheat diets. In contrast, it appears that AA contents were concentrated in the fermented wheat diets because of the loss of DM at the expense of fermentable carbohydrate in the wheat. In addition, microbial proliferation that synthesizes cellular protein, enzymes, and other cellular components may contribute to the change in AA concentration after fermentation (Zhang et al., 2007). In fact, various studies have reported the increase in CP or AA concentration after fermentation (Hong et al., 2004; Zhang et al., 2007; Chen et al., 2010; Hackl et al., 2010).

There is good consensus that the nutrient availability of feeds for pigs increases after the feeds are fermented (Yang et al., 2006; Kim et al., 2007; Chen et al., 2010; Hackl et al., 2010; Pieper et al., 2011; Shi et al., 2015). This is in agreement with our results that the inclusion of fermented wheat increases the AID and ATTD of nutrients. During fermentation, microbial enzymes and wheat endogenous enzymes hydrolyze the complex linkages that pigs cannot break down intrinsically (Scholten et al., 1999). This might have enhanced the digestibility in weaned pigs. Indeed, *Lactobacillus* produces various dietary enzymes such as carbohydrase, lipase, and protease (Crittenden et al., 2002). In addition, ANF such as NSP and phytates can be destroyed by microbial activity during the fermentation process (Lopez et al., 2000; Pal Vig and Walia, 2001; Canibe and Jensen, 2003; Skrede et al., 2007), and encapsulated nutrients such as starch, protein, and minerals are released (Bedford and Schulze, 1998). In fact, the fermented wheat diets

contained substantially lower levels of NSP than unfermented wheat diets. In particular, soluble NSP is negatively correlated with AA utilization (Zhu et al., 2005) because it increases the viscosity of the digesta, thereby increasing endogenous losses and decreasing its reabsorption (Nyachoti et al., 1997). Therefore, the increase in the available nutrients and the reduction in the indigestible components and ANF in fermented wheat diets might be a major reason for the higher AID and ATTD of nutrient and energy in the present study.

Improved ATTD of P might be because most strains of *Lactobacillus* are known to be capable of producing phytase, which liberates 6 P moieties and inositol by hydrolyzing phytate (Lopez et al., 2000). Apart from the microbial phytase produced during fermentation, the endogenous phytase, present in large amounts in wheat, would have contributed to phytate degradation because its optimal activity occurs at pH 4 (Brejnholt et al., 2011), the pH generally attained during *Lactobacillus* fermentation (Pieper et al., 2011). This is supported by other studies that have shown that fermentation improved the P availability for pigs (Pieper et al., 2011; Kraler et al., 2014). Furthermore, it is worthwhile to note that phytate is present in the form of complexes with dietary nutrients such as proteins, free AA, and starch (Selle et al., 2007), suggesting that the breakage of the complexes increases the availability of these bound nutrients. This partly contributes to the increase in starch and AA digestibility in fermented wheat diets. Indeed, the beneficial influence of phytase on AA digestibility has been reported in the study by Liao et al. (2005) wherein the pigs were fed a wheat-canola meal-based diet.

Apart from phytate-bound AA, carbohydrate-protein complexes such as glycoproteins and proteoglycans might have been hydrolyzed by the microbial enzymes during fermentation, enhancing AA digestibility (Meng et al., 2005; Ayoade et al., 2012). In addition, Yang et al. (2006) reported that fermentation significantly increased the content of small-size peptides (< 20 kDa) in

soybean meal, suggesting greater availability of AA for nursery pigs. Similarly, the content of trichloroacetic acid soluble protein, small peptides (2–20 residues), and free AA, in rapeseed cake was significantly reduced after fermentation (Shi et al., 2015). However, it is difficult to elucidate the reasons for improvements in the AID and SID of particular AA in pigs fed fermented wheat diets because of the complicated metabolism of AA during fermentation by *Lactobacillus* (Fernández and Zúñiga, 2006). Given that some strains of *Lactobacillus* are acid- and bile-tolerant (Liong and Shah, 2005), *Lactobacillus* assimilates selective AA in the body during fermentation (Taylor, 1947), and the AA might have bypassed the gastrointestinal tract, lowering their digestibility. This is partially supported by a human study by Johansson et al. (1998), which reported that *L. plantarum* in fermented oats survived in the gut, and identical strains were observed in the feces. However, no detailed analysis of microbes was performed in the present study.

Greater digestibility of Hetero L.-fermented wheat diets compared to that of Homo L.-fermented wheat diets was observed in the present study. The underlying reason is unclear. A possible reason might be the greater capability of *L. buchneri* to produce various dietary enzymes, liberating more available nutrients than *L. plantarum*. For instance, Nsereko et al. (2008) reported that *L. buchneri* strains showed greater activity of ferulate esterase, a hydrolase that degrades arabinoxylans, releasing more ferulic acid than other *Lactobacillus* spp. This suggests that enzymatic degradation of the cell wall occurs. In particular, based on the improved P and Ca digestibility in the present study, phytase activity of *L. buchneri* when inoculated in wheat seems to be greater than that of *L. plantarum*, liberating more P. Indeed, Fischer et al. (2014) reported that the phytate-degrading capability of *L. buchneri* was greater than that of *L. plantarum* in food fermentation. In addition, as reviewed by Holzer et al. (2003), acetic acid produced by *L. buchneri*

is more beneficial for aerobic stability during fermentation. It inhibits yeast and mold growth more effectively than the lactic acid solely produced by *L. plantarum*. This might have resulted in a more stable pH during fermentation where desirable reactions such as *Lactobacillus* enzyme activity and inherent phytase activity occur.

Exogenous enzymes are widely used as a fermentation additive. Added carbohydrases hydrolyze the cell wall components during fermentation, providing fermentable carbohydrates, which stimulate the proliferation of inoculated *Lactobacillus* (Yitbarek and Tamir, 2014). In the present study, the MC contained phytase, which, along with microbial and wheat-inherent phytase, broke down the phytate bonds during the fermentation period. This might have been an important reason for the increased P and Ca digestibility in the fermented wheat diets. However, it should be noted that enzyme supplementation was more effective for homo-fermentation in terms of starch, Ca, and P digestibility, showing the interactive effects between the inoculants and the MC. This might be due to the lower dietary enzyme activities of *L. plantarum.* However, to our knowledge, no studies have performed a complete comparison of the dietary enzyme activities of *L. buchneri* and *L. plantarum*.

With regard to AA digestibility in fermented wheat diets, enzyme supplementation for wheat fermentation was effective, regardless of the inoculants. Supplemental enzymes are associated with a rapid pH decline during fermentation wherein proteolysis is inhibited, lowering the ammonia-N production (Dean et al., 2005; Tibarek and Tamir, 2014). This is likely to preserve more available AA in the enzyme-treated fermented wheat, increasing AA digestibility. In addition, supplemental enzymes added for fermentation that accompanied the fermented wheat were also mixed with other ingredients; therefore, they might have acted as exogenous feed enzymes in the gut, contributing to the digestibility.

Lactic acid produced by *Lactobacillus* fermentation reduces dietary pH, and the acidified diet is likely to lower the pH in the gastrointestinal tract, benefiting gut health (Ravindran and Kornegay, 1993). In addition, *Lactobacillus* fermented diets are likely to positively alter the VFA concentrations by delivering *Lactobacillus* to the gut, as reported by Scholten et al. (1999). Therefore, we measured the pH as well as VFA and lactate concentrations of the gut (Nyachoti et al., 2006) as parameters of gut health. However, contrary to expectations, no effects of fermented diet were observed. This result is in partial agreement with a study by Le et al. (2016) where feeding diets containing *L. rueteri*-fermented wheat did not affect the VFA concentration in the ileal digesta. Also, Rateliffe et al. (1986) failed to observe increased Lactobacilli population in the gastrointestinal tract of weaned pigs fed *L. reuteri* fermented milk diets. This lack of effect may be related to the established microbiota in the weaned pigs. Inclusion of whey permeates as a source of lactose in all the experimental diets was likely to stimulate sufficient proliferation of *Lactobacillus* in all groups, which would have countered the effects of *Lactobacillus*-fermented diets.

5.4 CONCLUSIONS

Fermentation of wheat can be a practical strategy to improve preservability while using its high moisture content. Farmers would also benefit in terms of broader time windows for harvesting and lower drying costs. The presence of indigestible carbohydrates such as xylans in wheat is a limiting factor preventing the inclusion of large quantities of wheat in nursery diets. Based on our results, the use of fermented wheat in place of unfermented wheat in nursery diets reduces the content of NSP, particularly soluble NSP. In addition, AA contents were higher in the fermented wheat diets and the inclusion of fermented wheat increased the OM, starch, P, and GE

digestibility. Although GE content was lower in the fermented wheat diets, no difference in DE was observed compared to that in the control wheat diets. It seems that the benefits of fermented wheat inclusion are greater than those achieved by adding MC to the complete diet (PC) in terms of nutrient digestibility. Moreover, based on our results, Hetero L.-fermented wheat is preferable for weaned pigs, considering the higher digestibility of nutrients and energy. Adding an MC as a wheat fermentation additive enhances subsequent nutrient availability (e.g., mineral and AA) for pigs when the wheat is included in the nursery diet. However, although fermented wheat diets had a lower pH, they did not alter the pH of the ileum or feces. In addition, the fermented wheat diets did not alter the VFA and lactic acid concentration compared to the control wheat diets.

6.0 GENERAL DISCUSSION

In swine production, piglets have to be weaned at an early age (mostly at 21 d), when their digestive system is not developed enough to consume a solid, cereal-based type of diet. Moreover, weaned pigs likely experience anorexia caused by various stressors such as nutritional, psychological, and environmental stressors (Pluske et al., 1997). Therefore, nursery diets are formulated with highly digestible and palatable ingredients to minimize post-weaning growth retardation. Also, various additives are added to nursery diets to promote gut health and stimulate digestive function. Therefore, the price of nursery diets is much higher than grower or finisher diets. Many nutritional strategies have been researched to reduce feed costs during the nursery phase. If alternative feed ingredients can be included while maintaining growth performance, the unit price of feed ($/ton) will directly decrease. Also, if dietary modification can promote the gut function of weaned pigs by improving gut health, weaned pigs will utilize dietary nutrients and energy more efficiently for their growth, showing greater G:F. In this regard, a variety of nutritional strategies have been suggested to reduce costs for nursery diets while maintaining the beneficial function of conventional weaner diets. In the present research, four strategies that can be adopted practically in Western Canada have been chosen, and three studies have been conducted to investigate the potential benefits of the nutritional strategies on costs for nursery diets.

For the last decade, dietary fiber has received great attention due to its potential benefits on gut health. Various dietary fiber sources have been fed to weaned pigs to investigate enteric diseases, such as PWD (Kim et al., 2012). The beneficial effects of WB, a source of insoluble fiber, on the gut health of weaned pigs has been researched (Molist et al., 2009, 2010, 2011; Chen et al., 2013). The inclusion of WB positively modified the microbiota in the GIT, reducing the ability of

E. coli to grow and attach to the intestinal mucosa of early weaned piglets challenged with *E.coli* (Molist et al., 2010). Also, Chen et al. (2013) found that including WB enhanced intestinal barrier function in weaned pigs by increasing the concentration of intestinal barrier factors, such as diamine oxidase and trefoil factor family. In addition, an increase in individual VFA production has been reported by Molist et al. (2009, 2011). However, none of the studies available on these benefits can be further extended to nutrient digestibility. If inclusion of WB improves gut health, thereby increasing nutrient digestibility, particularly AA digestibility, then supplementing crystalline AA to meet their requirements could be avoided. Therefore, in Manuscript I, the influence of dietary WB inclusion on nutrient and energy digestibility and microbial metabolites in weaned pigs was studied. As expected, microbial metabolites such as VFA and pH were beneficially influenced by the inclusion of WB. The VFA concentration was greater and the pH in feces was lower when weaned pigs were fed a 4% WB diet. The VFA play crucial trophic roles in the gut. The VFA, particularly butyric acid, produced by microbes in the gut from dietary fiber are known to stimulate epithelial cell proliferation and growth of the intestine (Montagne et al., 2003). Moreover, the antimicrobial effect of VFA in the gut has been demonstrated (Ricke et al., 2003). However, these possible benefits were not further extended to nutrient and energy digestibility. Rather, reduced digestibility in weaned pigs fed a WB diet was observed. Also, DE was less in the WB diet than in the control diet. It seems that any possible benefit of WB inclusion does not override the antinutritional effect of NSP in WB in terms of energy and nutrient digestibility. Considering that energy is the most expensive component in swine diets, reducing energy availability for weaned pigs would be economically negative. Therefore, although inclusion of WB per se reduces the unit cost of nursery diets, it would not be a sound strategy to reduce costs for

nursery diets. However, it is obvious that weaned pigs maintain their gut health better under an experimental environment, suggesting a commercial environment might produce a different output.

Numerous studies have been conducted to identify opportunities to reduce diet complexity in order to lower nursery diet costs. If weaned pigs that were fed simplified diets show comparable growth performance to those fed conventional diets, feed costs would be substantially reduced. Also, if conventional ingredients such as animal-protein sources and dairy products are replaced with alternative feed ingredients, mostly plant-based, the unit price of complete diets will be substantially lower, and economic benefits will be realized to a certain extent, even though a slight reduction in growth performance is observed. Therefore, in Manuscript II, three diets were formulated with different complexities. Complex1 was formulated to mimic a conventional weaner diet, whereas complex2 partially or completely replaced blood meal, skim milk powder, and dried whey with alternative feed ingredients. The simple diets were corn-wheat-SBM-based. Based on the average price of feed ingredients for the last 5-year (Jan. 2012–Dec. 2016), the complex2 diet and the simple diets were substantially cheaper to formulate than Complex1 diet for both phase I and phase II (Table 6.1). As Dritz et al. (1996) stated, diet composition is a critical factor in determining growth performance of pigs for the first week of weaning. Greater ADG, ADFI, and G:F observed in pigs fed the complex1 diet at the beginning of post-weaning was supported by other studies (Dritz et al., 1996; Mavromichalis et al., 2001; Wolter et al., 2003; Mahan et al., 2004; Sulabo et al., 2010; Skinner et al., 2014). However, there is controversy regarding how long the positive effects of a conventional diet last post-weaning. In the present study, complex1 diet led to greater ADG, ADFI, and G:F only for the first week post-weaning. It is worthwhile to note that the complex2 and the simple diet contained fish meal and dried whey. This might have helped

Table 6.1 Economic analysis for diet complexity and multicarbohydrase supplementation in newly-weaned pigs[1]

Item	Diet Complexity			MC[3]		SEM	P-value		
	Complex1	Complex2	Simple	+	-		Diet	MC	Diet× MC
Feed cost, \$/ton[2]									
Phase I	642	510	502						
Phase II	459	362	365						
Total weight gain, kg	13.36[x]	12.33[y]	13.17[xy]	13.00	12.91	0.384	0.027	0.784	0.675
Total feed cost per pig, \$/pig	10.87[x]	7.85[y]	8.00[y]	8.82	9.00	0.243	<0.001	0.371	0.871
Feed cost/kg of BW gain, \$/kg/pig	0.82[x]	0.64[y]	0.61[y]	0.68	0.70	0.013	<0.001	0.077	0.843

[1]Average price of feed ingredients for 5 years (Jan. 2012–Dec. 2016) was calculated to estimate feed cost (Corn, \$185; SBM, \$483; Barley, \$170; Wheat, \$161; Oat, \$193; Field pea, \$295; Blood meal, \$1,080; Canola meal, \$253; Fishmeal, \$1,930; Dried whey, \$973; Skim milk powder, \$3,203; Corn DDGS, \$260; Wheat millrun, \$179; Vegetable oil, \$1,022; Monocalcium phosphate, \$1,011; Lysine, \$1,860; Methionine, \$7,370; Salt, \$310; Threonine, \$4,230; Limestone, \$190; Vitamin-mineral premix, \$4,000; Grains and Oilseeds market price, Manitoba agriculture).

[2]Canadian dollar, sum of the price of each ingredient.

[3]The price of MC was assumed at \$5/kg.

[x,y,z]Means within the main effect of diet complexity and within row lacking a common superscript letter differ (P <0.05).

weaned pigs adapt to weaner diets, showing comparable growth performance from the second week post-weaning. This is supported by studies where pigs fed simple diets containing fish meal and/or dried whey showed poorer growth performance, which were limited in the beginning of post-weaning, compared to those fed a conventional complex diet (Mavromichalis et al., 2001; Wolter et al., 2003; Pettey et al., 2002). At the end of the experiment (d 28 post-weaning), the BW of pigs fed the simple diet was not statistically different from that of the pigs fed the complex diet. Also, it was interesting to find that pigs fed the simple diet showed greater G:F during the overall 4-week of experimental period than those fed the complex1 diet. It seems that feeding the complex1 for 4 wks leads to overconsumption for their digestive capacity, resulting in a higher fecal score. Based on ADG, ADFI, and unit cost of experimental diets, total feed cost/pig and feed cost/kg of BW were compared among treatments (Table 6.1). The total feed cost per pig fed the simple diet was 26.4% lower than that fed Complex1, due to the lower unit cost of the simple diet. Also, greater G:F as well as lower feed cost in the simple diet resulted in a 21-cent reduction in feed cost/kg of BW gain when pigs were fed the simple diet rather than the complex1. Similarly, feeding complex2 diet compared to complex1 showed lower total feed cost/pig and feed cost/kg of BW gain. However, complex2-fed pigs showed reduced final BW compared with complex1-fed pigs. Therefore, it is logical to conclude that feeding weaned pigs the simple diet (containing fish meal and dried whey during phase I) rather than a conventional complex diet is economically beneficial with regard to lowering feed costs for the 4-week post-weaning period without compromising final BW. However, it should be noted that the small intestinal morphology was more desirable in weaned pigs fed a complex1 than in those fed either a complex2 or a simple diet, showing higher VH and VH:CD ratio. Gut architecture, such as VH and CD, as well as feed intake are crucial factors affecting gut barrier function of pigs after weaning (Wijtten et al., 2011). In this

regard, the present results might indicate that different results appear in more stressful environments, such as a commercial facility or heat stress in the summer.

It is imperative that a large proportion of cereal grains (e.g., corn, wheat, and barley) have to be formulated for nursery diets to meet energy requirements and to balance the feed costs. Apart from energy, cereal grains in nursery diets contribute to a substantial proportion of AA requirements as well as minerals. Therefore, improvement in the availability of nutrients and energy in cereal grains would provide great economic benefits by reducing the proportion of expensive supplemental feed ingredients (e.g., SBM and inorganic phosphorus). Wheat contains relatively high soluble NSP (e.g., arabinoxylans and β-glucan) compared with corn (Zijlstra et al., 1999). The negative effects of soluble NSP on nutrient availability in pigs have been demonstrated (Zhu et al., 2005). Further, 56.4% of total P in wheat is formed in phytate (NRC, 2012). A fermentation technique can be beneficially employed to eliminate ANF in wheat. Also, if the fermentation is initiated with beneficial inoculants (e.g., *Lactobacillus*), the inoculant bacteria and/or their products (e.g., lactic acid and exopolysaccharides) will be delivered to the gut, providing a potential prebiotic effect (Kedia et al., 2007). Therefore, in Manuscript III, the effects of inclusion of fermented wheat on nutrient digestibility and microbial metabolites in weaned pigs were studied. Also, two types of inoculants (Homo L. vs. Hetero L.) were compared. In this study, experimental diets were formulated to mimic the nursery diets widely used in Western Canada. Numerous studies have shown that fermentation can improve the nutritive values of feed ingredients by reducing ANF and modifying nutrient composition (Lopez et al., 2000; Pal Vig and Walia, 2001; Canibe and Jensen, 2003; Skrede et al., 2007). The inclusion of fermented wheat instead of unfermented wheat in a nursery diet decreased NSP content, but increased AA content. In addition, the inclusion of fermented wheat increased DM, OM, GE, starch, P, and AA

digestibility. During fermentation, Lactobacilli release various dietary enzymes such as carbohydrases, lipase, and protease (Crittenden et al., 2002). These enzymes might hydrolyze the outer cell membranes of wheat, mostly composed of NSP, and complex linkages in wheat that pigs cannot digest. In this process, encapsulated nutrients or bound nutrients that might be released as exogenous feed enzymes act in the gut may become available for pigs to digest. Fermented wheat diets not only had greater AA contents, but also feeding such diets led to greater AA digestibility. Moreover, although GE is lower in fermented wheat diets than in unfermented wheat diets, DE content was comparable due to the greater energy digestibility. Therefore, fermented wheat diets provide weaned pigs with more digestible nutrients without reductions in DE.

However, in contrast to expectations, VFA and lactate concentration, and pH in the gut were not affected by fermented wheat diets. It is considered that an acclimation period of at least 12 days is required for treatments to alter intestinal microflora (Collier et al., 2003; Agyekum et al., 2016). In this study, samples were collected following a 5-day adaption period. Therefore, pigs might have been assigned to a dietary treatment for too short a period for intestinal bacteria to be affected, thereby, thereby showing no differences in microbial metabolites. Also, it is noteworthy that whey permeate as a lactose source was included in all experimental diets. Given that lactose is known to stimulate the proliferation of *Lactobacillus* in the gut of weaned pigs (Pierce et al., 2006, 2007), *Lactobacillus* in the gut of pigs fed unfermented-wheat diets were also favoured by whey permeate, showing no differences in microbial metabolites across dietary treatments.

For the last decade, exogenous enzymes in swine diets have been researched to increase nutrient availability for pigs. These include carbohydrases (e.g., amylases, xylanase, cellulase, pectinase, mannanase, and so on) and phytase. In Manuscript II, 0.1% of MC addition to nursery diets increased G:F as well as DM, OM, and GE digestibility. Similarly, in Manuscript III, 0.05%

of enzyme supplementation increased starch and Cys digestibility. Several modes of action of supplemental enzymes in the gut might explain its beneficial influence of nutrients. Feed enzymes hydrolyze ANF, such as phytate and NSP, which negatively affects digestibility by altering digesta viscosity and endogenous losses (Nyachoti et al., 1997; Woyengo et al., 2013). Because cell wall components of feed ingredients mostly comprise NSP, hydrolysis of cell walls release encapsulated nutrients available for pigs (Bedford and Schulze, 1998). Furthermore, nutrients bound in a form of phytate, glycoproteins, and proteoglycans become more available for pigs by the action of exogenous feed enzymes (Meng et al., 2005; Woyengo et al., 2013). These actions of MC increase the contents of nutrients available for pigs, thereby increasing nutrient and energy digestibility. In Manuscripts II and III, tendencies of greater DE were observed when exogenous enzymes were added. These benefits might lead to greater feed efficiency, which could potentially reduce feed costs. Based on our economic analysis (Table 6.1), MC addition tended to decrease feed cost/kg of BW gain.

Apart from improved nutrient availability, the potential benefits of the addition of enzymes on gut health have been suggested (Kiarie et al., 2013). In Manuscripts II and III, it was found that feed enzymes improved gut health indicators. After weaning, both gut barrier function and the intestinal architecture of pigs are impaired (Wijtten et al., 2011), which often results in PWD. In this state, pigs poorly digest and absorb nutrients in their diets (Pluske et al., 1997). In this regard, intestinal morphology and fecal score partly indicate the gut health of weaned pigs. The addition of MC in Manuscript II increased the intestinal morphology and decreased fecal score for 3-week after weaning. Results from other studies (Kim et al., 2003; Fan et al., 2009; Jiang et al., 2015; Waititu et al., 2016) are in agreement with our results of improved intestinal morphology. Greater VH and VH:CD ratio are desirable because a broader surface indicates broader digestive and

absorptive sites (Pluske et al., 1997), and thus there are important gut health indicators in weaned pigs. Digesta viscosity is negatively correlated with intestinal morphology because viscous digesta are susceptible to be fermented by pathogenic bacteria-producing toxins, such as amines and NH_3 (McDonald et al., 2001). Added feed enzymes can hydrolyze viscous NSP, such as arabinoxylans and β-glucan, in nursery diets (Bedford and Schulze, 1998). This, in turn, might reduce digesta viscosity, thereby providing a beneficial gut environment for villus proliferation. In a study by Kiarie et al. (2010), potential prebiotic effects of feed enzymes were suggested. The authors reported enzyme hydrolysis products led to the greater fluid absorption in the segments of small intestine in weaned pigs challenged with ETEC. This is partially supported by results in Manuscript III that enzyme supplementation increased total VFA concentration and tended to increase lactic acid concentration in feces. These beneficial effects partly explain the low fecal score in pigs fed MC supplemented diets.

Feed enzymes can be also beneficially used for wheat fermentation. It seems that the mode of actions of feed enzymes described above also occurs during fermentation, and the nutrients are utilized by the proliferation of inoculated microbes (Yitbarek and Tamir, 2014). This might help approach low pH rapidly in the beginning of fermentation, where most of the spoilage by bacteria activity is inhibited, and thus the low pH by lactic acid produced by *Lactobacillus* is maintained throughout the fermentation. In Manuscript III, enzyme supplementation for wheat fermentation was particularly beneficial for Ca, P, and AA digestibility. However, it should be noted that the interactive effects of enzyme supplementation and inoculants were observed with regard to Ca and P digestibility. Enzyme complex seemed more effective with Homo L than Hetero L.. The reason is unclear. Possibly, the phytase activity of Homo L. is less than that of Hetero L. (Fischer et al., 2014), which might make effects of exogenous phytase greater in Homo L.-fermentation. Enzyme

supplementation as a wheat fermentation additive was beneficial for AA digestibility in weaned pigs, irrespective of type of inoculants. This might be explained by the fact that aerobic stability in fermentation is improved by the addition of enzymes (Tibarek and Tamir, 2014). Indeed, Dean et al. (2005) reported that NH_3 loss during Bermuda grass silage was reduced by the addition of exogenous fibrolytic enzymes.

Taken together, the addition of feed enzymes would be a beneficial feeding strategy because it improves G:F by improving nutrient and energy digestibility. This could potentially lead to a reduction in feed costs. In addition, enzyme supplementation of feed enzymes positively modified gut health indicators (e.g., fecal score, intestinal morphology, and VFA productions), which suggests its benefitial effects on growth performance might be greater in a commercial environment, where weaned pigs are more likely to develop gut disorder. Furthermore, feed enzymes can be advantageously added to wheat fermentation in order to increase nutrients, particularly AA digestibility in weaned pigs. Considering the price of feed enzymes for swine are affordable and the benefits can be produced with small qualities (0.05–0.10%), feed enzymes can be utilized profitably for weaned pigs, regardless of diet complexity.

7.0 CONCLUSIONS AND FUTURE STUDIES

CONCLUSIONS

The following conclusions can be drawn from the present research:

1. Inclusion of WB in nursery diets improved VFA concentration in feces and ileal digesta. However, nutrient and energy digestibility was reduced in pigs fed a WB diet compared with those that were fed a control diet.

2. Formulating a simple diet (corn-wheat-SBM-based) substantially reduced the unit cost of feed ($/ton) and feeding such a diet increased G:F for the entire experimental period of 4 weeks and reduced total feed cost/pig and feed cost/kg of BW gain, compared with a conventional nursery diet (Complex1). However, Complex1-fed pigs showed higher VH or VH:CD ratio in the small intestine.

3. Inclusion of fermented wheat instead of unfermented wheat reduced NSP contents, whereas AA contents were increased. Feeding fermented wheat diets increased nutrient (starch, P, and AA) and energy digestibility compared with the unfermented wheat diets.

4. The addition of MC containing cellulase, pectinase, mannanase, galactanase, xylanase, glucanase, amylase, and protease to nursery diets increased G:F, ATTD of DM, OM, NDF, and GE, intestinal morphology (VH or VH:CD), and total VFA concentration in ileal digesta. Also, the fecal score was reduced by the addition of MC.

FUTURE STUDIES

In the present research, the inclusion of WB positively modified microbial metabolites. Also, feeding simple diets to weaned pigs would directly reduce costs for nursery diets. Furthermore, the present research demonstrates nutrient and energy availability for weaned pigs can be improved by using fermented wheat or MC supplementation. These benefits would potentially control feed costs by improving feed efficiency or providing opportunities to reduce the level of supplements in nursery diets (e.g., SBM, inorganic phosphorus, crystalline AA). However, further studies are required to:

1. Investigate interaction between microbiota and growth performance in weaned pigs fed nursery diets containing WB.

2. Investigate the effects of diet complexity in a commercial environment on growth performance and the intestinal bacteria profile of nursery pigs, and their subsequent growth performance and carcass characteristics.

3. Evaluate standardized ileal digestibility of AA in pigs fed fermented gains and the energy value of fermented grains.

4. Explore the effects of fermented grains on intestinal microbiota in weaned pigs.

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