Prebiotics and Beta-Glucan in Modulation of Growth Performance, Nutrient Utilization and Alkaline Phosphatase Kinetics in the Weanling Pig

by

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A Thesis
presented to
The University of Guelph

In partial fulfillment of requirements
for the degree of
Master of Science
in
Animal and Poultry Science

Guelph, Ontario, Canada

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ABSTRACT

PREBIOTIC AND β-GLUCAN IN MODULATION OF GROWTH PERFORMANCE, NUTRIENT UTILIZATION AND ALKALINE PHOSPHATASE KINETICS IN THE WEANLING PIG

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University of Guelph, 2012

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This thesis examined effects of dietary supplementations (0.75%) of the prebiotics of retrograded resistant cornstarch, fibersol-2 and inulin, and oat β-glucan in replacing feed antibiotics on growth performance, plasma urea concentrations, total tract dry matter (DM) and lactose digestibility, fecal scores, proximal jejunal and serum alkaline phosphatase (AP) kinetics and large intestinal fermentation in weanling pigs fed corn and soybean meal-based diets. There were no differences ($P > 0.05$) in the growth performance, plasma urea concentrations, DM and lactose digestibility and the volatile short-chain fatty acid concentrations in the cecal and fecal samples among the treatment diets. Dietary lactose was completely digested in the weanling pigs. Supplementations of fibersol-2, inulin and β-glucan significantly affected some of the jejunal and serum AP kinetics. In conclusion, lactose was a highly digestible carbohydrate and dietary supplementations of the three prebiotic and β-glucan at 0.75% had little effects on growth performance and plasma urea concentration but might affect gut and the whole body health status via influencing the AP detoxification kinetics in the weanling pigs.
ACKNOWLEDGEMENTS

There are many people that I would like to thank and acknowledge for helping me through the process to complete this thesis. Firstly, I would like to thank my advisor, Dr. Ming Z. Fan. His guidance and insight along the way have been invaluable. I would also like to thank the members of my advisory committee, Dr. Kees de Lange and Dr. Gregory Bedecarrats for their additional support and assistance.

To my friends and colleagues that I have made along the way, I could not be more grateful for the many stimulating and informative discussion we have had. My lab friends, Tania Arcbold, Qi Wang, Xiaojian Yang and Weijun Wang, I cannot thank you enough for all your help with everything from animal work to developing, performing and perfecting laboratory protocols. I would also like to thank Doug Wey for his excellent assistance with one animal trial. Without his skilled handling of the pigs, our sample collection would have taken unacceptably long. In addition to my lab colleagues, I would like to express my thanks to my office colleagues. Steve Larmer, Lindsay Case and Danielle Glanc have all been an excellent source of stimulating conversation and helped me generate ideas from different perspectives. I cannot properly express how valuable these interactions have been to me.

Outside of Animal and Poultry Science I would like to thank my family and friends. Their continued support has been so important in this process and without it, completion of this project would have been very difficult. Specifically, I would like to thank my parents, Paul and Dayle Hayhoe for their love and assistance throughout and particularly in the final months of this endeavor.
I would also like to acknowledge Ontario Pork, the Agriculture Adaptation Council of Canada and the Ontario Ministry of Agriculture, Food and Rural Affairs (OMAFRA) for providing funding to allow me to complete this program. Specifically I would like to thank OMAFRA for the opportunity to participate in the Highly Qualified Personnel (HQP) program and internship. The opportunity this program gave me to work with the great staff at the Business Development Centre (now the Catalyst Centre) at the University of Guelph was truly life changing. In addition, I would like to thank the staff of Animal and Poultry Science for all their help and support.
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<td>AA</td>
<td>Amino acid(s)</td>
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<td>ADG</td>
<td>Average daily gain</td>
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<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
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<tr>
<td>AOAC</td>
<td>Association of Official Analytical Chemists</td>
</tr>
<tr>
<td>AP</td>
<td>Alkaline phosphatase</td>
</tr>
<tr>
<td>CP</td>
<td>Crude protein</td>
</tr>
<tr>
<td>Cu</td>
<td>Copper</td>
</tr>
<tr>
<td>$d$</td>
<td>day</td>
</tr>
<tr>
<td>DE</td>
<td>Digestible energy</td>
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<tr>
<td>DM</td>
<td>Dry matter</td>
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<tr>
<td>$E.\ coli$</td>
<td><em>Escherichia coli</em></td>
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<tr>
<td>ETEC</td>
<td>Enterotoxigenic <em>Escherichia coli</em></td>
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<tr>
<td>F-2</td>
<td>Fibersol-2</td>
</tr>
<tr>
<td>FOS</td>
<td>Fructooligosaccharide</td>
</tr>
<tr>
<td>G:F</td>
<td>Gain to feed ratio</td>
</tr>
<tr>
<td>$\beta$-G</td>
<td>$\beta$-Glucan</td>
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<tr>
<td>GOS</td>
<td>Galactooligosaccharide</td>
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<td>IMO</td>
<td>Isomalto-oligosaccharide</td>
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<td>IN</td>
<td>Inulin</td>
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<tr>
<td>$K_m$</td>
<td>Enzyme affinity</td>
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<td>LDC</td>
<td>Lactase digestive capacity</td>
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<td>N</td>
<td>Nitrogen</td>
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<tr>
<td>NAD⁺</td>
<td>Nicotinamide adenine dinucleotide</td>
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<tr>
<td>NADH</td>
<td>Nicotinamide adenine dinucleotide’s reduced form</td>
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<td>NC</td>
<td>Negative control</td>
</tr>
<tr>
<td>NK</td>
<td>Natural killer</td>
</tr>
<tr>
<td>NRC</td>
<td>National Research Council</td>
</tr>
<tr>
<td>PC</td>
<td>Positive Control</td>
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<tr>
<td>PMSF</td>
<td>Phenylmethysulfonyl fluoride</td>
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<tr>
<td>RCS</td>
<td>Resistant cornstarch</td>
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<tr>
<td>SBM</td>
<td>Soybean meal</td>
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<tr>
<td>SOS</td>
<td>Soya-oligosaccharide</td>
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<tr>
<td>SST™</td>
<td>Serum separating tubes</td>
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<tr>
<td>$V_{cap}$</td>
<td>Digestive capacity</td>
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<tr>
<td>$V_{max}$</td>
<td>Maximal enzyme activity</td>
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<tr>
<td>VFA</td>
<td>Volatile short-chain fatty acids</td>
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<tr>
<td>wk</td>
<td>Week</td>
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<td>XOS</td>
<td>Xylo-oligosaccharide</td>
</tr>
<tr>
<td>Zn</td>
<td>Zinc</td>
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<td>ZnO</td>
<td>Zinc oxide</td>
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CHAPTER 1

GENERAL INTRODUCTION AND LITERATURE REVIEW

1.1. BACKGROUND

Pork is the most highly consumed meat product worldwide. Currently, intake of pork represents more than 39 percent of the global consumption of animal protein (FAO, 2007). This is steadily increasing due to elevated consumption preference in large and increasingly wealthy marketplaces such as China (Speedy, 2003). Despite this world trend, pork consumption rates in Canada have been declining since the mid 1980’s (AAFC, 2011). This decline is compensated by increasing pork exports from Canada and the total export market value increasing by $100 million between 2009 and 2010 (AAFC, 2012). These trends are noteworthy due to the nature of the pork production industry in North America. In order to supply products to meet consumer demand at the desired price points, producers have become increasingly reliant on technological advances and husbandry techniques that afford increased efficiencies. The global recession, beginning in late 2008, along with rising costs of key grain products and several zoonotic disease scares, has caused substantial losses for many pig farmers. This is due to their inability to receive an adequate price per pig to cover the expenses required to raise the animal (CPC, 2009). Despite the high world demand for pork, the decrease in consumption patterns in Canada and the US has had an impact on most North American pork producers and has
intensified interest in husbandry practices that can be used to decrease operating costs and therefore increase the returns gained per animal (FAO, 2007).

Providing non-therapeutic levels of antibiotics in the diet of pigs has become a common practice to increase production efficiencies of pigs over the last several decades. In the 1960s, agriculture was booming and scientists were discovering new ways to improve the efficiency of production for many animal species. The discovery of vitamin B$_{12}$ supplementation directly leads to the realization that providing bacterial inhibitors at low levels in animal feeds could improve feed efficiency and growth (Summons, 1968). Although this technique is practiced in many species of production animals, the concern associated with feeding pigs non-therapeutic levels of antibiotics has been augmented due to discovery of antibiotic-resistant bacteria on pig farm workers (Chapin et al., 2005) and the publicity associated with some zoonotic diseases that originated from, or have been disseminated by pig production facilities (Karasin et al., 2004; Gottschalk et al., 2007; Dawood et al., 2009).

With levels of antibiotic resistance rising (Arias and Murray, 2009) and many regions and countries, such as the European Union in 2006 and South Korea in 2011, moving to ban the use of non-therapeutic levels of antimicrobials in animal feed, the search for viable alternatives has intensified. The aforementioned market stressors experienced by many pork producers have placed an emphasis on the search for alternatives to antibiotic feeding with many producers feeling that in order to maintain their livelihood, production boosting additives are necessary. This is especially true in the case of weanling pigs due to high stress associated with separation from the sow as well as mixing with new animals resulting in new pathogen exposure, psychological
stress and a decrease in circulating maternal immunoglobulins and the acquired passive immunity (Lalles et al., 2007).

Any alternatives to non-therapeutic levels of antibiotics in pig feed, and specifically weanling pig feed, will need to meet several criteria before they can be adopted widely. Stability and ease of use, acceptable cost of use and minimal negative repercussions associated with their applications, are all important considerations when evaluating antibiotic alternatives. In order to meet these criteria, a developed understanding of the changes that occur in association with weaning and the physiological functioning of the animal are necessary.

1.2. WEANLING PIG PHYSIOLOGY

At birth, the piglet’s immune system is immature and maternal immunoglobulins obtained through the consumption of sow’s colostrum are necessary to protect the animal from infection and disease (Vandeputte et al., 2001). When piglets are weaned, the level of circulating maternal immunoglobulins begins to deplete before endogenously secreted immunoglobulins are produced at adequate levels to adequately stave off invading microorganisms in the piglets (Miller et al., 1962). When weaning occurs at 21 days of age, the pig’s active immune system is still developing and becoming capable of producing immunoglobulins, which allows for better survival and increasingly efficient growth.

Many stressors, such as removal from the sow, mixing with new animals, a new environment with new pathogens and new feed of less-digestible plant nutrients, cause
the newly weaned pigs to experience a spike in cortisol that can last up to four weeks post weaning (Colson et al., 2012). Elevated cortisol inhibits proper immune function (Salak-Johnson et al., 2007) and decreases muscle protein deposition (LaPier, 1997). In the digestive tract, weaning is often accompanied by negative changes in intestinal morphology. These negative changes involve changes to the intestinal villi including decreased villus height, width and an increase in crypt depth, which further reduces absorptive capacity and brush-border enzyme expression (McCracken et al., 1999; Lackeyram et al., 2010). Furthermore, stress events associated with cortisol spikes are also directly related to substantial disturbances to intestinal barrier function such as increased intestinal permeability and reduction in transepithelial electrical resistance (Moeser et al., 2007). Villus atrophy has been linked to this cortisol spike as well as a decrease in feed intake that accompanies the weaning period (Beers-Schreurs et al., 1998; Fan, 2013).

Another marker of intestinal health and function that is receiving renewed interest is the expression of the alkaline phosphatase (AP) in the intestine and secretion of AP isomers into the blood. Expression of AP is enterocyte differentiation dependent and is down regulated in nutrient deprived animals (Goldberg et al., 2008). Nutritionally, intestinal AP is noted for its ability to hydrolyze nucleoside monophosphates (Carver and Walker, 1995). It also assists with enterocyte lipid transport by forming a surfactant-like molecule (Zhang et al., 1996) and helps maintain intestinal health and function by neutralizing the acidic gastric digesta by stimulating secretion of bicarbonate through hydrolyzing luminal phosphates (Akiba et al., 2007). Physiologically, intestinal AP is recognized as a maintainer of gut microbial homeostasis (Malo et al., 2010).
Furthermore, it protects against luminal pathogenic bacteria by hydrolyzing endotoxic lipopolysaccharides, which is a function unrelated to the innate or adaptive immune system (Geddes and Philpott, 2008). Because of the protective and nutritionally relevant functions of this enzyme, research has looked into its action at the time of weaning. An early study by Miller et al. (1986) found that intestinal AP activity was not affected by age or weaning in 4 to 6 wk old piglets. A more recent study showed a significant effect of early weaning, at 10 d of age, on intestinal AP digestive capacity, the total amount of the AP enzyme available, and activity as well as the affinity or efficiency of the enzyme to act on substrate (Lackeyram et al, 2010). This study explained the differences in results between their work and the early work by Miller et al. (1986) as being based on pig age at evaluation and on the sensitivity of the tests used to evaluate intestinal AP activity (Lackeyram et al., 2010).

In human medicine, the AP enzyme is often measured in the blood with changes used as an indicator of various types of disease processes. The primary source of this enzyme found in the blood is the liver or bone (Kendall et al., 1970). Little research has been done examining the changes in levels of AP in pig blood and how fluctuations can relate to gut or whole body functioning. The abundance of work on the topic in human medicine would indicate that this blood AP enzyme might be a useful early indicator of a multitude of bodily functions including hormonal shifts (Crilly et al., 1980), bone mineralization problems (Hessle et al., 2002) and cardiovascular-related illness (Abramowitz et al., 2010).

The dietary stress experienced at weaning can have a significant impact on the young animal. Sow’s milk has highly digestible casein protein, milk fat and lactose,
while weaner diets that are rich in more difficult to digest vegetable proteins and cereal starches. A shift from a diet rich in lactose sugar to a diet with less available carbohydrates can have a detrimental impact on muscle deposition, as glucose acts as a signaling molecule which is essential to the expression of amino acid (AA) transporters (Yang et al., 2008; Yang et al., 2011).

These weaning associated changes significantly impact the ability of the newly weaned pig to grow and thrive to its potential. The lag in production at this time has been noted for many years and stimulated the search for solutions, culminating with feeding antibiotics. The exact mechanisms that allow low levels of antibiotics added to the diet to be effective in improving piglet performance post weaning have not been completely elucidated but are expected to be related to gut function. Early studies have demonstrated that antibiotics do not have an effect on germ free animals (Coates et al, 1955; Coates et al., 1963). This has lead to speculation that growth promotion is obtained through decreased competition for nutrients by gut bacteria due to their severe reduction caused by antibiotics (Visek, 1978; Anderson et al., 1999). This is likely not the whole story as volatile short chain fatty acid (VFA) production is depressed proportionately to decreases in gut microbiota. VFAs are generated in the large intestine by microbial fermentation (Holtug et al., 1992). Acetate, propionate and butyrate represent 92% of the total VFAs produced in the ascending colon. Butyrate in particular has been studied and has been found to have important activities in the gut such as a trophic effect, being the primary energy source for the colonic epithelium (Scheppach, 1994). It may reduce oxidative stress through an increase in glutathione (Toden et al., 2007). Furthermore, butyrate has the ability to modulate the mucosal immune response (Segain et al., 2000) and exerts
anti-inflammatory effects (Klampfer et al., 2003). Antibiotics are rather non-selective and eliminate both harmful and helpful, such as butyrate-producing, bacteria. Despite the detriment in terms of VFA production, there is an overall boost in production due to the elimination of potentially deleterious bacteria. This evidence makes the issue of feed antibiotics complicated, as their application is expected to help growth by the elimination of gut bacteria but the VFAs that are produced in the hind-gut are required for growth. Therefore you would expect a decrease in intestinal health and growth related to antibiotic feeding with decreased hindgut fermentation and subsequent decreases in VFAs.

Theoretically, an additive that would allow the continued proliferation of helpful bacteria with a subsequent decrease in deleterious bacteria, could have great potential. By allowing the animal’s gut to stave off negative organisms through the over abundance of commensal bacteria would not only prevent the negative effects caused by harmful bacteria, but also boost production through the use of the positive attributes of helpful bacteria. This combination could hypothetically boost production to levels not seen with traditional non-therapeutic antibiotic treatments alone.

1.3. NUTRITIONAL STRATEGIES FOR MANAGING WEANED PIGS

Weanling-associated growth lag, as previously discussed, has been an issue for pig producers and therefore pig nutritionists and digestive physiologists for many years. Because of the scale and pervasive nature of the problem, many solutions have been
devised and attempted. This section reviews some of the most common and relevant methods developed.

1.3.1. Segregated Early Weaning

Early weaning is one practice that became popular to lower the cost of production through increasing the number of litters per sow per year. Weaning, typically defined as the cessation of suckling, occurs between 15 and 22 wk of age in a domestic pig that is allowed to remain with its mother un-inhibited (Jensen, 1995). This is starkly contrasted to the practice of early weaning, which began in the 1990s, when the piglet is typically removed from maternal contact between the ages of 10 and 18 d (Maxwell and Carter, 2001). This has been used with varying success rates that are largely dependent on the diligence and additional husbandry techniques used by the producer. Segregated weaning is one husbandry technique that has been employed to improve the success rates associated with early weaning. This practice associated with early weaning is known as segregated early weaning (SEW) that experienced popularity beginning in 1990. In 2000 over 4 percent of farms in the US adhered to this technique, which, because most of these farms were large, represented approximately 30 percent of the country’s pig population (NAHMS, 2000). The method was developed as a means to minimize vertical disease transfer between the sow and her offspring (Dritz et al., 1994; 1996). It was reported to improve growth by up to 10 percent, which was speculated to be a result of decreased energy partitioning for the development of immune responses to disease challenge (Dritz et al., 1994; 1996). Despite the positive outcomes associated with SEW, the practice has been met with concerns over animal welfare in relation to the piglets and the sow. Piglets have been noted to perform elevated belly and flank nosing activity to pen mates, which
is speculated to be a sign of frustration from lack of positive tactile contact with the sow (Weary et al., 1999). For the sow, the lactation period is significantly shortened. This is associated with slower return to estrus, reduced farrowing rates, and decreased subsequent litter size (Dritz et al., 1994). Furthermore, the intensive nature of the care required to maintain healthy piglets during the weaning period helps negate some of the benefits associated with SEW.

1.3.2. Creep Feeding

Creep feeding, the practice of providing diet similar in form and composition to weaning pig diet during the sucking phase, gained some popularity and is a method that can be easily added to any weaning program. It is reported to ease the transition of weaned pigs onto their new diet through acclimation of the animals to feed sources other than the sow while they are still nursing (Bruininx et al., 2002). Consumption of cereal grain starches at this time can also work to increase the intestinal expression of key enzymes such as maltase and isomaltase (Dahlqvist, 1961). Despite the potential for improved weaning pig performance, this system remains controversial to some and has been dismissed by others due to the limited to absent intake of the feed by some piglets and low impact on overall scouring rates and performance post weaning (Barnett et al., 1989). In general, the impact of creep feeding seems to be insignificant on early weaned pigs between 10 and 12 days of age (Kelly et al., 1991; Edmond et al., 1991).

1.3.3. Organic Acids

Another feeding regimen modification is acidification of the diet, primarily through adding organic acids. The weaning pig has an immature digestive system with an underdeveloped capacity to produce hydrochloric acid (Ravindran and Kornegay,
1993) and the shift in diet from a lactose rich liquid milk diet that is fermented into lactic acid to a solid diet further inhibits the creation of an ideally acidic environment (Cranwell et al., 1976). This is important because a stomach with a decreased pH is noted to have an antibacterial effect (Hansen et al., 2007). Unfortunately the stress of weaning can further complicate the situation. Often, immediately following weaning there is a marked decrease in food intake or temporary anorexia that is accompanied by an elevation in gastric pH (Coulton and Firth, 1988). Adding organic acids to the diet can help maintain an optimal gastric pH and has been shown to decrease the number of pathogenic bacteria in the stomach and small intestine (Hansen et al., 2007). Furthermore, noted improvements in feed intake and growth performance have been observed in relation to adding organic acids to the diet of weanling pigs (Biagi et al., 2006). At a microscopic level, improvements to gut morphology, specifically associated with villi structure, were noted to be associated with the feeding of organic acids (Biagi et al., 2006). The positive effects of organic acids are thought to arise in two ways. The first involves a decrease in pH, which makes the fore-gut less hospitable for pathogenic bacteria and allows penetration of gram-negative bacterial cell walls and subsequent disruption of the cellular function through enzyme denaturing and decreasing purine base integrity (Vondruskova et al., 2010). This first function is performed particularly well by organic acids with low numbers of carbon atoms such as formic acid (Partanen et al., 2001). The second function is through a physiological response to specific substances such as butyric acid, which has been well documented for its trophic effects and ability to improve fore and hindgut health (Kotunia et al., 2004; Opapeju et al., 2010). Furthermore, organic acids,
particularly in powder form, can have feed preservation effects, which can decrease feed costs by extending the stability of the diet (Pringle et al., 1983).

There have been a number of studies, examining a wide range of types of organic acids in pig feed. The results have been inconsistent and in need of additional evaluation. The variability in research results is likely related to the range in chemical and physical properties of the organic acids examined (Cherrington et al., 1991) as well as the different utilization protocols employed by researchers. There are differences in results but some indicate positive outcomes could warrant additional research be focused on this feed additive.

1.3.4. Plant Extracts

Plants and their extracts have been employed for prophylactic and therapeutic functions for years, although their applications in animal feeding industry are rather marginally in recent history. Interest in larger scale application has recently arisen as a result of the discovery of increasing numbers of antibiotic resistant bacteria. The research into these compounds has found that they can have anti-microbial properties (Deans and Ritchie, 1987; Dorman and Deans, 2000; Nascimento et al., 2000), anti-oxidative properties that are associated with anti-inflammatory effects (Benavente-Garcia and Castillo, 2008) and anti-parasitic properties (Magi et al., 2006). Furthermore, some results indicate a prophylactic effect against a series of enteric diseases (Liu et al., 2010). This is particularly interesting when compared with data showing that essential oils selectively reduce the proliferation of coliform bacteria (Namkung et al., 2004) and changes in fecal bacterial profiles with fewer pathogenic bacteria (Li et al., 2012) and.
In vitro studies have shown antibacterial properties for various plants including grape leaf and ginger and it is suspected that this is, in part, related to the polyphenols content (Hara-Kudo et al., 2004). Plant extracts with their potential for direct impact on diarrhea prevention, specifically multi-drug resistant E. coli originated diarrhea, include acacia nilotica, syzygium aromaticum and cinnamomum zeylanicum (Khan et al., 2009). Unfortunately study results are often contradictory or inconclusive, with many also reporting no observed impacts. This may be a result of the highly variable nature of the products. The plants are grown in diverse climates with variable harvesting practices that are likely responsible for the range in treatment effects. In order to properly assess the function of these additives, the growth, harvesting and preparation of these plant extract products needs to be well documented and standardized (Li et al., 2012).

1.3.5. Protein

It is recognized that urinary nitrogen (N) loss increases substantially from the suckling to the weaning phase in pigs (Fan et al., 2006). During suckling, N loss has been measured to be at about 9% but once in the post-weaning phase it can reach levels of around 40% in pigs fed corn and soybean meal (SBM) based diets (Fan et al., 2006). This is significant when considering the amount of unused AA associated with high levels of N loss.

Post-weaning diets that are formulated to meet the requirements laid out by NRC (1998) have higher levels of plant protein due to the need to meet essential AA requirements when feeding plant protein with less than ideal AA profiles and lower ileal digestibility. Unfortunately gut microbes digest the excessive AA and the excessive N is excreted primarily in the form of urea. Various studies have shown that several
potentially pathogenic bacteria predominately ferment protein (Le et al., 2005; Bauer et al., 2006). Diets formulated with decreased levels of crude protein (CP) were able to show a decrease in levels of *Clostridium perfringens* and a decrease in *Escherichia coli* and their endotoxins (Bauer et al., 2006). It is also important to note that type, ranging from soybean products to bovine serum albumin, and processing, such as fermentation and enzymatic treatment, of the protein source impact post-weaning health (Pluske et al., 2002).

SBM has been noted to be associated with an increase in diarrhea incidences (Li et al., 1991) and is suspected to be related to increased incidences of enterotoxigenic *Escherichia coli* (ETEC) infection due to less than ideal functioning of the intestines (Fairbrother et al., 2005). Also, feeding SBM based diets has been associated with lower rates of gain, decreased intestinal villi height and increased immunoglobulin levels in comparison with feeding milk protein-based diets (Li et al., 1990). Conversely, extrusion (Woodworth et al., 2001) or the combination of grains with exogenous enzymes (Owusu-Asiedu et al., 2002) was able to improve performance of plant protein-sourced diets in terms of digestibility when compared with less refined plant protein-based diets.

Generally, a decrease in dietary (plant) CP post-weaned pigs causes a decrease in protein fermentation and improved fecal consistency (Nyachoti et al., 2006). Furthermore, a decrease in total dietary (plant) protein has been associated with a decrease in intestinal inflammation (Opapeju et al., 2010). The primary concern associated with feeding a lower protein diet post-weaning is related to evidence showing compromised growth rates (Nyachoti et al., 2006; Wellock et al., 2006). This is a controversial topic with additional research showing that decreased dietary protein has no
significant impact on growth (Heo et al., 2008; Lordelo et al., 2008). These contradictory results may be associated with the protein sources used in individual trials or the processing techniques used on the protein sources, as discussed previously.

More work into elucidating required treatments for protein sources in order to allow the most favourable AA profile available will be vital to creating diets that consistently decrease the incidences of post-weaning diarrhea while maintaining growth. Therefore, interest in formulating lower CP diets with added crystalline limiting essential essential AA can be viewed as a method to reduce post-weaning diarrhea but formulation must be carefully controlled to avoid growth lag. Additional research into determining specific formulations with sources of protein and additional crystalline AA would be useful to make this technique more efficient for commercial application.

1.3.6. Minerals and Clays

There has been some interest in adding natural mineral compounds like zinc oxide (ZnO) to animal diets and also interest in adding clay supplements such as bentonite, zeolite or kaolinite. When zinc (Zn) is deficient, this can cause growth retardation and a decrease in tissue enzyme activity (Prasad et al., 1969; Prasad and Oberleas, 1971). Furthermore, and perhaps more nutritionally relevant, Zn has roles in the functioning of lipid and carbohydrate metabolism (Reeves and O’Dell, 1983). The NRC for Swine (1998) recommended 100 mg/kg diet required by a 5-10 kg weaned pig and further recommended that a pharmacological level of Zn up to 3000 mg/kg could be given with antimicrobial effects including decreasing post-weaning diarrhea and improving growth performance (Li et al., 2006; Castillo et al., 2008). The exact mode of action for this activity is not yet known. Increased gene expression of antimicrobial peptides in the
small intestine (Wang et al., 2004), improved stability of the microbiota (Katouli et al., 1999) and reduction in the electrolyte secretion from enterocytes (Carlson et al., 2006) are likely contributing factors. Unfortunately dietary supplementation of pharmacological levels of Cu and Zn, have been linked to antimicrobial resistance (Fard et al., 2011). This makes supplementation with these minerals a poor strategy for substitutes of antimicrobials as it has been demonstrated that pharmacological levels of Cu and Zn contribute to the development of these trace mineral specific antibiotic resistant bacteria in swine populations (Amachawadi et al., 2010; Cavaco et al., 2011; Fan, 2013).

There is also evidence for the positive effects of adding multi-mineral containing sources such as clays. Clay consumption has been practiced for hundreds of years by humans and animals (Williams and Haydel, 2010). Wild animals have been documented to seek out and consume clays, assumedly to alleviate various gastrointestinal or disease related symptoms (Johns and Duquette, 1991). Clay minerals are formed by a net of tetrahedral and octahedral layers which contain silicon, aluminum and oxygen molecules. These layers can be connected by hydrogen bonds or cations and these connections make up a component of the clay referred to as the interlayer. Classification of clays occurs according to the type of layers, interlayer contents, charge of the layers and the chemical formula. Naturally extracted clays are a mixture of various clay minerals (Vondruskova et al., 2010). Because of their porous structure, clays have a sorptive capacity, which allows them to draw in other substances, and this occurs as a result of the exchange of the already present metals cations such as Na\(^+\) or Ca\(^{2+}\) for other, potentially more harmful metal cations (Vondruskova et al., 2010). Once clay has taken up these other cations into
the interlayer they are excreted from the host’s system. This is aided by the ability of clay to expedite bowel evacuation by acting as a bulking agent (Vondruskova et al., 2010). This can also reduce incidence of post-weaning diarrhea through the movement of water into the interlayer space of clay, which causes mineral expansion. This expansion slows and regulates the passage rate of the digesta, allowing the body to pick up more water, which further compacts the feces (Vondruskova et al., 2010). In addition to the potential of clay for improving or maintaining weanling pig gut health is attributed to the capacity of clay to bind aflotoxins (Jaynes and Zartman, 2011), plant metabolites (Dominy et al., 2004), heavy metals (Hassen et al., 2003) and toxins (Knezevic and Tadic, 1994). In vivo studies in pigs and in vitro studies have shown that clays can absorb bacteria and endotoxins (Hu and Xia, 2006; Trcko et al., 2009). Supplementation with clays is recommended at levels of 1 to 3% (Vondruskova et al., 2010) but higher levels are readily received with no negative impact on growth (Castro and Iglesia, 1989) and are related to improved body weight gains and feed conversion (Trcko et al., 2009). The question of the exact amounts and types of clays added to weaning pig diets that can reduce diarrhea is still under consideration and needs additional research. Unfortunately, despite the positive effects associated with feeding clay, a feeding trial evaluating many different types of clay to E. coli challenged pigs showed no positive effects on growth performance (Song et al., 2012).

1.3.7. Probiotics and Synbiotics

Probiotics are described as live microbes that can impact host health and are stable enough to withstand the gastric pH and enzymes in the foregut in order reach the distal ileum, the caecum and the ascending colon where their primary activity is carried
out (Roselli et al., 2005). The most common types of bacteria used as probiotic include *Bifidobacterium* and *Lactobacillus* species (Williams, 2010). They are expected to act through competing with and subsequently decreasing numbers of harmful bacteria as well as producing higher levels of positive VFAs such as butyrate (Fuller, 1989; Bomba et al., 2002). Pathogenic bacteria are further inhibited by the ability of the probiotics to produce organic acids and antibacterials that work to destroy or prevent the growth of many pathogenic bacteria (Bomba et al., 2002; Marinho et al., 2007). They also increase nutrient availability for the host through the action of bacterial enzymes on components of feed that is indigestible to animal enzymes (Bomba et al., 2002). The probiotics influence the host’s immune system through increasing immunoglobulin production (Perdigon et al., 1995) and up-regulating macrophage, natural killer (NK) cells (Chiang et al., 2000; Matsuzaki and Chin, 2000) and pro- or anti-inflammatory cytokine production (Shu et al., 2001; Roselli et al., 2005). The effects of these microbiota associated with probiotic feeding are hard to specifically enumerate because they are dependent on other factors such as the dosage, the combination with any pharmaceuticals, the type of feed being given to the animal and the storage conditions prior to feeding (Kyriakis et al., 1999). There are numerous combinations of bacteria that have been administered in an effort to determine which provide the most benefit to the host. *Lactobacillus* and *Bifidobacterium* have been combined (Bomba et al., 2002). *Enterococcus* and *Streptococcus* and *Enterococcus faecium* are administered and found to prevent the K88 strain of ETEC from adhering to the intestinal mucous membrane of piglets (Bomba et al., 2002; Scharek et al., 2005). Furthermore, several treatments of probiotics have been shown to increase pig growth performance while decreasing morbidity and mortality
(Bombas et al., 2002). Unfortunately there are also data that report no effects and therefore results are quite inconsistent. Some possible reasons for the differences and discrepancies may be related to low and varying dosages of probiotics, interactions with other factors and the health status as well as the genetics of the host (Vondruskova et al., 2010). It is worth noting that it is speculated that the best outcome arrives when the supplement is given shortly after birth or to the late prepartum sow (Martin et al., 2009, Vondruskova et al., 2010).

Despite the many positive implications associated with probiotics alone, the discussion would not be complete without the consideration of combining prebiotics and probiotics. The prebiotics, generally are defined as a fermentable fiber source and will be discussed in detail at the end of this chapter, come in many forms and can be combined with a variety or commensal probiotic bacteria. Administration of these mixes has shown to decrease mortality of weaned pigs (Abe et al., 1995). They can also boost VFA production and decrease the production of noxious gas from fecal sources (Roselli et al., 2005). Different combinations of bacteria and prebiotics have been tried with some very positive results. Despite the many positive results observed, there is still controversy over whether the combination causes a synergistic or additive effect. The study by Piva et al. (2005) shown the synergistic impact of probiotics and prebiotics. Chapman et al. (2011) were able to show an additive effect of inulin and Bifidobacterium longum. It is speculative that the exact impact of synbiotics may be impossible to predict accurately and reliably due to the highly diverse nature of the pigs available in terms of genetics, environment, feed and gut bacterial profiles.
1.4. ROLE OF LACTOSE

Lactose is a disaccharide sugar that is composed of D-galactose and D-glucose units with a $\beta-1\rightarrow4$ glycosidic linkage. It is primarily found in milk and at variable levels depending on the species. The secretion of the enzyme lactase on the enterocytic apical membrane in the intestinal villi allows lactose to be broken down into the galactose and glucose subunits. Glucose and galactose are then readily absorbed by the epithelial cells and further metabolized for growth. In the weanling pig, lactose is an ideal source of glucose and its supplementation has been shown to increase weanling pig performance (Mahan and Newton, 1993). Other simple sugars such as sucrose have been examined and found to cause performance similar to weaned pigs supplemented with lactose (Mavromichalis et al., 2001) but later research by Burrin et al. (2003) showed that sucrose is not an effective replacement for lactose. This is likely related to lower expression of sucrase in the weanling pig compared with lactase.

There has been some recent discussion of the potential prebiotic effect of lactose (Szilagyi, 2004). Lactose has long been accepted for its importance as a growth promotant in weaning pigs and for its ability to spare protein and improve the efficiency of whole body N retention (Mahan and Newton, 1993; Cromwell et al., 2008). Supplementation of early post-weaned pig diets with other alternatives, such as cornstarch, were shown to be inferior to lactose (Mahan and Newton, 1993). Early post-weaned pigs fed gradient increases in lactose improved performance accordingly (Nessmith et al., 1997). Interestingly, improvements to the *Lactobacillus* levels in the ileum and cecum of 38-day-old pigs were not seen in association with 40% lactose
supplementation for 10 days (Krause et al., 1997). This would seem to indicate that the growth-promotion effect of lactose is not related to hindgut beneficial bacterial stimulation or its potential prebiotic effects in the weanling pig.

Deeper consideration of the structure and properties of lactose in comparison with the updated description of a prebiotic given by Gibson et al. (2004) should be able to more fully address this issue. The first characteristic of a prebiotic is that it should be capable of resisting gastric acidity and hydrolysis by the host enzymes and subsequent gastrointestinal absorption. The remaining characteristics are related to the ability of the feed ingredient to selectively stimulate gut bacteria and the growth of these specific bacteria resulting in positive effects on the host. This criteria therefore must be evaluated in association with age and lactose supplementation level. If lactose is provided at low enough levels at 8 wks to not overwhelm the small intestinal lactase digestive capacity then lactose will have no prebiotic effect.

The answer to the first criterion will, in part, be dependent on the species and age considered. In pigs, after the cessation of suckling, the expression of lactase activity begins to decrease with pigs having lactase activity decreased by 50% at 4 weeks of age and down to only 25% of it’s full expression potential by 8 weeks of age even when lactose content is sustained in the diet (Kelly et al., 1991). This would indicate that at least some lactose is subjected to enzymatic digestion by the mammalian enzyme. Furthermore, in the human population, there is a large segment that maintains the ability to easily digest lactose because of mutation that allows higher levels of lactase expression later in life (Troelsen, 2005). It is not unreasonable to suspect that this mutation could be found in some pigs with at least a few animals maintaining high lactase expression
throughout their lifetime. Furthermore, Lackeyram (2010) showed that the whole intestinal lactase digestive capacity remained significantly large in the early-weaned pig. These literature reports would negate the speculation of dietary supplemental lactose as a prebiotic for weanling pigs.

For the purposes of this dissertation research that is aimed at evaluating prebiotics in weanling pigs, lactose can be definitely removed from contention because the intestinal expression of lactase is much higher in the young, newly weaned pig (Kelly et al., 1991). Despite this, lactose can be considered against the next criteria laid out by Gibson et al. (2004), because some luminal lactose may escape the host enzymatic digestion in the foregut and therefore have some prebiotic like effects on the hind gut microflora. In 2004, after the publication of Gibson et al. (2004) paper that further revised the definition of a prebiotic, Szilagyi (2002) wrote a paper explaining how lactose could be classified as a prebiotic in lactase nonpersistent subjects.

The last two prebiotic criteria include fermentation by intestinal microflora and selective stimulation for specific bacteria with positive host effects that can be considered together. In general, lactose that escapes enzymatic digestion in the foregut will increase the water content of the stool and reduce transit time (Schaafsma, 2008). Furthermore, lactose that escapes enzymatic digestion in the foregut is fermented and selectively stimulates the growth of *Bifidobacterium* and *Lactobacillus* (Gomes and Malcata, 1999; Schaafsma, 2008). The positive effects of *Bifidobacterium* and *Lactobacillus* on host health have been accepted for a long time. These bacterial species are described as commensal bacteria and are frequently used in probiotic preparations and to treat various
conditions such as irritable bowel syndrome in humans and necrotizing enterocolitis in pigs (O’Mahony et al., 2005; Siggers et al., 2008).

This evidence would seem to indicate that lactose has a prebiotic effect once it escapes digestion by the small intestinal β-galactosidase (lactase) but more recent research may refute this claim. Research into the effect of lactose on *Bifidobacterium* growth stimulation has been largely related to human infant health and the impact formula feeding can have when chosen over breast milk. Original research suggested that the level of oligosaccharides, specifically lactose, were a primary determinate for the amount of *Bifidobacterium* in an infant’s large intestine, which was used as an indication of gut health (Westerbeek et al., 2011). More recent research is linking other components of breast milk such as lactoferrin, lysozyme, lactoperoxidase, immunoglobulins, and nucleotides that are related to *Bifidobacterium* levels in spite of lactose content (Venema, 2011). Despite this controversy, because of the high level of intestinal lactase activity available in piglets for up to 8 weeks of age, it is reasonable to expect that the prebiotic impact of dietary supplemental lactose on the freshly weaned, 3 wk old pig may be negligible.

In order to create a complete picture of lactose digestion in the pig, the activity of the foregut bacteria should be considered. Gastric and jejunal microbiota are not often considered due to the low levels relative to ileal and cecal microbiota. Despite their relative minimal abundance, consideration should be given for their impact on health. Early work has shown that *lactobacillus* can survive the gastric environment (Uonsson et al. 1985) and that lactic acid producing bacteria are acid tolerant and able to bind to microvillus surface of the proximal gastrointestinal tract (Camp et al., 2009). This would
point to the ability of these microbes to cause impact on health through their functioning in the foregut.

1.5. ROLES OF β-GLUCAN

In order to properly assess the role of β-glucan as a potential prebiotic it must be held up to the standards laid out by Gibson et al. (2004). In order to do this an accurate understanding of β-glucan must be developed. β-glucans are long chains of D-glucose monomers called polysaccharides and β-glycosidic bonds link these glucose monomers. β-glucans differ greatly in terms of molecular mass, solubility and viscosity. They can occur in the bran of cereal grains such as barely and oats or the cell wall of baker’s yeast and in certain fungi, mushrooms and bacteria. β-glucans are often found in cell walls and function to maintain the rigidity and shape of the cell (Sandula et al., 1995). It is recognized that mushrooms and yeast contain mainly (1-3)(1-6)-β-glucans, while cereals contain (1-3)(1-4)-β-glucans (Mantovani et al., 2008). The solubility of β-glucans are directly impacted by the degree of polymerization with highly branched (1-6)-β-glucans having the greatest solubility (Zekovic et al., 2005). The β-glucan used in this thesis is of oat origin with β-1-3 and 1-4 bonds. The desire to label these molecules as prebiotics arises from their highly fermentable nature, their ability to increase the viscosity of digesta in humans with positive effects (Dikeman et al., 2006) and their ability to promote the growth of Lactobacillus and Bifidobacterium in vitro when added at 0.5% (Jaskari et al., 1998) and in vivo at a rate of 5 MJ /kg (O’Connell et al., 2005).
It has been accepted that β-glucans can be described as a functional food at levels of 3 g β-glucan/d because of their cholesterol-lowering effects (FDA, 2008). Furthermore, they have the ability for immune modulation via the innate immune receptor Dectin-1 (Willment et al., 2001) that is expressed on gut-associated immune system like intraepithelial lymphocytes, neutrophils, macrophages and dendritic cells (Goodridge et al., 2009, Drummond and Brown, 2011; Esteban et al., 2011). Despite this, the description as a prebiotic still remains controversial. When weaning pigs were supplemented with yeast derived β-glucan at 0.01% to 0.1%, likely with β-1,3 and 1,6 branching (not specified), they did not show any improvements in growth performance or health status (Dritz et al., 1995; Hahn et al., 2006).

The first consideration to determine if β-glucans can be classified as a prebiotic is their ability to resist gastric acidity, host mammalian enzyme hydrolysis and gastrointestinal absorption (Gibson et al., 2004). Microbial β-glucanases are capable of breaking down β-glucans. There are a variety of forms of β-glucanase, which act on the specific bonds in the diverse forms of β-glucans, and it has been suggested that these enzymes are not secreted by monogastric animals (Zhang et al., 1997). Unfortunately this is not a clear answer because high ileal digestibility of β-glucan has been demonstrated by Wehlziehn et al. (1990). These data are not enough to indicate that β-glucans meet the first prebiotic designation criterion.

The remaining criteria can once again be considered together. The ability of the substrate to be fermented by intestinal microflora and cause selective growth of bacteria, which cause positive host effects, is the final requirements for a prebiotic. In this area, the evidence for β-glucans becomes more convoluted. Some studies have suggested that
cereal grains (Brennan and Cleary, 2005) and mushrooms or yeast (Tzianabos, 2000) β-glucans have prebiotic-like effects. There is also a substantial amount of data indicating that β-glucans do not have the ability to selectively stimulate specific bacteria. These studies have shown a variety of bacteria including *Bifidobacterium* and *Lactobacillus* (Jaskari et al., 1998; Metzler-Zebeli et al., 2011), *Clostridium histolyticum* (Hughes et al., 2008) and *Enterobacter* (Murphy et al., 2012) showing increases in growth in response to the intake of β-glucans, which falls outside of the typical bacterial genera correlated with prebiotics. An interesting note to the diversity seen in altered bacterial profiles in response to β-glucan treatment is related to the findings by Murphy et al. (2012) showing that oat derived β-glucans alone shifted the bacterial profile towards *Enterobacter* while this did not occur when a supplement of cereal β-glucan with an β-glucanase additive was provided to the pigs. It is a direct result of this type of conflicting *in vivo* evidence that disallows the term prebiotic from being applied to β-glucans.

The different types of β-glucans and their various characteristics may be responsible for the inconsistency in the results associated with β-glucan feeding trials. A major challenge associated with elucidating the exact effect of these molecules is not only related to the diversity in structure but also related to our ability to develop pure, controlled samples for experimental and subsequent commercial application (Tzianbos, 2000). For the time being, the immune-modulating (Estrada et al., 1997), cholesterol-lowering through absorption inhibition (Naumann et al., 2006) and anti-carcinogenic effects (Salminen et al., 1998) of β-glucans can easily place this group of carbohydrate in the category of functional foods but further study is needed to determine, which, if not all, forms of β-glucan can be classified as prebiotics.
1.6. PREBIOTICS

Prebiotics are host indigestible food ingredients that selectively stimulate the growth of one or a limited number of specific bacterial species that cause changes in the gastrointestinal tract, which positively impacts the animal (Gibson et al., 2004). The recent interest in prebiotics extends from health application in human nutrition to fish feed but has received a significant amount of attention as a prophylactic substrate for farm animal feed. This is particularly the case in light of the recent growth in incidence of antibiotic-resistant bacteria and subsequent public outcry against feeding of non-therapeutic levels of antibiotics that are expected to contribute to the development of antibiotic-resistant bacteria.

Defining a prebiotic has become an interesting and somewhat complicated task. In 1994 when the topic of prebiotics was originally becoming of increasing interest, Gibson and Roberfroid wrote what is now a very seminal paper in which they created a clear set of criteria that describe a prebiotic. In 2004 they updated their criteria in a new paper that examined several common food ingredients and labeled them as prebiotics, not prebiotics or certain ones in need of more research.

Several of the existing prebiotics have been classified by Mitchell (2006) and are outlined below. Fructooligosaccharides (FOS), are D-fructose polymers that can be produced by the degradation of inulin (polyfructose). FOS can be synthesized by disaccharide sucrose or processed from inulin derived from the chicory root. FOS and inulin, which will be discussed in more detail in the next section, can be defined as
prebiotics because of their ability to stimulate the growth of health-promoting bacteria such as the lactic acid producing bacteria, *Lactobacilli* and *Bifidobacterium*, in the hind-gut (Kaplan and Hutkins, 2000; Bird et al., 2009).

Galactooligosaccharides (GOS) are made up of either mono or polysaccharides, usually D-galactose units that are formed via transgalactosylation by lactase on lactose with the final structure being dependent on the source of the lactase enzyme (Chen et al., 2003). These carbohydrates can be described as prebiotics based on their ability to reduce adherence and proliferation of harmful gut bacteria (Tzortzis et al., 2005; Silk et al., 2008). They also increase the proliferation of commensal bacteria such as *Bifidobacterium* in the colon (Tzortzis et al., 2005).

Polydextrose is a synthetic soluble fiber that is produced from dextrose and sorbitol. It is a molecule containing randomly polymerized branched chains with various types of glycosidic bonds that cannot be hydrolyzed by human enzymes (Raninen et al., 2011). The evidence for the prebiotic effects of this molecule is related not only to its ability to avoid digestion by animal enzymes but also because of its capacity to promote beneficial gut changes by impacting bacterial proliferation (Fava et al., 2007). Furthermore, not all polydextrose is fermented by hind-gut bacteria and a portion of this carbohydrate escapes microbial fermentation and is excreted in the feces (Stowell, 2009).

Lactulose is another synthetic food additive comprised of galactose and fructose that is fully broken down by microbes (Mitchell, 2006). It is commonly used in the treatment of constipation and works through osmotic action, drawing water into the bowel and resulting in a cathartic effect. Its classification as a prebiotic is related to its
ability to offer a protective effect to bowels by altering the bacterial profile (Tuohy et al., 2001; Rumi et al., 2004).

Xylo-oligosaccharides (XOS), soya-oligosaccharides (SOS), lactitol and isomalto-oligosaccharides (IMO) have been classified as emerging prebiotics by Mitchell (2006). Despite the time that has elapsed since Mitchell (2006) published these classifications, little additional research has been done that can firmly label these substances as prebiotics notwithstanding the presence of some convincing research (Anderson et al., 1999; Rycroft et al., 2001; Piva et al., 2005; Aachary and Prapulla, 2011). With further study into the specific repercussions associated with the application of these substances in monogastic animal diets, it is anticipated that they can be fully classified as prebiotics.

1.6.1. Resistant Starch

Resistant starch is a group of carbohydrates containing high amylose starch and its degradation products that escape major digestion in the foregut and then is fermented by hindgut bacteria to produce VFA's and healthful effects on the host through the proliferation of selective microorganisms. Englyst et al. (1982) reported the separation of a starch that is resistant to α-amylase and referred to it as “resistant starch.” Englyst and Hudson (1996) set a classification for the different types of resistant starch, ranging from resistant starch produced by heat to resistant starch produced by enzymatic processing.

Since the early 1980’s, resistant starch has received some interest for its ability to modulate the gut by causing the proliferation of healthy bacteria. Resistant starch, applied at a rate of 10% of the fermentation media, was able to increase the levels of Bifidobacterium and Atopobium and alter the production of VFA production in favour of increasing butyrate (Lesmes et al., 2008). This effect of increased butyrate was also
noted in a study examining consumption of stale maize porridge that showed increased levels of resistant starch compared with fresh, with subsequent health preserving effects on consumers (Ahmed et al., 2000).

Unfortunately the amount of research in pigs is limited. There is some data that have examined feeding pigs resistant starch in order to extrapolate information to humans. In many of these studies the pigs were given diets similar to what a human would be consuming, plus the resistant starch. Many of these, including Brown et al. (1997) showed the prebiotic ability of high amylose resistant starch to increase *Bifidobacterium*, but in these studies high amylose resistant starch was often supplemented at very high levels (50% of the diet). There was also evidence indicating that not all resistant starch products have the same positive impact, with resistant starch containing rice fed to baby pigs showing no impact on gut health parameters (Topping et al., 2003).

Dietary inclusion levels of resistant starch should be viewed in a similar manner to the approach described for inulin. The diverse nature of individual’s bacterial profile in their foregut and hindgut makes reliable prediction of the impact that a specific dose of resistant starch has very difficult at this stage of research. Evidence for this is found with the variations in resistant starch excretion levels in human feeding trials, indicating that individual’s bacterial flora treated the same dose of resistant starch responded differently (Hylla et al., 1998).

**1.6.2. Fibersol-2**

Fibersol-2 is a commercial prebiotic supplement that was developed and is marketed by Matsutani Chemical Industry. Because of the proprietary nature of the
product, data on this additive are not readily available in the literature. This carbohydrate is produced from cornstarch processing and is a type of soluble resistant starch. It is maltodextrin that is modified to resist digestion through the elimination of α-1-4 linkages in favour of β-1,2- and 1,3- linkages that are not cleaved by mammalian enzymes (REF).

Rat model studies with this additive have confirmed a protective effect through the up-regulation of protective bacteria and epithelial defense mechanisms but the levels of the supplement given are very high, up to 62.5% of the diet (Rodriguez-Cabezas et al., 2010). The evidence in pigs for the prebiotic impact of this additive seems to be lacking. Additional research is likely needed, or requires dissemination for any accurate assessment of its potential for commercial application in swine feeding.

1.6.3. Inulin

As previously described, inulin is made of D-fructose polymers. The degree of polymerization (which defines the number of fructosyl monomers) varies from 2 to approximately 60 units (Gibson and Roberrroid, 2008). These linear fructans consist primarily of β-(1-2) fructosyl-fructose linkages (Roberfroid, 2005). The main source of inulin or inulin-type fructans is the chicory root. Because of β-conformation of the anomeric C₂, inulin is able to resist hydrolysis by mammalian enzymes due to their specificity for α-glycosidic bonds (Roberfroid, 2005). Inulin-type fructose with prebiotic function can also be obtained by enzymatic hydrolysis with Aspergillus niger’s β-fructosidase (Gibson and Roberfroid, 2008). Furthermore, inulin-type fructose can be produced by mixing oligofructose and long-chain inulin, which can be a by-product of the food industry when specific separation techniques are applied. All these products
have differing degrees of polymerization but quite similar biological activities (Gibson and Roberfroid, 2008).

In general, after escaping digestion by mammalian enzymes in the upper intestinal tract, inulin and FOS are fermented and selectively stimulate the growth of specific bacteria to the benefit of the host. They are well known to stimulate the growth of *Bifidobacterium* in rats and humans, but with less consistency in pigs (Loh et al., 2006). Inulin-type fructans have also been shown to elevate *lactobacilli*, although to a lesser extent than *Bifidobacterium* (Roberfroid, 2006) and possibly other bacterial species that are known butyrate producers such as *Clostridium coccoides* and *Eubacterium rectale* (Kleessen et al., 2001). Interestingly, the degree of inulin digestion by pig enzymes is under some debate. Loh et al. (2006) reported that 20 to 50% of inulin-type fructan was shown to be degraded by the time it reached the jejunum and interestingly Houdijk et al. (1998) reported that just under 90% of the inulin-type fructan was digested before it reached the cecum. This information is in direct opposition to results indicating under a 5% loss of inulin-type fructans are digested before reaching the cecum (Yasuda et al., 2007). The reason for this may be related to the sampling methodology employed in which some investigators only sampled from one location in the cecum resulting in a non-representative sample.

Another important area of consideration, particularly for production animal application, is the dose, or level of inclusion in the diet. There is a large amount of research showing the effect of large differences in levels of inulin-type fructans. Farnworth et al. (1992) reported levels as low as 1.5% having a positive effect on digestion. Tako et al. (2008) reported levels as high as 4% having a positive effects on
gene expression. The reason for the similar responses to differing dose levels is described by Robefroid (2005). This description looks primarily at human studies, but because data are more limited in pigs, it can be extrapolated to treatment of the pig. Studies that compared patients with similar levels of Bifidobacterium at the start were unable to find a correlation in effects of treatments with inulin (Bouhnik et al., 1999). Furthermore, meta analysis of populations dosed with varying levels of inulin saw no correlation in Bifidobacterium level changes (Roberfroid et al., 1998; Rao, 2001; Rycroft et al., 2001). The reason for this, as described by Roberfroid (2005), is related to the highly variable population of microbes that exists in the gut. With the extensive level of diversity that exists between different individuals gut bacterial populations it becomes very difficult to assign a generalized volume of prebiotic, and specifically inulin-type fructan to take in order to have a specific positive impact.

1.6.4. Concluding Notes

An important observation associated with the application of most prebiotics is that upon cessation of their ingestion, the changes seen in bacterial profiles revert to prior states (Gibson and Robefroid, 1995; Roberfroid, 2005). Depriving the host of the specific prebiotic results in a progressive decrease to disappearance of the elevated populations of health-promoting bacteria (Roberfroid, 2005). This should be a consideration as to cost is always a factor in feeding production animals. This evidence indicates that the application of prebiotics in pig diets would need to continue for the duration of time that the prophylactic impact is expected to be required. While this is not likely considered unusual, specifically in light of the fact that current practices that involve antibiotic applications require continual application, it may become a more
interesting piece of information as prebiotics that are more effective and more predictable are developed. Adding a probiotics and prebiotic mix right after birth to a malleable digestive system may be an effective way to cause positive changes to the digestive tract.

1.7. RESEARCH HYPOTHESES AND OBJECTIVES

The potential for prebiotics and β-glucans to modulate the gut microbiota and impart a positive impact on the host is large. The large amount of research on this topic has opened many avenues that need exploring. With the use of antibiotic growth promoters in pigs, and particularly, weaning pigs becoming more of a concern, looking into alternatives such as prebiotics is a highly desirable pursuit. Unfortunately the line between acceptable cost and functionality is a difficult one to tread. The best method to determine this precise ratio is with continual trials and the collection of a large amount of data. It is with these considerations that the following hypotheses and research objectives were developed.

Research Hypotheses
In relation to the following stated objective, this thesis aimed to investigate the following hypotheses:
1.) Retrograded resistant cornstarch,fibersol-2, inulin and β-glucan supplementation at 0.75% will increase growth performance traits in weaning pigs in comparison with corresponding measurements from pigs fed the control diet containing no growth-promoting antibiotics.
2.) Retrograded resistant cornstarch, fibersol-2, inulin and β-glucan supplementation at 0.75% will improve fecal scores in weaning pigs in comparison with these endpoints from pigs fed the control diet containing no growth-promoting antibiotics.

3.) Retrograded resistant cornstarch, fibersol-2, inulin and β-glucan supplementation at 0.75% will decrease AP kinetics in proximal small intestinal and serum samples in comparison to the corresponding measurements from pigs fed the control diet containing no growth promoting antibiotics.

4.) Retrograded resistant cornstarch, fibersol-2, inulin and β-glucan supplementation at 0.75% will improve volatile compound profiles in weanling pigs with decreases in odor-causing molecules such as indole and skatole in comparison with these endpoints from pigs fed the control diet containing no growth-promoting antibiotics.

**Research Objectives**

This thesis research aimed to investigate the effects of four diet additives of retrograded resistant cornstarch, fibersol-2, inulin and β-glucan on the following responses in the weanling pigs fed corn and soybean meal (SBM)-based diets:

1.) changes in weaning pig growth performance traits including initial and final body weights, average daily gain (ADG), average daily feed intake and gain to feed ratio;

2.) changes in fecal scores in weaning pigs in responses to the dietary additives;

3.) changes in AP kinetics in the proximal jejunal and serum samples of pigs fed these additives compared with the prebiotics; and

4.) changes in volatile compound concentrations in freshly collected cecal and fecal samples at wk two and three of weanling pigs fed the dietary additives.
As a final note, an inclusion level of 0.75% was chosen for each additive based on past research and the desire to identify an economically viable option for producers. Because the main goal was to develop useful alternative additives for producers, growth performance was one of our primary area of considerations when evaluating other studies to determine an appropriate inclusion level. Retrograded resistant cornstarch has limited literature data on changes to growth performance. As this was the case for several additives, results indicating a positive host response were considered. When resistant starch was fed up to 50% of the diet it showed positive impacts on bacterial profiles (Lesmes et al., 2008). But feeding 50% of resistant starch in diets for pigs is not relevant to industry practices. Lower levels, at 0.16% of the diet, showed improvements in swine dysentery rates (Durmic et al., 2002). It was hoped that increasing the dietary level to 0.75% would be enough to impart positive growth performance responses.

Similarly, there were little data on growth performance in animals supplemented with β-glucan. Weanling pigs supplemented with 8.95% β-glucan showed changes to gene expression in the cecum (Metzler-Zebeli et al., 2012). Pigs challenged with Salmonella Typhimurium had reduced shedding when fed 3.75% β-glucan (Pieper et al., 2012). Both these studies used relatively high levels of inclusion, but interestingly, an early study by Hahn et al. (2006) showed that β-glucan supplemented in weanling pigs at 0.02% showed no positive effects on growth performance endpoints.

Since fibersol-2 is an additive with limited available data, due to its proprietary status, it was more difficult to find evidence to factor in when deciding on an inclusion level. One study in rats used a 5% inclusion level but saw no effects on growth performance (Kishimoto et al., 2009) but because of species and basal diet differences
these results seemed to offer little significance to our study. This lack of data led us to create an inclusion level based on what levels were suspected to be most efficient for the other substrates.

Inulin was the substrate where the majority of the data was found. Changes in *bifidobacteria* were noted in pigs supplemented with 3% inulin (Loh et al., 2006). An experiment by Muhl and Liebert (2007) found that an inulin containing supplement added between 0.05% and 0.15% was not able to elicit a growth performance response in the pig. Conversely, increasing inulin supplementation up 0.08% in weaned pigs challenged with *B. hyodysenteriae* was able to alleviate the overall impact of the disease in a linear fashion (Hansen et al., 2011). Despite the lack of data in showing a significant impact on growth performance, there are ample data indicating that inulin is able to alter bacterial populations at levels below 0.5% of the diet. By including inulin at 0.75% of the diet we hoped to not only elicit positive changes in the gut but also have those translate into growth performance improvements.

In general, the research results that exist on our chosen additives and prebiotics in general are rather inconsistent and less conclusive. Often the exact type and sources of the additives is difficult to determine, or not possible. Furthermore, there is research that looks at the impacts of the addition of certain foodstuffs to a diet, with the specific foodstuffs containing unknown quantities of potentially beneficial carbohydrates. The researchers often attribute positive experimental outcomes to the anomalous compounds in the foodstuffs but because it has not been separated and added at a control level, these findings become questionable sources of information. Because of this, not only did we attempt to choose levels that might elicit positive growth, nutritional and physiological
responses but we also hoped to provide clear results associated with feeding these additives at well-defined supplementation levels to create a clear path for future research.
CHAPTER 2

THE EFFECTS OF PREBIOTICS AND \( \beta \)-GLUCAN ON GROWTH PERFORMANCE, WHOLE BODY PROTEIN UTILIZATION STATUS AND LACTOSE DIGESTIBILITY IN WEANLING PIGS FED CORN AND SOYBEAN MEAL-BASED DIETS

2.1. ABSTRACT

This study was conducted to determine effects of dietary supplementations of three prebiotics and \( \beta \)-glucan on growth performance, whole body protein utilization status and lactose digestibility in comparison with two control diets containing an antibiotic growth promoter and one without antibiotics in weanling pigs fed corn and soybean meal (SBM)-based diets. Six experimental diets were formulated with corn (40\%), SBM (28\%) and supplemented with dried whey powder (20\%) and fish meal (9\%) as the bulky ingredients. Diet 1, being a negative control (NC), was the basal diet, containing no antibiotics and supplemental prebiotics or \( \beta \)-glucan. Diet 2, being a positive control (PC), was formulated by adding an antibiotic premix (lincomix 44 at 0.10\%) in the basal diet at the expense of cornstarch. Diets 3 to 6 were formulated to contain 0.75\% of the three test prebiotics of retrograded resistant cornstarch (diet 3), Fibersol-2 (a modified digestion-resistant maltodextrin) (diet 5) and inulin (diet 6), and the viscous soluble fiber oat \( \beta \)-glucan (diet 4), respectively, at the expense of cornstarch.
Titanium oxide (0.30%) was included as a digestibility marker. The diets were formulated to meet the National Research Council (1998) recommended nutrient requirements. A total of 144 Yorkshire pigs, at the age of 21 d and an average initial body weight (BW) at about 5.5 kg, were allocated to 12 floor pens with 6 pigs per pen, balanced for barrows and gilts in the pens and fed one of the 6 diets for 21 d in two study blocks according to a completely randomized block design. Initial and final individual pig BW, pen average daily feed intake and representative pig plasma urea concentrations were measured. Fecal samples were collected mid trial on d 12 on a per pen basis. Dunnett’s and Tukey’s tests were conducted on the endpoints by using the SAS procmixed model. There were no differences ($P > 0.05$) in average daily gain (ADG), average daily feed intake, gain to feed ratio, plasma urea concentration, total tract dry matter (DM) and lactose digestibility and the calculated lactase digestive capacity among the diets, as examined by the Tukey’s test. Furthermore, there were no differences ($P > 0.05$) in these endpoints between each of the four treatment diets and the NC or PC diet as examined by the Dunnett’s test. The total tract lactose digestibility was determined to be at 100%. The calculated lactase digestive capacity was about eight times the daily lactose intake when dietary lactose content was at 10-12%. These results suggest that the supplemental lactose was completely digested in the weanling pigs. In conclusion, dietary supplementation of the prebiotics and the oat β-glucan at 0.75% of the diets did not significantly affect the major growth performance endpoints, the status of whole body protein utilization, as well as fecal DM and lactose digestibility in the weanling pigs fed the corn SBM-based diets. The growth-promoting effect associated with dietary supplemental lactose is likely due to the fact that lactose is a highly
digestible carbohydrate rather then acting as an effective prebiotic in weanling pig nutrition.

Key Words: Antibiotics, β-Glucan, Lactose, Prebiotics, Weanling Pigs

2.2. INTRODUCTION

The modern practice of feeding weaning pigs antibiotics as growth promoters has been established for a long time. In the late 1940s, the development of supplemental vitamins, specifically vitamin B₁₂, led to the discovery that antibiotics can be used to improve growth and feed conversion efficiency (Summons, 1968). But the rise of the antibiotic resistance has helped encourage the search for alternative sources of growth-promoting substrates (Pluske et al., 2002; Heo et al., 2012). Numerous alternative feed additives have been evaluated for their impact on gut and overall health with prebiotics and other soluble fibers arising as viable options for improving livestock health.

Prebiotics are described as novel ingredients that resist gastrointestinal digestion and absorption by host animal enzymes and can be selectively fermented by intestinal microflora and have a positive impact on the host (Gibson et al., 2004). The classified prebiotics, including retrograded resistant cornstarch, fibersol-2 (a commercial supplement that is a resistant maltodextrin) and inulin, have been well documented for their prebiotic effects. Resistant starch can increase fecal bulk, increase the molar ratio of the trophic and signaling nutrients like butyrate and dilute fecal bile acids (Kendal et al., 2004). Fibersol-2 has been shown to shift fecal populations of Bifidobacterium to higher proportions, as well as to increase the production of butyrate (Fastinger et al., 2008) and
inulin has also been shown these positive effects (Seifert and Watzl, 2007). Furthermore, the aforementioned prebiotics fall easily into the classification as outlined in the Gibson et al. (2004) seminal paper.

Prebiotics are often soluble fiber but not all soluble fiber can be described as a prebiotic. The viscous soluble fiber, β-glucan, has received some interest because of the recognition that some forms of β-glucan have immune modulating effects (Wilment et al., 2001). Because of the heterogenous nature of β-glucans that arise from a variety of sources, its activity is diverse and not completely confirmed through sufficient research, which makes β-glucan ineligible to receive a prebiotic designation (Gibson et al., 2004). Despite this, the research that has shown the positive effects of specific forms of β-glucan would suggest that dismissing the use of β-glucan to improve health parameters would be premature. Oat β-glucan has been shown to have better ability to modulate bile acid secretion (Lia et al., 1995) as well as modulate the immune system with anti-inflammatory capabilities (Chang et al., 2006).

The role of lactose in improving weanling pig growth performance and efficiency of whole body nitrogen (N) retention has received considerable attention. It has been well established that the lactase enzyme activity is highest immediately after birth and during the neonatal suckling period. Following this period, the enzyme activity decreases dramatically during the weaning transition (Kelly et al., 1991; Lackeyram, 2012). Crystalline lactose and lactose from dried whey powder are well documented to be the essential dietary carbohydrate for improving growth performance and efficiency of whole body N retention in weanling pigs (Mahan, 1992; Nessmith et al., 1997). Mahan and Newton (1993) demonstrated that dextrose was effective but cornstarch was ineffective to
replace lactose in improving growth performance and efficiency of whole body N utilization in the weanling pigs, suggesting that lactose is still likely the rapidly digestible carbohydrate in weanling pigs. However, Fuller (1992) suggested that lactose likely garnered its positive prebiotic effects by modulating the gut microflora such as the commensal bacteria lactobacilli. Conversely, studies by Krause et al. (1995; 1997) did not observe improvements in the adherent lactobacillus counts in the ileum and the cecum in response to lactose supplementation in weanling pigs. Thus, examination of responses in the lactose digestibility and lactase digestive capacity in the absence and presence of feed antibiotics, as well as the effects of prebiotics and β-glucan supplementations will help further reveal the role of dietary lactose in the support of growth performance and efficiency of N utilization in the weanling pig.

Therefore the objectives of this study were to investigate the effects of three prebiotics, retrograded resistant cornstarch, fibersol-2 and inulin and a viscous soluble fiber oat β-glucan, on growth performance, responses in fecal lactose digestibility and the whole gut lactase digestive capacity, as well as the whole body N utilization status as indicated by changes in blood urea concentrations in the absence and presence of feed antibiotics in weanling pigs fed corn and soybean meal (SBM)-based diets.

2.3. MATERIALS AND METHODS

2.3.1. Animals and Management

A total of 144 Yorkshire piglets, with a ratio of 1 barrow to 1 gilt, were used for this study. The study was done in two replicates with 72 pigs per replicate. Each
replicate contained two blocks with 36 pigs per block. Piglets were divided into pens with six animals per pen. Pigs were weaned at 21 d of age and given *ad libitum* access to their test diets and water. Diets were provided in stainless-steel trough feeders measuring 26.5 cm deep by 76 cm long. Water was provided continuously through one dish per pen measuring 17 cm across and 15 cm deep with a push activated water dispenser.

Pigs were obtained from the University of Guelph Arkell Swine Research Station and transported to the animal research wing in the Department of Animal and Poultry Science at the University. The groups of six piglets were housed in floor pens (160 cm in length by 130 m in width and 13 cm off the ground) with rubberized woven wire floors called Tenderfoot® with openings measuring 3.0 cm by 1.5 cm. The room was kept at 24°C and the pens were supplemented with heating lamps elevated about 65 cm above the piglet sleeping area. Each floor pen was equipped with a rubber mat measuring approximately 60 cm by 95 cm that was placed in the corner under the heating lamp to provide a comfortable sleeping area.

The pens were cleaned on a biweekly basis. Due to the design of the pens and the room in which the animals were housed, fewer cleanings would have greatly impacted the sanitary conditions and welfare of the animals due to unreasonable exposure to fecal contamination. Conversely, more frequent cleanings were expected to poorly represent the disease challenge experienced by pigs in a typical commercial facility.

Environment enrichment was provided in the form of bocce balls. Furthermore, pigs were given 5 min per pen per d of positive contact time with a human. Positive contact included scratching and interacting with the piglets using the bocce balls.
Animals were fed the experimental diets for 21 d with *ad libitum* access being allowed. Feeders and water dispensers were checked twice per d to ensure proper functioning, adequate fill and to clean and replace diets as needed. Soiled and waste diets were collected in foil trays (120 cm by 90 cm) that were placed beneath the feeders, as well as being removed from the feeders and then dried to an air-dry basis using drying ovens set at 65°C, if necessary, and weighed to determine how much test diet was consumed.

The Animal Care Committee at the University of Guelph approved the experimental protocol, sampling procedure and procedures for the care and treatment of the animals in this study. The piglets used in this study were cared for in accordance with the guidelines set out by the Canadian Council on Animal Care (CCAC, 2009).

**2.3.2. Experimental Diets and Design**

Corn and SBM-based diets were formulated to meet NRC (1998) nutrient requirements for pigs of 5 to 10 kg body weight (BW) (*TABLE 2.1*). Dried whey (19.86 g/kg of diets), with an average analyzed lactose content of 54%, was included in the weanling pig diets by following standard practices (Mahan and Newton, 1993; Cromell et al., 2008). A negative control (NC) diet was formulated with no antibiotics and a positive control (PC) diet was formulated with 0.1% Lincomix® 44 premix according to the standard commercial practice. Various prebiotics and the soluble viscous fiber oat β-glucan, with β-1,3/1,4 branching, were added at a rate of 0.75% at the expense of cornstarch. Titanium oxide was included in the diets (0.30%) as a digestibility marker. The study was conducted according to a complete randomized block design with 6 treatments (diets), 3 blocks and 2 replicates per block. Each pen, containing 6 piglets,
represented an experimental unit. Supplements were included at a rate of 0.75% of the diet to meet numbers shown in the literature to elicit positive changes in growth or health endpoints while also being economically feasible for industry should commercial application be attempted.

2.3.3. Measurements, Sample Collection and Sample Preparation

Body weights were measured upon arrival on d one of the trial when the piglets were 21 d old and then again at the end of the trial, on d 21, when the piglets were 42 d old. These data were used to calculate growth rates of the weanling pigs over the duration of the trial. Feed intake was measured daily on a per pen basis by weighing back any remaining diets at 0900 and 1700 h. Feeders were checked twice per d to ensure diets were continuously available and to remove soiled diets from the feeders. The collection trays placed below the feeders in each pen to catch spilled diets were also checked and emptied at 0900 and 1700 h every day. Wet diets removed from the feeders and collection trays were dried to an air-dry basis in drying ovens set at 65°C to allow removal of free water before weighing back to facilitate accurate calculations of diet consumption.

Diet and fecal samples were collected on d 8 and d 15 of the trial. Fresh fecal samples were collected from at least two pigs per pen and homogenized. Fecal samples were freeze-dried. To create homogenized samples, samples of the pelleted diet and dried fecal samples were ground with a mortar and pestle according to Rideout et al. (2004). Aliquots of freshly collected fecal samples from d 8 and d 15 of the study were stored at -80°C for later processing and the determination of lactose content. Diet samples were stored at 4°C for further analysis.
Venous blood samples were collected by puncture of the orbital sinus on the right or left side of one animal per pen on d 12 of the trial from physically immobilized pigs (Bregendahl et al., 2004). Blood was collected into pre-chilled plastic centrifuge tubes containing heparin for the separation of plasma. After collection, the blood samples were placed on ice and transferred to the lab where they were centrifuged at 2000 x g for 20 min with a centrifugation temperature of 4°C to separate the plasma fractions. These fractions were then removed, divided into aliquots and stored at -80°C for later analyses of plasma urea concentration.

2.3.4. Chemical and Biochemical Analysis

Dietary and fecal dry matter (DM) content was analyzed according to AOAC (1993). The digestibility marker titanium oxide content in the diet and fecal samples was analyzed by following the procedure of Leone (1973) and Myers et al. (2004). Resulting sample absorbances were measured at 410 nm by using an Epoch microplate spectrophotometer (BioTek, Winooski, VT).

Lactose content in diet and fecal samples was analyzed by using solutions from a commercial kit (Megazyme, Wicklow, Ireland) that measures β-D-galactose after lactose was hydrolyzed with the enzyme β-galactosidase. The kit works through measuring the absorbance of sample solutions at 340 nm, which is a reflection of NADH content. NADH is produced proportionately in response to the content of lactose in samples. Diet and fecal samples (~0.2 g) were accurately weighed out and transferred into 50 mL plastic centrifuge tubes. After thoroughly mixing with 12.5 mL of distilled and deionized water, the tubes were centrifuged at 1,000 x g for 15 min at 4°C to remove particulate substances. The supernatants were transferred into 50 mL volumetric flasks and brought
to volume with distilled and deionized water. The prepared aliquot samples (0.400 mL) were deproteinized via incubation of the sample tubes in a boiling water bath, followed by centrifugation at 1,500 x g for 15 min at 4°C. The further prepared supernatant samples were then measured for free β-D-galactose content as a background correction prior to further determination of lactose content by using the assay kit solution.

Plasma urea content was determined using premade kits (Stanbio Laboratory, Boerne, TX) with solutions that utilized the enzyme urease to hydrolyze urea to ammonia and carbon dioxide. Ammonia aminates α-ketoglutarate to glutamate while the concurrent oxidation of NADH to NAD$^+$ is catalyzed by glutamate dehydrogenase. Stanbio Diagnostics blood urea (endpoint) reagent is formulated in such a way that the decrease in absorbance at 340 nm, resulting from the oxidation of NADH to NAD$^+$, is directly proportional to urea concentration in the plasma samples.

2.3.5. Calculations and Statistical Analysis

A linear calibration equation was generated by using respective pure compounds in reaction with reagents for each batch of sample analyses of titanium, lactose and urea. The daily whole body intestinal lactase digestive capacity (LDC) was calculated according to the concept of Weiss et al. (1998) and Lackeyram et al. (2010). Weaning reduces gut lactase specific activity and whole gut lactase digestive capacity in the pig (Kelly et al., 1991; Lackeyram, 2012). Thus the daily whole body lactase digestive capacity associated with the weanling pigs fed the experiential diets was calculated according to formula 1.

$$LDC = (342.3 \times V_{cap} \times BW)/1000 \quad (1)$$
Where LDC is the daily whole body lactase digestive capacity (g lactose/pig·d); 342.30 is the molar mass of lactose representing 342.30 g lactose per mole; $V_{cap}$ is the small intestinal lactase digestive capacity of $151.42 \pm 15.79$ mmol/(kg·BW·d) measured by our group in the weanling Yorkshire pig (Lackeyram, 2012), and BW is the average final live body weight of experimental pigs (kg/pig).

Data were analyzed by using the Statistical Analysis Software 9.2 (SAS Institute, Cary, NC) for a mixed model of ANOVA according to the completely randomized block design.

The statistical model for the ANOVA according to a completely randomized block design with 6 diets (treatments), 6 replications (blocks) and 24 experimental units (pens) of a total of 144 animals, according to the following:

$$
\gamma_{ij} = \mu + t_i + \rho_j + \epsilon_{ij}
$$

Where $\mu$ is the general mean, $t_i$ is the treatment effect, $\rho_j$ is the block effect and $\epsilon_{ij}$ is the experimental error.

Comparisons between each test diet and each of the control diets were conducted by using the Dunnett-Hsu’s test. Comparisons among all the diets were carried out by using the Tukey-Kramer’s test for pairwise comparisons. Differences between and among the treatment diets were considered to be significant for $P < 0.05$. The animals were sorted completely at random within each block without taking gender or family factors into account.

### 2.4. RESULTS
The effects of three prebiotic and β-glucan supplementations on the major growth performance endpoints are summarized and compared in TABLE 2.2. There were no differences ($P > 0.05$) in the initial and final BW and the average daily gain (ADG) of the test weanling pigs among the 6 experimental diets, as well as between each supplemental diet and the positive control and the negative control diets. Furthermore, the three prebiotic and β-glucan supplementations had no effects ($P > 0.05$) on the average daily feed intake and the gain to feed ratio (G:F) in the weanling pigs.

The effects of three prebiotic supplements and β-glucan on dietary and fecal DM content, the apparent fecal DM digestibility and plasma urea concentration were summarized and compared in TABLE 2.3. Dietary supplementation of inulin at 0.75% increased ($P < 0.05$) dietary DM content in comparison with the NC diet when examined by the Dunnett-Hsu’s test. However, fecal DM contents were not affected ($P > 0.05$) by the dietary treatments. β-Glucan supplementation at 0.75% and the absence of the feed antibiotic in the PC diet resulted in numerically lower fecal DM digestibility and the plasma urea concentration values, however, these differences were not significant when compared using the Tukey-Kramer’s test and the Dunnett-Hsu’s test. The dietary treatments had no effects ($P > 0.05$) on the plasma urea concentrations in the weanling pigs.

There were no differences ($P > 0.05$) in dietary lactose content among the diets (TABLE 2.4). Dietary lactose content ranged from 10.21 (Diet 4) to 11.53% (Diet 2). Analyses of fecal lactose content revealed that dietary lactose was 100% digested. For all the test diets, fecal lactose content was found to be 0%. To prove if the dietary lactose was enzymatically digested, average daily lactose intake in the weanling pigs was
determined and was found to be not different \((P > 0.05)\) among the diets. Furthermore, lactase digestive capacity (LDC) in the weanling pigs was also calculated and was found to be not different \((P > 0.05)\) among the diets (\textbf{TABLE 2.4}). The calculated small intestinal LDC averaged at 500.49 g lactose/pig\(\cdot\)d and was about eight times of the estimated average daily lactose intake (63.59 g lactose/pig\(\cdot\)d) in the weanling pigs, further proving that dietary lactose was likely to be completely digested via enzymatic hydrolysis by the small intestinal lactase in these weanling pigs fed the test diets.

\section*{2.5. DISCUSSION}

The major objectives of this study were to evaluate the effects of retrograded resistant cornstarch, fibersol-2, inulin and \(\beta\)-glucan on growth performance and blood urea levels and to further investigate the role of lactose in weanling pig nutrition. No significant differences were detected between any of the diets for the growth performance parameters measured (\textbf{TABLE 2.2}). Comparison of results from this study with other studies is difficult due to limited research reports available on specific prebiotic supplements that look at growth performance in the weanling pig. There is also variability in inclusion levels of prebiotics used, which further complicates the comparison process.

\subsection*{2.5.1. Growth Performance Responses}

Retrograded resistant cornstarch is one prebiotic that has been well reported for its effects on human and animal gut health endpoints. Unfortunately the available research results have not delivered clear answers regarding its effects on growth performance.
Studies looking at the growth performance of weanling pigs fed retrograded resistant cornstarch are scarce. De Schrijver et al. (1999) noted that pigs fed 6% retrograded cornstarch had a decreased ability to digest fat, however, they did not evaluate changes in growth performance. Rideout et al. (2007) reported responses in the cecal butyrate concentration in grower pigs fed diets containing different forms of resistant cornstarch but the animals’ growth performance endpoints were not measured.

Fibersol-2 supplementation also did not reveal any improvements in growth performance in the weanling pigs in this study. There is a scarcity of literature reports on fibersol-2 supplementation in the weanling pig. Rodriguez-Cabezas et al. (2010) reported the effects of fibersol-2 supplementation (at 63%) on immune responses and found significant improvements in rats. Unfortunately, changes in growth performance were not presented and discussed in their report.

According to the results from this study, supplementation of inulin in the diet for up to 0.75% was not sufficient to cause improvements in growth performance characteristics in the weanling pigs fed corn and soybean meal-based diets. This was in agreement with Pierce et al. (2005) who added inulin to weaning pig diets at 0.015% and observed no effects on growth performance endpoints. Furthermore, Maire et al. (2010) added inulin to weaning pig diets at 0.4% and could not find any improvements in growth performance.

β-Glucan supplementation did not impact the growth performance parameters of the weanling pigs in this study. This is contrary to Dritz et al. (1995) findings that diets supplemented with 0.025% β-glucan did show improvements in ADG and average daily feed intake. In agreement with our findings, later work done by Hiss and Sauerwein
(2003) who found that supplementing up to 0.03% of yeast-derived β-glucan slightly increased average daily feed intake but did not improve any other growth performance parameters. The reason for differences between these studies could be related to the source for β-glucan with it ranging from barley and oat β-glucan to yeast β-glucan. Furthermore, the differences in levels of supplemented β-glucan would be a natural area for discussion. Nevertheless, the intuitive explanation that higher level of dietary supplementation of β-glucan would be related to a greater improvement in growth performance seems to be refuted by results of these aforementioned studies.

2.5.2. Dry Matter Content and Digestibility

Consideration of the prebiotic and β-glucan supplementations in conjunction with dietary DM digestibility is the next area for discussion. Once again, there is a lack of significant data with respect to the supplementation of retrograded resistant cornstarch, β-glucan and fibersol-2 in this study. The test diet containing retrograded resistant cornstarch was shown to have no significant difference in DM digestibility values in comparison with the NC or the PC diets, which is corroborated by the findings of Rideout et al. (2007). Similarly, β-glucan, fibersol-2 and inulin supplementations were found not result in differences in the whole tract DM digestibility compared with the NC or the PC diet. It is interesting to observe that DM content in the inulin-supplemented diet (diet 6) was significantly higher then that in the NC diet (TABLE 2.3). This is likely due to the fact that inulin is a good source of soluble fiber and likely holds more bound water. Whereas conventional cornstarch is known to form a semi-crystalline granular structure and likely holds less bound water because of a relatively less available hydrogen-bonding capacity.
2.5.3. Plasma Urea Concentrations

Our results suggested that dietary supplemenations of the prebiotics and β-glucan did not have a significant effect on plasma urea concentrations in weanling pigs in this study. It has been well established that changes in plasma urea concentrations are biomarkers of gut tissue hyperplastic growth, availability of blood circulating amino acids to peripheral muscle growth, and whole body status of N utilization in pigs (Coma et al., 1995; Jiang et al., 2000). Plasma urea concentrations have been further correlated with G:F ratios in weanling pigs (Whang et al., 2000). Because no dietary effects were seen on growth performance parameters, it aligns well that no changes in the plasma urea concentrations were noted in the weanling pigs of this study. Houdijk et al. (1998) corroborated these findings in showing no effects on growth performance from the feeding of prebiotic supplements to pigs. Despite the noted positive effects of prebiotic supplements on hind-gut bacterial profile in the literature, there is a continual deficit of data collected in showing positive responses in growth performance in conjunction with prebiotic or fermentable soluble fiber feeding in the weanling pig.

2.5.4. Lactose Digestibility and Lactase Digestive Capacity

Lactose is known to have a growth-promoting effect when fed to weanling pigs (Mahan et al., 1992) and this has been suggested to be the result of a prebiotic effect (Fuller, 1992; Pierce et al., 2006). Contrary to this, the series of studies by Krause et al. (1995; 1997) did not show significant evidence of the prebiotic effect resulting from dietary supplementation of lactose in the weanling pigs. Our results showed that despite the well established concept that the small intestinal lactase activity decreases dramatically during the weaning transition and the post-weaning growth in the pig,
dietary lactose supplemented at 10-12% was completely digested in the weanling pig (TABLE 2.4), suggesting that lactose is a highly digestible carbohydrate in promoting weanling pig growth performance. This observation is further substantiated by our calculations that the whole body LDC was about eight times of the determined dietary lactose intake, proving that the measured 100% dietary lactose fecal digestibility was likely enzymatic hydrolysis by the gut residual lactase activity in the weanling pigs from this study. Furthermore, our results suggest that dietary supplementations of feed antibiotics, representative prebiotics and β-glucan did not affect lactose digestibility in the weanling pigs. This observation of lactose as a highly digestible carbohydrate in the weanling pigs is consistent with the conclusion made by Lackeyram (2012) through in vitro small intestinal lactase kinetic analysis in the weanling pigs. To the best of our knowledge, this is the first in vivo study showing that dietary supplemental lactose is completely digested at the fecal level in the weanling pig.

2.5.5. Concluding Remarks

It can be concluded that dietary supplementations of the prebiotics, of retrograded cornstarch, fibersol-2 and inulin at 0.75%, and oat β-glucan at 0.75%, did not significantly improve the major growth performance endpoints, whole body status of N utilization as indicated by changes in plasma urea concentrations, and total tract DM digestibility in the weanling pigs. Dietary supplemental lactose at 10-12% was completely digested, which was not affected by dietary supplementations of feed antibiotics, prebiotics or the viscous soluble fiber β-glucan, in the weanling pigs. Therefore, the growth-promoting effect associated with dietary lactose supplementation is
likely due to the fact that supplemental lactose is a highly digestible carbohydrate rather than serving as an effective prebiotic in the weanling pig.
### TABLE 2.1. Composition of experimental diets for the weanling pigs

<table>
<thead>
<tr>
<th>Item</th>
<th>Diet 1 (NC)</th>
<th>Diet 2 (PC)</th>
<th>Diet 3 (RCS)</th>
<th>Diet 4 (β-G)</th>
<th>Diet 5 (F-2)</th>
<th>Diet 6 (IN)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cornstarch</td>
<td>1.70</td>
<td>1.80</td>
<td>0.01</td>
<td>0.73</td>
<td>1.05</td>
<td>1.05</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>27.80</td>
<td>27.80</td>
<td>27.80</td>
<td>27.80</td>
<td>27.80</td>
<td>27.80</td>
</tr>
<tr>
<td>Fish meal (herring)</td>
<td>9.00</td>
<td>9.00</td>
<td>9.00</td>
<td>9.00</td>
<td>9.00</td>
<td>9.00</td>
</tr>
<tr>
<td>Animal fat blend</td>
<td>0.18</td>
<td>0.18</td>
<td>0.18</td>
<td>0.18</td>
<td>0.18</td>
<td>0.18</td>
</tr>
<tr>
<td>Lysine-HCl&lt;sup&gt;3&lt;/sup&gt;</td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
</tr>
<tr>
<td>DL-Methionine&lt;sup&gt;4&lt;/sup&gt;</td>
<td>0.09</td>
<td>0.09</td>
<td>0.09</td>
<td>0.09</td>
<td>0.09</td>
<td>0.09</td>
</tr>
<tr>
<td>Limestone</td>
<td>0.36</td>
<td>0.36</td>
<td>0.36</td>
<td>0.36</td>
<td>0.36</td>
<td>0.36</td>
</tr>
<tr>
<td>Iodized salt&lt;sup&gt;5&lt;/sup&gt;</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>Min-Vit premix&lt;sup&gt;6&lt;/sup&gt;</td>
<td>0.50</td>
<td>0.50</td>
<td>0.50</td>
<td>0.50</td>
<td>0.50</td>
<td>0.50</td>
</tr>
<tr>
<td>Feed antibiotic&lt;sup&gt;7&lt;/sup&gt;</td>
<td>0.00</td>
<td>0.10</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>RCS-RS&lt;sup&gt;8&lt;/sup&gt;</td>
<td>0.00</td>
<td>0.00</td>
<td>1.79</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>β-Glucan&lt;sup&gt;9&lt;/sup&gt;</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>1.07</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Fibersol-2&lt;sup&gt;10&lt;/sup&gt;</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.75</td>
<td>0.00</td>
</tr>
<tr>
<td>Inulin&lt;sup&gt;11&lt;/sup&gt;</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.75</td>
</tr>
<tr>
<td>Titanium oxide&lt;sup&gt;12&lt;/sup&gt;</td>
<td>0.30</td>
<td>0.30</td>
<td>0.30</td>
<td>0.30</td>
<td>0.30</td>
<td>0.30</td>
</tr>
</tbody>
</table>

Dietary nutrient contents (on as-fed basis)<sup>13</sup>:  

---

56
<table>
<thead>
<tr>
<th></th>
<th>Diet 1</th>
<th>Diet 2</th>
<th>Diet 3</th>
<th>Diet 4</th>
<th>Diet 5</th>
<th>Diet 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude protein, %</td>
<td>23.51</td>
<td>23.51</td>
<td>23.51</td>
<td>23.51</td>
<td>23.51</td>
<td>23.51</td>
</tr>
<tr>
<td>Total calcium, %</td>
<td>0.78</td>
<td>0.78</td>
<td>0.78</td>
<td>0.78</td>
<td>0.78</td>
<td>0.78</td>
</tr>
<tr>
<td>Total phosphorus, %</td>
<td>0.71</td>
<td>0.71</td>
<td>0.71</td>
<td>0.71</td>
<td>0.75</td>
<td>0.71</td>
</tr>
<tr>
<td>Total amino acid levels:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arginine</td>
<td>1.43</td>
<td>1.43</td>
<td>1.43</td>
<td>1.43</td>
<td>1.43</td>
<td>1.43</td>
</tr>
<tr>
<td>Histidine</td>
<td>0.62</td>
<td>0.62</td>
<td>0.62</td>
<td>0.62</td>
<td>0.62</td>
<td>0.62</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>1.02</td>
<td>1.02</td>
<td>1.02</td>
<td>1.02</td>
<td>1.02</td>
<td>1.02</td>
</tr>
<tr>
<td>Leucine</td>
<td>1.97</td>
<td>1.97</td>
<td>1.97</td>
<td>1.97</td>
<td>1.97</td>
<td>1.97</td>
</tr>
<tr>
<td>Lysine</td>
<td>1.50</td>
<td>1.50</td>
<td>1.50</td>
<td>1.50</td>
<td>1.50</td>
<td>1.50</td>
</tr>
<tr>
<td>Methionine</td>
<td>0.43</td>
<td>0.43</td>
<td>0.43</td>
<td>0.43</td>
<td>0.43</td>
<td>0.43</td>
</tr>
<tr>
<td>Cysteine</td>
<td>0.37</td>
<td>0.37</td>
<td>0.37</td>
<td>0.37</td>
<td>0.37</td>
<td>0.37</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>1.06</td>
<td>1.06</td>
<td>1.06</td>
<td>1.06</td>
<td>1.06</td>
<td>1.06</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>0.80</td>
<td>0.80</td>
<td>0.80</td>
<td>0.80</td>
<td>0.80</td>
<td>0.80</td>
</tr>
<tr>
<td>Threonine</td>
<td>0.98</td>
<td>0.98</td>
<td>0.98</td>
<td>0.98</td>
<td>0.98</td>
<td>0.98</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>0.29</td>
<td>0.29</td>
<td>0.29</td>
<td>0.29</td>
<td>0.29</td>
<td>0.29</td>
</tr>
<tr>
<td>Valine</td>
<td>1.12</td>
<td>1.12</td>
<td>1.12</td>
<td>1.12</td>
<td>1.12</td>
<td>1.12</td>
</tr>
</tbody>
</table>

1Pigs weaned at 21 d of age and fed the diets for 3 wk post-weaning to meet or exceed the NRC (1998) nutrient requirements for pigs of 5-10 kg BW.

2Diet 1 as the negative control (NC); diet 2 as the positive control (PC); diet 3 with retrograded resistant cornstarch (RCS); diet 4 with β-glucan (β-G); diet 5 with fibersol-2 (F-2); and diet 6 with inulin (IN).

3Crystalline lysine-HCl of 79% purity commercially available.
Crystalline DL-Methionine of 99% purity commercially available.

Supplied by the Windsor Salt Co. (Toronto, ON, Canada). Composition (g/kg): NaCl, 965.0; ZnO, 40.0; FeCO$_3$, 1.6; MnO, 1.2; CuO, 0.33; Ca(IO$_3$)$_2$, 0.07; and CaO, 0.04.

The trace mineral and vitamin premix supplied the followings per kg of diet provided by the DSM Nutritional Products Inc. (Ayr, ON, Canada) with guaranteed analyses of the followings: copper, 15.0 mg; iodine, 0.5 mg; iron, 100 mg; manganese, 20.0 mg; selenium, 0.30 mg; and zinc, 105.0 mg; vitamin A, 10000 IU, vitamin D$_3$, 1000 IU, vitamin E, 40 IU; vitamin K, 2.5 mg; thiamine, 1.5 mg; riboflavin, 5.0 mg; pyridoxine, 1.5 mg; vitamin B$_{12}$, 0.025 mg; niacin, 25 mg; d-pantothenic acid, 15.0 mg; folic acid, 2.0 mg; d-biotin, 0.200 mg; and choline, 500 mg.

Feed antibiotic of Lincomix® 44 supplied by Elanco Canada Inc. (Guelph, ON, Canada) for providing 0.044 g lincomycin per kg of the positive control diet.

Retrograded high amylose cornstarch (RCS) with resistant starch (RS) content at about 42% from the National Starch (Bridgewater, NJ).

β-glucan with β-(1-3) and β-(1-4) branching extracted from oats at 70% purity donated by Garuda International Inc. (Lemon Cove, CA).

A commercial trade name for resistant maltodextrin marketed and donated by DSM, Matsutani LLC (Clinton, IA).

Inulin (100% purity) marketed by Nealanders International Inc. (Mississauga, On, Canada).

Nutrient digestibility marker purchased from Fisher Scientific (Ottawa, ON, Canada).

Calculated according to NRC (1998).
**TABLE 2.2.** The effects of three prebiotics and $\beta$-glucan on growth performance in weanling pigs fed corn and soybean meal-based diets

<table>
<thead>
<tr>
<th>Item</th>
<th>Diet 1 (NC)</th>
<th>Diet 2 (PC)</th>
<th>Diet 3 (RCS)</th>
<th>Diet 4 (β-G)</th>
<th>Diet 5 (F-2)</th>
<th>Diet 6 (IN)</th>
<th>SEM$^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight</td>
<td>kg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial</td>
<td>7.0</td>
<td>6.6</td>
<td>6.7</td>
<td>6.8</td>
<td>6.4</td>
<td>6.6</td>
<td>0.50</td>
</tr>
<tr>
<td>Final</td>
<td>13.2</td>
<td>12.9</td>
<td>12.7</td>
<td>12.6</td>
<td>12.5</td>
<td>11.9</td>
<td>0.80</td>
</tr>
<tr>
<td>ADFI$^3$</td>
<td>0.6</td>
<td>0.6</td>
<td>0.6</td>
<td>0.6</td>
<td>0.6</td>
<td>0.6</td>
<td>0.12</td>
</tr>
<tr>
<td>ADG$^4$</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
<td>0.04</td>
</tr>
<tr>
<td>G:F$^5$</td>
<td>0.49</td>
<td>0.52</td>
<td>0.56</td>
<td>0.54</td>
<td>0.53</td>
<td>0.46</td>
<td>0.124</td>
</tr>
</tbody>
</table>

$^1$See **TABLE 2.1** for details of diet formulations. Diet 1 as the negative control (NC); diet 2 as the positive control (PC); diet 3 with retrograded resistant cornstarch (RCS); diet 4 with $\beta$-glucan ($\beta$-G); diet 5 with fibersol-2 (F-2); and diet 6 with inulin (IN).

$^2$Pooled standard errors of means ($n = 4$).

$^3$ADFI, average daily feed intake.

$^4$ADG, average daily gain.

$^5$G:F, gain to feed ratio.

Endpoints without common or different superscript letters do not differ ($P > 0.05$).
TABLE 2.3. The effects of three prebiotics and β-glucan on apparent fecal dry matter (DM) digestibility and plasma urea concentration in weanling pigs fed corn and soybean meal-based diets

<table>
<thead>
<tr>
<th>Item</th>
<th>Diet 1 (NC)</th>
<th>Diet 2 (PC)</th>
<th>Diet 3 (RCS)</th>
<th>Diet 4 (β-G)</th>
<th>Diet 5 (F-2)</th>
<th>Diet 6 (IN)</th>
<th>SEM²</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM in diets</td>
<td>89.3a</td>
<td>89.9a,b</td>
<td>90.4a,b</td>
<td>90.5a,b</td>
<td>89.8a,b</td>
<td>91.0b*</td>
<td>0.56</td>
</tr>
<tr>
<td>Fecal DM</td>
<td>90.2</td>
<td>90.6</td>
<td>90.9</td>
<td>91.0</td>
<td>90.7</td>
<td>91.1</td>
<td>2.13</td>
</tr>
<tr>
<td>DM digestibility</td>
<td>80.2</td>
<td>77.7</td>
<td>85.1</td>
<td>66.2</td>
<td>81.2</td>
<td>82.3</td>
<td>5.52</td>
</tr>
<tr>
<td>Urea³</td>
<td>2.5</td>
<td>2.0</td>
<td>2.3</td>
<td>2.0</td>
<td>2.7</td>
<td>2.2</td>
<td>0.85</td>
</tr>
</tbody>
</table>

¹See TABLE 2.1 for details of diet formulation. Diet 1 as the negative control (NC); diet 2 as the positive control (PC); diet 3 with retrograded resistant cornstarch (RCS); diet 4 with β-glucan (β-G); diet 5 with fibersol-2 (F-2); and diet 6 with inulin (IN).

²Pooled standard errors of means (n = 4).

³Plasma urea concentration.

a,b Means that diets with different superscript letters differ (P < 0.05) when compared with the Tukey-Kramer’s test.

*Difference (P < 0.05) from diet 1 (NC) as compared with the Dunnett-Hsu’s test.

Endpoints without common or different superscript letters do not differ (P > 0.05).
TABLE 2.4. The effects of three prebiotics and \( \beta \)-glucan on fecal lactose digestibility and the lactase digestive capacity in weanling pigs fed corn and soybean meal-based diets

<table>
<thead>
<tr>
<th>Experimental diets(^1)</th>
<th>Diet 1</th>
<th>Diet 2</th>
<th>Diet 3</th>
<th>Diet 4</th>
<th>Diet 5</th>
<th>Diet 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Item</td>
<td>(NC)</td>
<td>(PC)</td>
<td>(RCS)</td>
<td>((\beta)-G)</td>
<td>(F-2)</td>
<td>(IN)</td>
</tr>
<tr>
<td>Lactose</td>
<td>%</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dietary content(^3)</td>
<td>11.0</td>
<td>11.5</td>
<td>10.4</td>
<td>10.2</td>
<td>10.6</td>
<td>10.5</td>
</tr>
<tr>
<td>Fecal content(^3)</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Digestibility(^4)</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
</tr>
</tbody>
</table>

\( g \) lactose/pig \( d \)

| ADLI\(^5\) | 63.1 | 67.9 | 62.3 | 62.6 | 59.5 | 65.6 | 12.89 |
| LDC\(^6\)   | 522.2 | 503.9 | 501.6 | 489.8 | 505.0 | 479.9 | 30.14 |

\(^1\) See TABLE 2.1 for details of diet formulation. Diet 1 as the negative control (NC); diet 2 as the positive control (PC); diet 3 with retrograded resistant cornstarch (RCS); diet 4 with \( \beta \)-glucan (\( \beta \)-G); diet 5 with fibersol-2 (F-2); and diet 6 with inulin (IN).

\(^2\) Pooled standard errors of means (\( n = 4 \)).

\(^3\) Analyzed lactose content, %, on as fed basis.

\(^4\) Fecal lactose digestibility.

\(^5\) Average daily lactose intake obtained by multiplying average daily feed intake with the analyzed dietary lactose contents.

\(^6\) Lactase digestive capacity.

Endpoints without common or different superscript letters do not differ (\( P > 0.05 \)).
CHAPTER 3

THE EFFECTS OF PREBIOTICS AND β-GLUCAN ON GROWTH PERFORMANCE, WHOLE BODY PROTEIN UTILIZATION STATUS, FECAL SCORE, JEJUNAL AND PLASMA ALKALINE PHOSPHATASE KINETICS AND THE LARGE INTESTINAL FERMENTATION IN WEANLING PIGS FED CORN AND SOYBEAN MEAL-BASED DIETS

3.1. ABSTRACT

This study was conducted to determine effects of dietary supplementations of three prebiotics and β-glucan in replacing feed antibiotics on growth performance, blood urea concentration, fecal scores, jejunal and serum alkaline phosphatase (AP) kinetics and large intestinal fermentation in weanling pigs fed corn and soybean meal (SBM)-based diets. Six experimental diets were formulated with corn (49%), SBM (28%) and fishmeal (9%) as the major bulky ingredients. Diet 1, being a negative control (NC), was the basal diet, containing no antibiotics or supplemental prebiotics or β-glucan. Diet 2, being a positive control (PC), was formulated by adding an antibiotic premix (lincomix 44 at 0.10%) in the basal diet at the expense of cornstarch. Diets 3 to 6 were formulated to contain 0.75% of the three test prebiotics including retrograded resistant cornstarch (diet 3), Fibersol-2 (a modified digestion-resistant maltodextrin) (diet 5) and inulin (diet 6), and the viscous soluble fiber oat β-glucan (diet 4), respectively, at the expense of cornstarch. The diets were formulated to meet the National Research council (1998)
recommended nutrient requirements. A total of 216 Yorkshire pigs, at 21 d of age, with an average initial body weight (BW) of 7.0 kg were allocated to 12 floor pens with 6 pigs per pen, balanced for gender and litter. They were fed one of the 6 diets for 21 d in three study blocks according to a completely randomized block design. Weekly individual pig body weight (BW), pen average daily feed intake, daily pen fecal scores, and plasma urea concentrations at the ends of wk 2 and 3 were recorded. Fresh fecal samples were collected at the end of wk 2 and 3 for each pen, respectively. Fresh cecal digesta samples were collected from one representative pig of each pen at the ends of wk 2 and wk 3. Proximal jejunal and serum samples were also collected from one representative pig from each pen at the end of wk 3. Volatile short-chain fatty acids (VFA) in the extracted cecal and fecal samples were analyzed by gas chromatography-mass spectrometry. Kinetics of AP activity in proximal jejunal homogenates and serum were conducted with P-nitrophenyl phosphate (0 – 10 mM), pH 7.4 at 37ºC. Dunnett’s and Tukey’s tests on the endpoints were conducted by using the SAS procmixed model. There were no differences ($P > 0.05$) in the growth performance endpoints, plasma urea concentration and the VFA concentrations in the cecal and fecal samples among the treatment diets and between each of the treatment diets and the NC or the PC diet. Dietary supplementation of 0.75% β-glucan (Diet 4) increased ($P < 0.05$) the wk 1-3 overall fecal score compared with the NC (Diet 1), suggesting fecal water-holding capacity was enhanced by the 0.75% β-glucan supplementation. Dietary supplemtations of 0.75% fibersol-2 (Diet 5) and inulin (Diet 6) reduced ($P < 0.05$) the $V_{max}$ of the proximal jejunal AP compared with the NC diet (Diet 1). The $V_{max}$ of serum AP was numerically much large in the β-glucan-supplemented diet (Diet 4) among the test diets and the difference was significant ($P <$
0.05) between the β-glucan-supplemented diet (Diet 4) and the PC diet (Diet 2). Furthermore, serum AP affinity $K_m$ values were at least 10-fold higher than the $K_m$ values of the jejunal AP, suggesting that the proximal jejunal AP is much efficient in detoxification of lipopolysaccharides. In conclusion, dietary supplementations of the three prebiotics and oat β-glucan at 0.75% had little effects on growth performance but might affect the gut and whole body health status via influencing the AP detoxification kinetics in the weanling pig.

**Key Words:** Alkaline Phosphatase Kinetics, Antibiotics, β-Glucan, Gut Health, Prebiotics, Volatile Short-Chain Fatty Acids, Weanling Pigs

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### 3.2. INTRODUCTION

The use of antibiotics in commercial diets for weanling pigs continues to be a standard practice in most major marketplaces, but the concern over increasing instances of antibiotic resistance has placed emphasis on the search for viable alternatives (Aminov and Mackie 2007). The discussion around the use of soluble fibers to improve gut and therefore animal health and production has been steadily growing over the past several decades. The accumulating evidence suggests that some key health-promoting effects are attributed to prebiotics and soluble fibers (Gibson and Roberfroid, 1994). Ingestion of prebiotics such as inulin and resistant starch can have positive consequences such as enhancing butyrate production and beneficial gut microflora without affecting digesta
viscosity and thus digesta passage rate (Mihatsch et al., 2006; Westerbeek, 2011). Specific investigations into the use of soluble fibers in animal diets are needed to evaluate the impact on growth performance as well as the impact on changes to the intestinal environment and blood bio-markers for health.

β-Glucan and specifically, oat β-glucan, although not identified as a prebiotic, is thought to exert positive biological effects in monogastric animals (Gallois et al., 2009; Rieder and Smuelsen, 2012). Some of these positive effects include increasing the viscosity of the digesta in the intestine (Jenkins et al., 1978), which contributes to a myriad of other outcomes such as modulation of the immune system (Ripsin et al., 1992; Davis et al., 2004; Rieder and Smuelsen, 2012) and alteration of blood lipid profiles (Brown et al., 1999; Reyna-Villasmi et al., 2007). Unfortunately, the enhanced digesta viscosity by β-glucan may also have negative impacts on weanling pigs through decreasing sugar and other nutrient digestion and absorption that is thought to be resulted from a decreased digesta passage rate in association with the disturbance of the gut mucosal intestinal unstirred water layer (Maki, 2007). Furthermore, although β-glucan has been shown to stimulate some positive hind-gut bacteria such as *Bifidobacterium* and *Lactobacillus* (Jaskari, 1998; Snart, 2006), other studies showed that feeding weanling pigs diets enriched with β-glucan also increased *E. coli* binding to the gut mucosa (Ewaschuk et al., 2012). These noted negative implications β-glucans can have on the host negate them as a true prebiotic.

Besides being responsible for much of the offensive odors associated with swine manure (Spoelstra, 1980), VFA and other volatile compounds are also associated with several changes in the gut and subsequently changes in host health (Meijer, 2010). A
shift in the profile of these volatile compounds in the hindgut may be accomplished through dietary modulations with various types of fiber including prebiotics (Berggren, 2006; Loh, 2006), which can stimulate several health-promoting effects. It is also worth noting that evidence suggests that shifts towards accepted healthful gut bacteria such as *Lactobacillus* and possibly *Eubacteria* may decrease the production of odorous compounds and therefore improve public acceptance of close-by swine production facilities (Zhu, 2000).

Conversely, alkaline phosphatase (AP) is an interesting enzyme that has been identified as a bio-marker for gut health in the weaning pig (Lackeyram et al., 2010). In the small intestine, this enzyme plays a detoxification role and helps maintain luminal pH (Lackeyram et al., 2010) as well as assisting with fat absorption (Zhang et al., 1996). Changes to abundance, digestive capacity and enzyme affinity have been associated with early weaning in pigs and can be taken as a sign of decreased productivity due to the roles of AP in association with gut health. Furthermore, AP activity in the blood can also be used as a potential bio-marker for poor health, as its elevation is associated with poor liver health, bone resopotion, enhanced peripheral immune activation and general blood acidification (Geddes and Philpott, 2009; Lackeyram et al., 2010; Meidinger et al., 2012). Thus, responses in intestinal and serum AP activity kinetics may be a valuable biomarker to reflect effects of prebiotics and soluble fiber on the gut and whole body health status.

Due to the preliminary evidence suggesting many positive effects of β-glucan, and in spite of the few noted negative effects, and the accepted attributes of the prebiotics; retrograded resistant cornstarch, Fibersol-2™, and inulin, these soluble fibers were added to a standard corn-SBM diet that was formulated to meet NRC (1998) nutrient
requirements for weanling pigs of 5 to 10 kg of body weights. The impacts of these dietary additives were compared in terms of the production, nutrition and physiological responses. Each dietary additive was also compared with a diet formulated with the industry standard of 0.1% of a commercial antibiotic and one diet with no soluble fiber additive or antibiotic. The major objectives of this study was to identify positive attributes of these dietary additives in terms of the aforementioned endpoint responses and to use the findings to suggest alternatives to the current practice of feeding subtherapeutic levels of antibiotic to weanling pigs.

3.3. MATERIALS AND METHODS

3.3.1. Animals and Management

A total of 216 Yorkshire piglets, with a ratio of 1 barrow to 1 gilt, were used for this study. Piglets were divided into pens with six animals per pen. The study was done in three replicates with 72 pigs per replicated. Each replicate contained two blockes with 36 pigs per block. Pigs were weaned at 21 d of age and given *ad libitum* access to feed and water. Diets were provided in stainless-steel trough feeders measuring, 26.5 cm wide by 76 cm long and were 10 cm deep at the front and 14 cm deep at the back. These troughs were fitted with wire grates that were designed to prevent excessive spilling and contamination through piglets laying and jumping into feeders. Water was provided continuously through one dish per pen that measured 17 cm across and 15 cm deep, with a push activated water dispenser.
Pigs were obtained from the University of Guelph Arkell Swine Research Station and transported to the animal research wing in the Department of Animal and Poultry Science at the University. The groups of six piglets were housed in floor pens (160 cm in length by 130 m in width and 13 cm off the ground) with rubberized woven wire floors called Tenderfoot® with openings measuring 3.0 cm by 1.5 cm. The room was kept at 24°C and the pens were supplemented with heating lamps elevated about 65 cm above the piglet sleeping area. Each floor pen was equipped with a rubber mat measuring approximately 60 cm by 95 cm that was placed in the corner under the heating lamp to provide a comfortable sleeping area.

The pens were cleaned on a biweekly basis. Due to the design of the pens and the room in which the animals were housed, fewer cleanings would have greatly impacted the sanitary conditions and welfare of the animals due to unreasonable exposure to fecal contamination. Conversely, more frequent cleanings were expected to poorly represent the disease challenge experienced by pigs in a typical commercial facility.

Environment enrichment was provided in the form of bocce balls, Ping-Pong balls for the first two weeks and hanging chains with \( \frac{3}{4} \) cm diameter and 3 cm links. Furthermore, pigs were given 5 min per pen per d of positive contact time with a human. Positive contact included scratching and interacting with the piglets using the bocce balls and Ping-Pong balls.

Animals were fed the experimental diets for 21 d with \textit{ad libitum} access being allowed. Feeders and water dispensers were checked twice per d to ensure proper functioning, adequate fill and to clean and replace diets as needed. Soiled and waste diets were collected in foil trays (120 cm by 90 cm) that were placed beneath the feeders as
well as being removed from the feeders and then dried to an air-dry basis using drying ovens set at 65°C, if necessary, and weighed to determine how much test diet was consumed.

The Animal Care Committee at the University of Guelph approved the experimental protocol, sampling procedure and methods or techniques for the care and treatment of the animals in this study. The piglets used in this study were cared for in accordance with the guidelines set out by the Canadian Council on Animal Care (CCAC, 2009).

3.3.2. Experimental Diets and Design

A corn and SBM-based diet was formulated to meet NRC (1998) nutrient requirements for pigs of 5 to 10 kg body weights (BW) (TABLE 3.1). A negative control (NC) diet was formulated with no antibiotics and a positive control (PC) diet was formulated with 0.1% Lincomix® 44 premix, according to the standard commercial practice. Three prebiotics and β-glucan were added at a rate of 0.75% at the expense of cornstarch. The study was conducted according to a completely randomized block design with 6 treatments (diets), 3 replicates and 2 blocks per replicate. A single pen, containing 6 piglets, represented an experimental unit.

3.3.3. Measurements, Sample Collection and Sample Preparation

Body weights were measured upon arrival at d one of the trial when the piglets were 21 d old, d 8 of the trial when the piglets were 29 d old, d 15 of the trial when the piglets were 36 d old and then again on d 21, the end of the trial when the piglets were 42 d old. These data were used to calculate average daily gains (ADG) of the piglets over the duration of the trial.
Feed intake was measured daily on a per pen basis by weighing back any remaining diets at 0900 and 1700 h. Feeders were checked twice per d to ensure diets were continuously available and to remove soiled diets from the feeders. The collection trays placed below the feeders in each pen to catch spilled diets were also checked and emptied at 0900 and 1700 h every day. Wet diets removed from the feeders and collection trays were dried to an air-dry basis in drying ovens set at 65°C to allow removal of free water before weighing back to facilitate accurate calculations of diet consumption.

Fecal scoring was conducted on a per pen basis and was recorded daily. It was done on a scale of 0 to 3. Zero represented a healthy and normal stool and three represented an abnormal stool with signs of gastrointestinal distress. A detailed description of the scoring is listed in a footnote to TABLE 3.4. Diet and fecal samples were collected on d 11 (end of wk 2) and d 18 (end of wk 3) of the trial. Diet and fecal samples were freeze-dried and ground with a mortar and a pestle according to Rideout et al. (2004).

Venous blood samples were collected by puncture of the orbital sinus on the right or left side of one animal per pen on d 12 (end of wk 2) of the trial from physically immobilized pigs (Bregendahl et al., 2004). Blood was collected into pre-chilled plastic centrifuge tubes with half of these tubes containing SST™ and silica clot activator for separation of serum and the other half containing heparin for separation of plasma. After collection, the blood samples were placed on ice and transferred to the lab where they were centrifuged at 2000 x g for 20 min with a centrifugation temperature of 4°C to separate the serum and plasma fractions. These fractions were then removed, divided
into aliquots and frozen at -80°C for later analyses of plasma urea concentration and serum AP activity kinetics.

The proximal jejunal samples were collected from one pig per pen on d 15 of the trial when the pigs were 36 d old as an adequate window of opportunity to examine adaptive responses in the gut (Lackeyram et al., 2010). Cecal digesta was also collected on d 15 (beginning of wk 3) of the trial. The cecal digesta was collected after making a small incision after which the contents were poured into chilled, sterilized tubes, which were immediately flash frozen in liquid N\textsubscript{2} for later analysis. In order to obtain the necessary samples, each piglet was placed under general anesthesia using isoflurane (Bimed-MTC Animal Health Inc., Cambridge, ON, Canada) with inhalation through a facial mask at a scale of 3 – 4 with an oxygen flow rate of 2.0 – 2.5 L/min on a Bain circuit anesthetic machine through a passive scavenger system. Piglets were euthanized by an intra-cardiac injection of sodium pentobarbital (50 mg/kg BW) (Schering Canada Inc., Pointe-Claire, QC, Canada). The segmentation of the small intestine was prepared and itemized according to Lackeyram et al. (2010). The abdomen was opened and the entire small intestine distal to the ligament of Treitz and proximal to the ileo-cecal ligament was removed and was immediately flushed with an ice-cold saline solution containing 0.1 mmol/L phenylmethysulfonyl fluoride (PMSF). The segment of small intestine proximal to the ligament of Treitz was designated as the duodenum. The mesentery-free small intestinal segment proximal to the ileo-cecal ligament was designated to be the ileum. The remainder of the small intestinal segment was divided into 2 equal portions as the proximal and the distal jejunum (Lackeyram et al., 2010). Each of the four segments were removed and stored separately after being flash-frozen in
liquid N₂. The frozen proximal jejunal samples were subsequently pulverized under liquid N₂ using a mortar and a pestle and stored at -80°C.

3.3.4. Chemical and Biochemical Analyses

Both diet and fecal samples were analyzed for their dry matter (DM) content according to AOAC (1993). For the analyses of volatile short-chain fatty acids (VFA) and other volatile odor compounds, including phenols and indoles, pulverized freshly frozen cecal and fecal samples (2 g) were thawed and extracted with 100% methanol (6 mL). Decanol (0.1244 g per tube) was added at the beginning of the extraction as an internal standard for quantification. Following the extraction procedure, the samples were homogenized with a Power Gen homogenizer (700D, Fisher Scientific) at 10,000 rpm for 2 min and centrifuged at 800 × g for 20 min (Rideout et al., 2004). The supernatants (2 mL) were mixed with about 2 g of anhydrous Na₂SO₄ to remove moisture in order to minimize damage to the gas chromatography capillary column. The processed supernatant aliquots were analyzed by gas chromatography-mass spectrometry (GC-MS) by using a 6890-GC coupled with a Hewlett-Packard 5973N mass selective detector (Agilent Technologies Inc., Wilmington, DE). The mass spectrometer was operated on the electron impact mode at 70 eV, with typical scanned ranges of 35-400. The GC-MS system was programmed and controlled using a computer with a Hewlett-Packard ChemStation software. A HP-1 capillary GC column (30 m × 0.32 mm i.d. × 5 μm film thickness, Agilent Technologies Inc.) was used for the chromatographic separation (Rideout et al., 2004). The injection port of the GC was maintained at a temperature of 220°C. The column temperature was programmed to increase from 60 to 225°C at 5°C/min Sample injection volume was 1 μL with a 1:2 split ratio. Target compounds
were identified by matching the mass spectra of the total ion chromatographic peaks to reference spectra from a Wiley NBS mass spectral reference library carried by the GC-MS system. Compound identities were further confirmed by comparing the retention time and mass spectra to those of authentic standard compounds (Rideout et al., 2004).

Plasma urea concentration was determined using premade kits (Stanbio Laboratory, Boerne, TX) with commercial solutions that utilized the enzyme urease to hydrolyze urea to ammonia (NH$_3$) and carbon dioxide (CO$_2$). The ammonia then aminates $\alpha$-ketoglutarate to form glutamate with the concurrent oxidation of NADH to NAD$^+$ in the reaction catalyzed by glutamate dehydrogenase. The Stanbio Diagnostics blood urea reagent is formulated in such a way that the decrease in absorbance at 340 nm, resulting from the oxidation of NADH to NAD$^+$, is linearly proportional to blood urea concentration in the samples.

Pulverized proximal jejunal samples (about 1.3g) were thawed in an ice-cold homogenized buffer (50 mmol/L D-mannitol and 0.1 mmol/L PMSF at a pH of 7.4) at a ratio of 20 mL homogenizing buffer per g frozen intestinal tissue sample and homogenized using a polytron homogenizer. Protein content in the tissue homogenate samples was analyzed by using a commercial kit (Bio-Rad, Hercules, CA) and bovine serum albumin (fraction V) as the protein standard (Lackeyram et al., 2010). Enzyme activities for intestinal AP were conducted according to our previously established procedures (Fan et al., 2002). Potassium fluoride (KF) (2.0 mmol/L) was used in all AP activity assays to inhibit acid phosphatase activity (Fan et al., 2002). Kinetics of AP specific activities in the proximal jejunal tissue homogenate and the serum samples were carried out at 37°C for 10 min in a final volume of 1 mL suspension containing about 10
µg of sample protein, 2.0 mmol/L KF, 4.0 mmol/L MgCl₂, and varying levels of ρ-nitrophenyl phosphate (0–10.0 mmol/L) except that the substrate buffers were adjusted to pH 7.4.

### 3.3.5. Calculations and Statistical Analyses

The cecal and fecal contents of the VFA and odor-causing volatile compounds were calculated according to the internal standard method and were expressed per unit of samples DM. Plasma urea concentrations (mmol/L) were calculated by using a linear standard curve with absorbances measured at 340 nm, when samples were reacted with the urea working kit reagent. Each batch of plasma samples were run in association with a standard curve and the working reagent. The plasma urea concentration was then calculated through using the linear calibration curve.

Data were analyzed by using the Statistical Analysis Software 9.2 (SAS Institute, Cary, NC) for a mixed model of ANOVA according to the completely randomized block design.

The statistical model for the ANOVA according to a completely randomized block design with 6 diets (treatments), 6 replications (blocks) and 36 experimental units (pens) of a total of 216 animals, according to the following:

\[ y_{ij} = \mu + t_i + \rho_j + \varepsilon_{ij} \]

Where \( \mu \) is the general mean, \( t_i \) is the treatment effect, \( \rho_j \) is the block effect and \( \varepsilon_{ij} \) is the experimental error.

Comparisons between each test diet and each of the control diets were conducted by using the Dunnett-Hsu’s test. Comparisons among all the diets were carried out by using the Tukey-Krammer’s test for pairwise comparisons. Differences between and
among the treatment diets were considered to be significant for \( P < 0.05 \). The animals were sorted completely at random within each block without taking gender or family factors into account.

### 3.4. RESULTS

The effects of three prebiotic and \( \beta \)-glucan supplementations on the major growth performance endpoints are summarized and compared in Table 3.2. There were no differences \( (P > 0.05) \) in the initial and final BW and ADG of the test weanling pigs among the 6 experimental diets and between each of the supplemental diets and the NC or the PC diets for the study duration. Furthermore, the three prebiotic and \( \beta \)-glucan supplementations had no effects \( (P > 0.05) \) on average daily feed intake and gain to feed ratio (G:F) in the weanling pigs for the study duration.

The effects of three prebiotic supplements and \( \beta \)-glucan on plasma urea concentrations are summarized and compared in Table 3.3. Plasma urea concentration was evaluated in wk 1, wk 2 and wk 3 of the trial, respectively. The dietary treatments had no effects \( (P > 0.05) \) on the plasma urea concentrations in the weanling pigs in any of the three experimental weeks.

Fecal scores were evaluated on a daily basis and are summarized for wk 1, wk 2, wk 3, wk 1 to 2, and wk 1 to 3, respectively (Table 3.4). The fecal score during wk 1 for pigs fed the \( \beta \)-glucan-supplemented diet (diet 4) was higher \( (P < 0.05) \) than that of the animals fed the NC diet, the PC diet, the diet 3 containing retrograded resistant cornstarch, the diet 5 containing fibersol-2 and the diet 6 containing inulin when
compared by using the Tukey-Kramer’s test. When further compared with the Dunnett-Hsu’s test, the fecal score of pigs during wk 1 fed the β-glucan-supplemented diet (diet 4) was higher \( (P < 0.05) \) than that of pigs fed the NC or the PC diet. At the end of wk 2, the fecal score of pigs fed the diet 4 containing β-glucan was higher \( (P < 0.05) \) than that of the animals fed the NC diet, the diet 3 containing retrograded resistant cornstarch, the diet 5 containing fibersol-2 and the diet 6 containing inulin. For wk 3, the fecal score of pigs fed the diet 6 containing inulin was lower \( (P < 0.05) \) than the fecal scores of the animals fed the NC and the PC diets and the diet 4 containing β-glucan. The cumulative fecal score for wk 1 to 2 was higher \( (P < 0.05) \) in animals fed the diet 4 containing β-glucan than the fecal scores of the animals fed the other diets, including the NC, the PC and the three prebiotic-supplemented diets. The cumulative fecal score for wk 1 to 3 in animals fed the diet 4 containing β-glucan was higher \( (P < 0.05) \) than the fecal scores of the animals fed the NC diet, the retrograted resistant cornstarch-supplemented diet (diet 3), the diet 5 containing fibersol-2 and the diet 6 containing inulin. Furthermore, the cumulative fecal score for wk 1 to 3 in animals fed the diet 6 containing inulin was lower \( (P < 0.05) \) compared with the fecal scores of pigs consuming the PC and the β-glucan-supplemented diet 4.

Kinetics of AP activity in the proximal jejunum and serum are summarized in TABLE 3.5 and are further illustrated in Figures 3.1 – 3.3. Jejunal AP affinity \( K_m \) was higher \( (P < 0.05) \) in the retrograded resistant starch-supplemented diet (diet 3) than the fibersol-2-supplemented diet (diet 5). However, there were no significant differences in the \( K_m \) values among the other experimental diets. Jejunal AP maximal activity \( V_{max} \) was lower \( (P < 0.05) \) in the fibersol-2 containing diet (diet 5) and the inulin-containing diet
(diet 6) than the NC diet (diet 1) when these differences were examined by the Tukey’s and the Dunnett’s tests. When AP kinetics in serum samples of the tests pigs were analyzed by expressing AP specific activity in nmol/mg serum protein-min, there were no significant differences in the estimated $K_m$ and $V_{max}$ values among the dietary treatments. When AP specific activity was expressed as nmol/mL serum-min, differences in the $K_m$ values were observed ($P < 0.05$) between the retrograded resistant starch-supplemented diet (diet 3) and the β-glucan-supplemented diet (diet 4), and difference in the $V_{max}$ values was detected ($P < 0.05$) between the β-glucan-supplemented diet (diet 4) and the PC (diet 2).

Dietary effects on the concentrations of VFA and other volatile compounds in cecal digesta and fecal samples are summarized in TABLE 3.6 and TABLE 3.7. At the end of wk 2 and 3 of the trial, there were no significant dietary treatment effects on the individual VFA and other volatile compounds in the cecal digesta. At the fecal level, indole concentration was higher ($P < 0.05$) in pigs fed the PC diet than that of pigs fed the NC diet at the end of wk 2. There were generally no significant differences in the cecal and the fecal VFA and other volatile compound concentrations among the test diets largely due to very large variability in the endpoint measurements, as reflected by the very large standard errors associated with these endpoint means (TABLE 3.6 and TABLE 3.7).

3.5. DISCUSSIONS
The purpose of this study was to examine the impact of supplementations of the three prebiotics of retrograded resistant cornstarch, fibersol-2 and inulin and the viscous soluble fiber oat β-glucan on several growth performances, nutrient utilization, and large intestinal fermentation parameters of the weanling pigs fed corn and SBM-based diets in the absence of dietary supplemental lactose. In this study, the endpoints were evaluated on a weekly basis to more closely monitor the dietary effects. Furthermore, daily fecal scoring, and intestinal and serum biochemical biomarker AP activity kinetics were measured to create a more comprehensive understanding of changes that might have occurred as a result of consumption of these test diets by the weanling pigs fed and housed in groups similar to swine production conditions.

3.5.1. Growth Performance Responses

There were no significant differences in the major growth performance endpoints among the diets in this study. This was true for each dietary supplement in every weekly period examined. The reason for expecting improvements in growth performance is related to the well-known concept of commensal bacteria in stimulating attributes of prebiotics (Gibson et al., 2004) and positive immune modulation by some viscous soluble fibers like β-glucan (Ewaschuk et al., 2012; Murphy et al., 2012).

Retrograded resistant cornstarch is the first prebiotic under consideration for this study as its effects on human and animal gut health endpoints have been well reported. Unfortunately the available research results have not delivered clear answers regarding its effects on growth performances. Studies looking at the growth performance of weanling pigs fed retrograded resistant cornstarch are limited. De Schrijver et al. (1999) noted that pigs fed 6% retrograded cornstarch had a decreased ability to digest fat, however, they
did not evaluate changes in growth performance. Rideout et al. (2007) reported responses in the cecal butyrate concentration in grower pigs fed diets containing different types of resistant cornstarch but the animal’s growth performance endpoints were not measured in that study.

Fibersol-2 was also used as a prebiotic in this study. Its application did not reveal any improvements in growth performance in the weanling pigs. Fibersol-2 is a patented supplement, and because of this, there is a scarcity of available literature reports testing this product in the weanling pig. Rodriguez-Cabezas et al. (2010) reported the effects of fibersol-2 supplementation on immune responses and found significant improvements in rats. Unfortunately, changes in growth performances were not presented and discussed in their report (Rodriguez-Cabezas, 2010).

The results from this study showed that supplementation of inulin in the diet for up to 0.75% was not sufficient to cause improvements in growth performance characteristics in the weanling pigs fed corn and SBM-based diets. Pierce et al. (2005) also found no effects on growth performance endpoints when inulin was added to weaning pig diets at 0.015%. Furthermore, Maire et al. (2010) added inulin to weaning pig diets at 0.4% and could not find any improvements in growth performance.

Finally, oat β-glucan supplementation was also not able to impact the growth performance endpoints in the weanling pigs. This is different from Dritz et al. (1995) findings that diets supplemented with 0.025% yeast β-glucan did show improvements in ADG and average daily feed intake. Work done by Hiss and Sauerwein (2003) found that supplementing 0.03% of yeast-derived β-glucan slightly increased average daily feed intake but found no other improvements in any other growth performance endpoints. The
reason for differences between these studies could be related to the sources of β-glucan with it ranging from oat β-glucan to yeast β-glucan. Furthermore, the differences in levels of β-glucan supplemented should be an area of consideration. Although, the intuitive explanation that higher level of dietary supplementation of β-glucan would be related to a greater improvement in growth performances seems to be refuted by results of these aforementioned studies.

It is important to note that in our trial, growth performance traits were evaluated on a weekly basis to determine if the supplements had impacts that could be observed over short terms but whose effects became lost when evaluated over a much longer study duration. The basis for this reasoning is related to the rapid changing nature of the weanling pig digestive system. As the pig undergoes post-natal development, the activities of specific gut mucosal enzymes such as lactase and maltase change differentially (Miller et al., 1986; Lackeyram, 2012). Because these changes can be rapid at the age of weaning and with the introduction of a different weanling diet, the evaluation of the test diets’ impacts on the pig’s growth performance over shorter durations when metabolic processes such as digestive functions were in a state of large dynamic flux was a logical avenue for exploration. Our results found a lack of significant differences noted in these major growth performance endpoints between weeks in the weanling pigs.

3.5.2. Plasma Urea Concentration Responses

Responses in plasma urea concentrations were used as a measure of whole body crude protein and nitrogen utilization status. In this study, plasma urea concentrations were determined each wk of the study period to establish if there were potential
differential responses to these dietary treatments over three weeks. In general, plasma urea concentrations were numerically higher for all diets in wk 2 of the study but there were no significant differences between the test diets in any of the studied weeks.

Changes in plasma urea concentrations in the pig are the recognized biomarkers of whole body status of N utilization (Coma et al., 1995) and gut tissue hyperplastic growth, availability of blood circulating amino acids to peripheral muscle growth (Jiang et al., 2000) in pigs. Furthermore, plasma urea concentrations had been correlated with G:F rates in weanling pigs (Whang et al., 2000). The lack of dietary effects seen on the growth performance parameters aligned well with our findings that no changes in the plasma urea concentrations among the diets were noted in the weanling pigs of this study. Thus, the lack of significant differences in serum urea concentrations suggest that dietary supplementalations of the three prebiotics and β-glucan at 0.75% did not affect the whole body N utilization and the gut mucosal growth status in the weanling pigs in this study.

3.5.3. Fecal Score Responses

In this study, fecal scores were measured as an index to observe a more direct impact of the test diets on gut health responses. Prebiotics have been assessed in human trials for their ability to relieve constipation (Quigley, 2011) and regulate bowel movements (Sartor, 2004). Inulin has been noted for its gastrointestinal and bowel movement regulation in humans (Kleesen et al., 1997). It was expected that the immune modulating activity of the prebiotics (Schley and Field, 2002) and the soluble viscous fiber β-glucan (Chan et al., 2009) would cause an improvement in fecal characteristics, working as a diarrhea prevention strategy by improving the pigs’ ability to handle bacterial challenge. This may be due to a change in physical digesta form or a positive
change in the gut causing a decrease in diarrhea. Dietary inulin supplementation at 0.75% reduced the 3-wk cumulative fecal score and the difference between the inulin-supplemented diet (diet 6) and the PC diet was significant. These results of β-glucan on fecal score in the weanling pigs from this study seem to align well with other findings that have indicated that β-glucan-containing diets can decrease the consistency of the stool (Lund et al., 1989). However, the increase in the fecal score due to β-glucan supplementation was small in magnitude likely due to an increase in the fecal water-holding capacity and did not suggest an abnormal fecal status. Thus, dietary supplementations of oat β-glucan and inulin at 0.75% might have differentially influenced fecal manure characteristics and gut health status in the weanling pigs fed corn and SBM-based diets.

3.5.4. Alkaline Phosphatase Activity Kinetics

The significant difference in the AP affinity $K_m$ between the retrograded resistant cornstarch-supplemented diet (diet 3) and the fibersol-2 diet (diet 5) is interesting. In general, fibersol-2 supplementation at 0.75% numerically increased the jejunal AP enzyme affinity by about one fold but the difference was only significant between the retrograded resistant cornstarch-supplemented diet and the fibersol-2 diet. Furthermore, while the jejunal homogenate AP enzyme affinity $K_m$ values measured from this study are close to $K_m$ value specific to the jejunal apical membrane reported in the growing pigs (Fan et al., 1999; 2002), these jejunal homogenate AP enzyme affinity $K_m$ values measured from this study are much lower than the $K_m$ values specific to the apical membrane reported in the suckling and early-weaned pigs in our previous studies (Fan et al., 2002; Lackeyram et al., 2010). Gut mucosal homogenate AP specific activities
measured in this study are largely contributed by AP isomers associated with enterocytes and the intraepithelial lymphocytes. In enterocytes, AP is primarily expressed on the apical membrane via covalent bonds and is highly glycosylated (Fan et al., 1999). Furthermore, AP activities in the jejunal homogenates were conducted at a physiological pH of 7.4 in this study, whereas AP activity kinetics were conducted at an optimal pH of 10.5 in our previous studies (Fan et al., 2002; Lackeyram et al., 2010). Thus, changes in the intraepithelial lymphocyte population and status of enterocyte differentiation in the gut mucosa, as affected by fibersol-2, as well as the enzyme assay pH conditions might have influenced the $K_m$ values of the jejunal homogenate AP activities in the weanling pigs.

On the other hand, the significant decreases in the jejunal homogenate AP $V_{max}$ values in the fibersol-2-supplemented diet (diet 5) and the inulin-supplemented diet (diet 6) in comparison with the NC and/or PC diets are unexpected. Prebiotic and soluble fiber can improve whole body and gut local immunity by suppressing the activation of the major immune organs and this may be mediated via indirectly modulating pathogenic microflora in the gut mucosa (Schley and Field, 2002). Gut mucosal homogenate maximal AP specific activities measured in this study are collectively contributed by AP isomers associated with both enterocytes and the intraepithelial lymphocytes. In enterocytes, AP expression is cell differentiation dependent (Fan et al., 1999). It has been well documented that dietary prebiotics and soluble fibre contribute to enterocyte differentiation (Rideout et al., 2008). As the largest secondary immune organ, changes in the intraepithelial lymphocyte population and cell types, as potentially affected by prebiotic supplementations in diets, will likely affect gut mucosal immunity such as
cytokine profiles and other cellular and molecular responses such as AP activity associated with these immune cells. Thus, decreases in the jejunal homogenate AP $V_{max}$ values associated with the fibersol-2 and the inulin supplementations in the diets for the weanling pigs from this study might have been largely contributed to by the changes of adaptative immunity in the gut. It is also interesting to note the significant elevation of serum AP $V_{max}$ under betag-glucan supplementation compared with the PC feeding pigs. Serum AP activity are collectively contributed to by immune organs, liver, bones and the gut. The β-Glucans are highly fermentable and viscous soluble fiber components, and it has been recognized as an immune modulator when an innate immune pattern recognition receptor, Dectin-1, was identified as a β-glucan receptor (Volman et al., 2008; Willment et al., 2001). Dectin-1 is not expressed in enterocyte but is expressed in various cells of the gut associated innate immune system such as intraepithelial lymphocytes (Volman et al., 2010). Thus, the increase in the serum AP $V_{max}$ in response to the beta-glucan supplementation in this study might have been mediated via the Dectin-1 receptor in the gut immune cells in the weanling pigs.

3.5.5. Volatile Compounds

Changes in the short-chain fatty acids and other volatile compounds in cecal digesta and fecal samples were measured to determine if potential test dietary effects on gut heath and functions were mediated through changes in these volatile compounds as metabolites and signaling nutrient such as butyrate. Generally speaking, dietary supplementations of the three prebiotics and oat beta-glucan did not result in significant differences in the cecal and fecal concentrations of the volatile compound endpoints. It is noteworthy pointing out that the volatile compounds measured in the weanling pigs fed in
groups in this study were associated with much larger variability than the values in reported in our previous studies in grower pigs individually housed in metabolic crates (Rideout et al., 2004; 2008). Thus, the group-housing condition similar to swine production practices for the weanling pigs in this study is likely responsible for the large variability in the cecal and fecal concentrations of the volatile compound endpoints measured in this study.

There has been some interest in the potential for reduction in odor-causing compounds such as indole and skatole in pig feces related to prebiotic application. Feeding chicory root, which is rich in inulin, to boars was shown to decrease in skatole concentrations in blood and backfat (Hansen et al., 2006). Dietary supplementation of chicory inulin extract at 5% dramatically reduced fecal skatole content in growing pigs (Ridout et al., 2004). Furthermore, Kim (2010) found that application of several types of prebiotics to pig fecal slurries was able to decrease the concentrations of indole and skatole, as well as some other odor causing compounds. The reasons for the differing results may be related to dietary inclusion levels with inulin applied at very high levels, up to 14% of the diet in the first study, which could account for the noted differences. In the second study, the differences observed may be related to the in vitro application.

There was also some expectation that butyrate concentration might increase in association with consumption of a test diet containing one of the test prebiotics or β-glucan. Prebiotics are fermented into VFAs in the hindgut and butyrate has been well studied and noted for its positive attributes as a signaling nutrient in stimulating mucosal cell differentiation. Butyrate concentration was found to be increased in weanling pigs feed 10% fructooligosaccharides (Tsukahara et al., 2003), which is contrary to the
findings of this study. Again, this may be related to inclusion levels of the prebiotics used in the study by Tsukahara et al. (2003) were much higher than the level (0.75%) of prebiotics used in this study. One practical concern of including high levels of prebiotics in weanling pig diets is the high cost associated with the supplementation.

3.5.6. Concluding Remarks

Dietary supplementations of the three prebiotic and oat β-glucan at 0.75% had little effects on growth performances but might affect the gut and whole body health status via influencing the AP detoxification kinetics in the weanling pig. The changes in AP affinity and maximal activity in the jejunal homogenate and serum in responses to feeding fibersol-2, inulin and β-glucan support the concept that prebiotics and β-glucan may modulate whole body gut health status by affecting alkaline phosphatase activity kinetics.
TABLE 3.1. Composition of experimental diets for the weanling pigs

<table>
<thead>
<tr>
<th>Item</th>
<th>Diet 1</th>
<th>Diet 2</th>
<th>Diet 3</th>
<th>Diet 4</th>
<th>Diet 5</th>
<th>Diet 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Item</td>
<td>(NC)</td>
<td>(PC)</td>
<td>(RCS)</td>
<td>(β-G)</td>
<td>(F-2)</td>
<td>(IN)</td>
</tr>
<tr>
<td><strong>g/kg</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td>27.80</td>
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<td>0.20</td>
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<td>0.09</td>
<td>0.09</td>
<td>0.09</td>
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<td>0.25</td>
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<tr>
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<td>Solka-Flock⁹</td>
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<td>6.00</td>
<td>6.00</td>
<td>6.00</td>
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<tr>
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<td>0.00</td>
<td>1.07</td>
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1. Composition of experimental diets for the weanling pigs
2. Experimental diets
3. SPC
4. Lysine-HCl
5. DL-Methionine
6. Iodized salt
7. Min-Vit premix
8. Sweetner
9. Solka-Flock
10. Feed antibiotic
11. RCS-RS
12. β-Glucan
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<td>0.00</td>
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<td>Titanium oxide</td>
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Dietary nutrient contents (on a air-dry basis)\(^6\):

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<tr>
<td>DE, MJ/kg</td>
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<td></td>
<td></td>
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<tr>
<td>Crude protein, %</td>
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<td>23.23</td>
<td>23.23</td>
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<tr>
<td>Total calcium, %</td>
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<td>0.80</td>
<td>0.80</td>
<td>0.80</td>
<td>0.80</td>
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<tr>
<td>Total phosphorus, %</td>
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<td>0.65</td>
<td>0.65</td>
<td>0.65</td>
<td>0.65</td>
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<tr>
<td>Total amino acids:</td>
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<tr>
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<td>1.52</td>
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<td>Histidine</td>
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<td>0.63</td>
<td>0.63</td>
<td>0.63</td>
<td>0.63</td>
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<tr>
<td>Isoleucine</td>
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<tr>
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<td>1.95</td>
<td>1.95</td>
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<tr>
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<td>1.43</td>
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<tr>
<td>Methionine</td>
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<td>Cysteine</td>
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<td>0.36</td>
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<td>1.09</td>
<td>1.09</td>
<td>1.09</td>
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<tr>
<td>Tyrosine</td>
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<td>0.83</td>
<td>0.83</td>
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<td>0.83</td>
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<tr>
<td>Threonine</td>
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<td>0.92</td>
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<td>0.92</td>
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<tr>
<td>Valine</td>
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<td>1.10</td>
<td>1.10</td>
<td>1.10</td>
<td>1.10</td>
</tr>
</tbody>
</table>
Pigs weaned at 21 d of age and fed the diets for 3 wk post-weaning to meet or exceed the NRC (1998) nutrient requirements for pigs of 5-10 kg BW.

Diet 1 as the negative control (NC); diet 2 as the positive control (PC); diet 3 with retrograded resistant cornstarch (RCS); diet 4 with β-glucan (β-G); diet 5 with Fibersol-2™ (F-2); and diet 6 with inulin (IN).

Soybean protein concentrate (SPC) supplied by Floradale Feed Mill (Floradale, ON, Canada).

Crystalline lysine-HCl of 79% purity, commercially available.

Crystalline DL-Methionine of 99% purity, commercially available.

Supplied by the Windsor Salt Co. (Toronto, ON, Canada). Composition (g/kg): NaCl, 965.0; ZnO, 40.0; FeCO₃, 1.6; MnO, 1.2; CuO, 0.33; Ca(IO₃)₂, 0.07; and CaO, 0.04.

The trace mineral and vitamin premix supplied the followings per kg of diet commercially available from the DSM Nutritional Products Inc. (Ayr, ON, Canada) with guaranteed analyses of the followings: copper, 15.0 mg; iodine, 0.5 mg; iron, 100 mg; manganese, 20.0 mg; selenium, 0.30 mg; and zinc, 105.0 mg; vitamin A, 10000 IU, vitamin D₃, 1000 IU, vitamin E, 40 IU; vitamin K, 2.5 mg; thiamine, 1.5 mg; riboflavin, 5.0 mg; pyridoxine, 1.5 mg; vitamin B₁₂, 0.025 mg; niacin, 25 mg; d-pantothenic acid, 15.0 mg; folic acid, 2.0 mg; d-biotin, 0.200 mg; choline, 500 mg.

Saccharine sweetener commercially available from Lucta (Barcelona, Spain).

Solka-Floc®, pure cellulose commercially available from International Fiber Corporation (North Tonawanda, NY).

Feed antibiotic of Lincomix® 44 premix supplied by Elanco Canada Inc. (Guelph, ON, Canada) for providing 0.044 g lincomycin per kg of the positive control diet.
Retrograded high amylose cornstarch (RCS) with resistant starch (RS) content at about 42% from the National Starch (Bridgewater, NJ).

β-glucan with β-(1-3) and β-(1-4) branching extracted from oats at 70% purity donated by Garuda International Inc. (Lemon Cove, CA).

A commercial trade name for resistant maltodextrin (100%) donated by DSM, Matsutani LLC (Clinton, IA).

Inulin (100% purity) marketed by Nealanders International Inc. (Mississauga, On, Canada).

Nutrient digestibility marker purchased from Fisher Scientific (Ottawa, ON, Canada).

Calculated according to NRC (1998).


**TABLE 3.2.** The effects of three prebiotics and β-glucan on growth performance in weanling pigs fed corn and soybean meal-based diets

<table>
<thead>
<tr>
<th>Experimental diets&lt;sup&gt;1&lt;/sup&gt;</th>
<th>Diet 1</th>
<th>Diet 2</th>
<th>Diet 3</th>
<th>Diet 4</th>
<th>Diet 5</th>
<th>Diet 6</th>
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<tbody>
<tr>
<td>Item</td>
<td>(NC)</td>
<td>(PC)</td>
<td>(RCS)</td>
<td>(β-G)</td>
<td>(F-2)</td>
<td>(IN)</td>
</tr>
<tr>
<td>Body weight</td>
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<tr>
<td>Initial</td>
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<td>Average daily gain</td>
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<tr>
<td>Average daily feed intake</td>
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Gain:feed $\text{kg/kg}$

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<td>0.9</td>
<td>0.6</td>
<td>0.3</td>
<td>0.7</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>0.8</td>
<td>0.7</td>
<td>0.4</td>
<td>0.7</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>0.7</td>
<td>0.6</td>
<td>0.4</td>
<td>0.6</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>0.44</td>
<td>0.08</td>
<td>0.14</td>
<td>0.14</td>
<td>0.06</td>
</tr>
</tbody>
</table>

$^1$See **TABLE 3.1** for details of diet formulations. Diet 1 as the negative control (NC); diet 2 as the positive control (PC); diet 3 with retrograded resistant cornstarch (RCS); diet 4 with $\beta$-glucan ($\beta$-G); diet 5 with Fibersol-2™ (F-2); and diet 6 with inulin (IN).

$^2$Pooled standard errors of means ($n = 6$).

Endpoints without common or different superscript letters do not differ ($P > 0.05$).
**TABLE 3.3.** The effects of three prebiotics and β-glucan on blood plasma urea concentrations in weanling pigs fed corn and soybean meal-based diets

<table>
<thead>
<tr>
<th>Item</th>
<th>Diet 1 (NC)</th>
<th>Diet 2 (PC)</th>
<th>Diet 3 (RCS)</th>
<th>Diet 4 (β-G)</th>
<th>Diet 5 (F-2)</th>
<th>Diet 6 (IN)</th>
<th>SEM²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wk 1</td>
<td>1.0</td>
<td>1.0</td>
<td>1.1</td>
<td>1.0</td>
<td>0.9</td>
<td>1.0</td>
<td>0.16</td>
</tr>
<tr>
<td>Wk 2</td>
<td>1.7</td>
<td>1.1</td>
<td>1.9</td>
<td>2.0</td>
<td>1.7</td>
<td>1.8</td>
<td>0.18</td>
</tr>
<tr>
<td>Wk 3</td>
<td>1.4</td>
<td>1.3</td>
<td>1.5</td>
<td>1.6</td>
<td>1.4</td>
<td>1.4</td>
<td>0.12</td>
</tr>
</tbody>
</table>

¹See TABLE 3.1 for details of diet formulations. Diet 1 as the negative control (NC); diet 2 as the positive control (PC); diet 3 with retrograded resistant cornstarch (RCS); diet 4 with β-glucan (β-G); diet 5 with Fibersol-2™ (F-2); and diet 6 with inulin (IN).

²Pooled standard errors of means (n = 6).

Endpoints without common or different superscript letters do not differ (P > 0.05).
TABLE 3.4. The effects of three prebiotics and β-glucan on fecal scores\(^1\) of weanling pigs fed corn and soybean meal-based diets

<table>
<thead>
<tr>
<th>Item</th>
<th>Diet 1 (NC)</th>
<th>Diet 2 (PC)</th>
<th>Diet 3 (RCS)</th>
<th>Diet 4 (β-G)</th>
<th>Diet 5 (F-2)</th>
<th>Diet 6 (IN)</th>
<th>SEM(^3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wk 1</td>
<td>0.6(^a)</td>
<td>0.6(^a)</td>
<td>0.7(^a)</td>
<td>0.8(^b)#</td>
<td>0.6(^a)</td>
<td>0.6(^a)</td>
<td>0.19</td>
</tr>
<tr>
<td>Wk 2</td>
<td>1.3(^a)</td>
<td>1.4(^ab)</td>
<td>1.3(^a)</td>
<td>1.5(^b)*</td>
<td>1.3(^a)</td>
<td>1.2(^a)</td>
<td>0.28</td>
</tr>
<tr>
<td>Wk 3</td>
<td>1.2(^a)</td>
<td>1.3(^a)</td>
<td>1.1(^ab)</td>
<td>1.3(^a)</td>
<td>1.2(^ab)</td>
<td>1.1(^b)#</td>
<td>0.10</td>
</tr>
<tr>
<td>Wk 1 to 2</td>
<td>1.0(^a)</td>
<td>1.0(^a)</td>
<td>1.0(^a)</td>
<td>1.2(^b)*</td>
<td>1.0(^a)</td>
<td>0.9(^a)</td>
<td>0.13</td>
</tr>
<tr>
<td>Wk 1 to 3</td>
<td>1.0(^ac)</td>
<td>1.1(^ab)</td>
<td>1.0(^ac)</td>
<td>1.2(^b)*</td>
<td>1.0(^ac)</td>
<td>1.0(^c)#</td>
<td>0.10</td>
</tr>
</tbody>
</table>

\(^1\)Fecal scoring done on a scale of 0 to 3. 0 = no abnormalities, feces have normal shape and colour; 1 = mild abnormality with signs of running fecal consistency and abnormal stool is happening 1 to 2 times daily; 2 = moderate abnormality with running feces being deposited frequently; and 3 = severe abnormality with bloody running feces occurring and being deposited at least once a day.

\(^2\)See TABLE 3.1 for details of diet formulations. Diet 1 as the negative control (NC); diet 2 as the positive control (PC); diet 3 with retrograded resistant cornstarch (RCS); diet 4 with β-glucan (β-G); diet 5 with Fibersol-2™ (F-2); and diet 6 with inulin (IN).

\(^3\)Pooled standard errors of means (n = 6).

\(^a,b,c\) Means that diets with different superscript letters differ (P < 0.05) when compared with the Tukey-Kramer test.

\(^*\)Difference (P < 0.05) from diet 1 (NC) as compared with the Dunnett-Hsu’s test.

\(^#\)Difference (P < 0.05) from diet 2 (PC) as compared with the Dunnett-Hsu’s test.
Endpoints without common or different superscript letters do not differ ($P > 0.05$).
**TABLE 3.5.** The effects of three prebiotics and β-glucan on jejunal and serum alkaline phosphatase activity kinetics\(^1\) in weanling pigs fed corn and soybean meal-based diets

<table>
<thead>
<tr>
<th>Item</th>
<th>Experimental diets(^1)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Diet 1</td>
</tr>
<tr>
<td>Jejunal alkaline phosphatase</td>
<td></td>
</tr>
<tr>
<td>(K_m) (^3)</td>
<td>0.17(^{abc})</td>
</tr>
<tr>
<td>(V_{max}) (^4)</td>
<td>40.41(^a)</td>
</tr>
<tr>
<td>Serum alkaline phosphatase</td>
<td></td>
</tr>
<tr>
<td>(K_m) (^3)</td>
<td>2.78</td>
</tr>
<tr>
<td>(V_{max}) (^4)</td>
<td>0.90</td>
</tr>
<tr>
<td>Serum alkaline phosphatase</td>
<td></td>
</tr>
<tr>
<td>(K_m) (^3)</td>
<td>3.05(^{abc})</td>
</tr>
<tr>
<td>(V_{max}) (^5)</td>
<td>54.02(^{abc})</td>
</tr>
</tbody>
</table>

\(^1\)See **TABLE 3.1** for details of diet formulations. Diet 1 as the negative control (NC); diet 2 as the positive control (PC); diet 3 with retrograded resistant cornstarch (RCS); diet 4 with β-glucan (β-G); diet 5 with Fibersol-2™ (F-2); and diet 6 with inulin (IN).

\(^2\)Pooled standard errors of means (\(n = 6\)).

\(^3\)\(K_m\), mmol/L.

\(^4\)\(V_{max}\), nmol/mg jejunal or serum protein.min.

\(^5\)\(V_{max}\), nmol/mL serum.min.
\textsuperscript{a,b,c} Means that diets with different superscript letters differ \((P < 0.05)\) when compared with the Tukey-Kramer’s test.

* Difference \((P < 0.05)\) from diet 1 (NC) as compared with the Dunnett-Hsu’s test.

# Difference \((P < 0.05)\) from diet 2 (PC) as compared with the Dunnett-Hsu’s test.

Endpoints without common or different superscript letters do not differ \((P > 0.05)\).
**TABLE 3.6.** The effects of three different prebiotics and β-glucan on the concentrations$^1$ of volatile short-chain fatty acids (VFA) and other volatile odor compounds in cecal digesta of weanling pigs fed corn and soybean meal-based diets for 2 and 3 weeks

<table>
<thead>
<tr>
<th>Item</th>
<th>Diet 1</th>
<th>Diet 2</th>
<th>Diet 3</th>
<th>Diet 4</th>
<th>Diet 5</th>
<th>Diet 6</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(NC)</td>
<td>(PC)</td>
<td>(RCS)</td>
<td>(β-G)</td>
<td>(F-2)</td>
<td>(IN)</td>
</tr>
<tr>
<td>Wk two$^3$</td>
<td>µmol/g DM of cecal digesta</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetic acid</td>
<td>202</td>
<td>271</td>
<td>287</td>
<td>158</td>
<td>286</td>
<td>215</td>
</tr>
<tr>
<td>Propionic acid</td>
<td>160</td>
<td>225</td>
<td>217</td>
<td>128</td>
<td>235</td>
<td>170</td>
</tr>
<tr>
<td>Isobutyric acid</td>
<td>26</td>
<td>33</td>
<td>43</td>
<td>20</td>
<td>60</td>
<td>28</td>
</tr>
<tr>
<td>Butyric acid</td>
<td>224</td>
<td>299</td>
<td>311</td>
<td>174</td>
<td>991</td>
<td>250</td>
</tr>
<tr>
<td>MA$^4$</td>
<td>51</td>
<td>68</td>
<td>89</td>
<td>45</td>
<td>104</td>
<td>55</td>
</tr>
<tr>
<td>Isovaleric acid</td>
<td>72</td>
<td>114</td>
<td>142</td>
<td>74</td>
<td>154</td>
<td>88</td>
</tr>
<tr>
<td>Valeric acid</td>
<td>164</td>
<td>233</td>
<td>248.</td>
<td>142</td>
<td>259</td>
<td>171</td>
</tr>
<tr>
<td>Hexanoic acid</td>
<td>148</td>
<td>244</td>
<td>295</td>
<td>145</td>
<td>280</td>
<td>173</td>
</tr>
<tr>
<td>P-cresol</td>
<td>10</td>
<td>7</td>
<td>11</td>
<td>5</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>4-Ethylphenol</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Indole</td>
<td>3</td>
<td>6</td>
<td>6</td>
<td>5</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>Skatole</td>
<td>20</td>
<td>10</td>
<td>21</td>
<td>10</td>
<td>4</td>
<td>11</td>
</tr>
<tr>
<td>Total</td>
<td>1318</td>
<td>1806</td>
<td>1913</td>
<td>1135</td>
<td>2631</td>
<td>1478</td>
</tr>
<tr>
<td>Wk three$^5$</td>
<td>µmol/g DM of cecal digesta</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetic acid</td>
<td>89</td>
<td>77</td>
<td>106</td>
<td>702</td>
<td>140</td>
<td>84</td>
</tr>
</tbody>
</table>

$^1$ Concentrations are expressed as µmol/g DM of cecal digesta. $^2$ Differences in volatile compounds were observed between (PC), (RCS), (β-G), (F-2), (IN) and SEM. $^3$ Wk two were performed after 2 weeks of treatment. $^4$ MA, acetamide. $^5$ Wk three were performed after 3 weeks of treatment.
<table>
<thead>
<tr>
<th>Acid</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Propionic acid</td>
<td>64</td>
<td>57</td>
<td>85</td>
<td>60</td>
<td>136</td>
<td>63</td>
<td>30.4</td>
</tr>
<tr>
<td>Isobutyric acid</td>
<td>11</td>
<td>12</td>
<td>19</td>
<td>13</td>
<td>40</td>
<td>10</td>
<td>12.2</td>
</tr>
<tr>
<td>Butyric acid</td>
<td>84</td>
<td>82</td>
<td>123</td>
<td>80</td>
<td>161</td>
<td>85</td>
<td>27.4</td>
</tr>
<tr>
<td>MA&lt;sup&gt;4&lt;/sup&gt;</td>
<td>19</td>
<td>27</td>
<td>41</td>
<td>27</td>
<td>90</td>
<td>16</td>
<td>27.9</td>
</tr>
<tr>
<td>Isovaleric acid</td>
<td>34</td>
<td>41</td>
<td>64</td>
<td>42</td>
<td>105</td>
<td>30</td>
<td>29.4</td>
</tr>
<tr>
<td>Valeric acid</td>
<td>60</td>
<td>58</td>
<td>94</td>
<td>62</td>
<td>140</td>
<td>55</td>
<td>32.6</td>
</tr>
<tr>
<td>Hexanoic acid</td>
<td>59</td>
<td>53</td>
<td>93</td>
<td>62</td>
<td>135</td>
<td>53</td>
<td>31.9</td>
</tr>
<tr>
<td>P-cresol</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>24</td>
<td>0</td>
<td>9.2</td>
</tr>
<tr>
<td>4-Ethylphenol</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>52</td>
<td>0</td>
<td>21.1</td>
</tr>
<tr>
<td>Indole</td>
<td>1</td>
<td>3</td>
<td>4</td>
<td>4</td>
<td>39</td>
<td>2</td>
<td>15.2</td>
</tr>
<tr>
<td>Skatole</td>
<td>4</td>
<td>0</td>
<td>3</td>
<td>2</td>
<td>553</td>
<td>0</td>
<td>224.7</td>
</tr>
<tr>
<td>Total</td>
<td>503</td>
<td>509</td>
<td>741</td>
<td>1152</td>
<td>1688</td>
<td>494</td>
<td>534.7</td>
</tr>
</tbody>
</table>

<sup>1</sup>Values are means and pooled SEM (<i>n</i> = 6).

<sup>2</sup>See **TABLE 3.1** for details of diet formulations. Diet 1 as the negative control (NC); diet 2 as the positive control (PC); diet 3 with retrograded resistant cornstarch (RCS); diet 4 with β-glucan (β-G); diet 5 with Fibersol-2™ (F-2); and diet 6 with inulin (IN).

<sup>3</sup>Samples were collected on Thursday of wk two, d 11 of the study.

<sup>4</sup>MA, 2-methylbutyric acid.

<sup>5</sup>Samples were collected on Thursday of wk three, d 18 of the study.

Endpoints without common or different superscript letters do not differ (<i>P</i> > 0.05).
**TABLE 3.7.** The effect of three different prebiotics and β-glucan on the concentrations\(^1\) of volatile short-chain fatty acids (VFA) and other volatile odor compounds in feces of weanling pigs fed corn and soybean meal-based diets for 2 and 3 weeks

<table>
<thead>
<tr>
<th>Experimental diets(^2)</th>
<th>Diet 1 (NC)</th>
<th>Diet 2 (PC)</th>
<th>Diet 3 (RCS)</th>
<th>Diet 4 (β-G)</th>
<th>Diet 5 (F-2)</th>
<th>Diet 6 (IN)</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Item</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wk two(^3) (\mu mol/g DM of feces)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetic acid</td>
<td>2159</td>
<td>1673</td>
<td>1697</td>
<td>2193</td>
<td>2071</td>
<td>1791</td>
<td>1178.9</td>
</tr>
<tr>
<td>Propionic acid</td>
<td>1142</td>
<td>961</td>
<td>881</td>
<td>1356</td>
<td>1251</td>
<td>1086</td>
<td>702.1</td>
</tr>
<tr>
<td>Isobutyric acid</td>
<td>298</td>
<td>572</td>
<td>283</td>
<td>366</td>
<td>294</td>
<td>297</td>
<td>212.4</td>
</tr>
<tr>
<td>Butyric acid</td>
<td>2277</td>
<td>1631</td>
<td>1807</td>
<td>2300</td>
<td>2195</td>
<td>1807</td>
<td>1290.6</td>
</tr>
<tr>
<td>MA(^4)</td>
<td>316</td>
<td>316</td>
<td>321</td>
<td>464</td>
<td>291</td>
<td>350</td>
<td>217.2</td>
</tr>
<tr>
<td>Isovaleric acid</td>
<td>540</td>
<td>553</td>
<td>520</td>
<td>724</td>
<td>534</td>
<td>583</td>
<td>363.0</td>
</tr>
<tr>
<td>Valeric acid</td>
<td>860</td>
<td>795</td>
<td>781</td>
<td>1154</td>
<td>968</td>
<td>1004</td>
<td>591.5</td>
</tr>
<tr>
<td>Hexanoic acid</td>
<td>388</td>
<td>420</td>
<td>348</td>
<td>667</td>
<td>229</td>
<td>542</td>
<td>353.9</td>
</tr>
<tr>
<td>P-cresol</td>
<td>360</td>
<td>289</td>
<td>368</td>
<td>410</td>
<td>341</td>
<td>323</td>
<td>200.8</td>
</tr>
<tr>
<td>4-Ethylphenol</td>
<td>21</td>
<td>13</td>
<td>11</td>
<td>13</td>
<td>21</td>
<td>19</td>
<td>8.7</td>
</tr>
<tr>
<td>Indole</td>
<td>121(^a)</td>
<td>54(^b)</td>
<td>74(^ab)</td>
<td>92(^ab)</td>
<td>91(^ab)</td>
<td>85(^ab)</td>
<td>65.6</td>
</tr>
<tr>
<td>Skatole</td>
<td>238</td>
<td>235</td>
<td>314</td>
<td>318</td>
<td>287</td>
<td>266</td>
<td>158.8</td>
</tr>
<tr>
<td>Total</td>
<td>9073</td>
<td>7839</td>
<td>7768</td>
<td>10409</td>
<td>8921</td>
<td>8502</td>
<td>5431.0</td>
</tr>
<tr>
<td>Wk three(^5) (\mu mol/g DM of feces)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetic acid</td>
<td>338</td>
<td>286</td>
<td>237</td>
<td>258</td>
<td>254</td>
<td>602</td>
<td>129.4</td>
</tr>
<tr>
<td>Compound</td>
<td>Diet 1</td>
<td>Diet 2</td>
<td>Diet 3</td>
<td>Diet 4</td>
<td>Diet 5</td>
<td>Diet 6</td>
<td>Total</td>
</tr>
<tr>
<td>-------------------</td>
<td>--------</td>
<td>--------</td>
<td>--------</td>
<td>--------</td>
<td>--------</td>
<td>--------</td>
<td>-------</td>
</tr>
<tr>
<td>Propionic acid</td>
<td>210</td>
<td>182</td>
<td>160</td>
<td>170</td>
<td>170</td>
<td>535</td>
<td>137.1</td>
</tr>
<tr>
<td>Isobutyric acid</td>
<td>53</td>
<td>54</td>
<td>40</td>
<td>45</td>
<td>45</td>
<td>202</td>
<td>60.7</td>
</tr>
<tr>
<td>Butyric acid</td>
<td>366</td>
<td>294</td>
<td>256</td>
<td>275</td>
<td>274</td>
<td>628</td>
<td>132.0</td>
</tr>
<tr>
<td>MA&lt;sup&gt;4&lt;/sup&gt;</td>
<td>80</td>
<td>89</td>
<td>68</td>
<td>67</td>
<td>71</td>
<td>358</td>
<td>110.4</td>
</tr>
<tr>
<td>Isovaleric acid</td>
<td>123</td>
<td>140</td>
<td>106</td>
<td>110</td>
<td>194</td>
<td>455</td>
<td>133.4</td>
</tr>
<tr>
<td>Valeric acid</td>
<td>232</td>
<td>216</td>
<td>187</td>
<td>196</td>
<td>190</td>
<td>586</td>
<td>144.9</td>
</tr>
<tr>
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<td>6</td>
<td>5</td>
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<td>7</td>
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<td>9</td>
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</table>

<sup>1</sup>Values are means and pooled SEM, (<i>n</i> = 6).

<sup>2</sup>See TABLE 3.1 for details of diet formulations. Diet 1 as the negative control (NC); diet 2 as the positive control (PC); diet 3 with retrograded resistant cornstarch (RCS); diet 4 with β-glucan (β-G); diet 5 with Fibersol-2™ (F-2); and diet 6 with inulin (IN).

<sup>3</sup>Samples were collected on Thursday of wk two, d 11 of the study.

<sup>4</sup>2-methylbutyric acid.

<sup>5</sup>Samples were collected on Thursday of Wk Three, d 18 of the study.

<sup>a,b</sup>Means that diets with different superscript letters differ (<i>P</i> < 0.05) when compared with the Tukey-Kramer’s test.

<sup>*</sup>Difference (<i>P</i> < 0.05) from diet 1 (NC) as compared with the Dunnett-Hsu’s test.

Endpoints without common or different superscript letters do not differ (<i>P</i> > 0.05).
Figure 3.1. Kinetics of AP activity in the proximal jejunum from weaning pigs fed the experimental diets for three weeks. See Table 3.1 for details of the diet formulations. Diet 1 as the negative control (NC); diet 2 as the positive control (PC); diet 3 with retrograded resistant cornstarch (RCS); diet 4 with β-glucan (β-G); diet 5 with Fibersol-2™ (F-2); and diet 6 with inulin (IN). Each point represents mean ± pooled SEM, n = 6 representative pigs from 6 replicate pens.
Figure 3.2. Kinetics of serum AP activity per mg serum protein from weaning pigs fed the experimental diets for three weeks. See Table 3.1 for details of the diet formulations. Diet 1 as the negative control (NC); diet 2 as the positive control (PC); diet 3 with retrograded resistant cornstarch (RCS); diet 4 with β-glucan (β-G); diet 5 with Fibersol-2™ (F-2); and diet 6 with inulin (IN). Each point represents mean ± pooled SEM, n = 6 representative pigs from 6 replicate pens.
Figure 3.3. Kinetics of serum AP activity per mL of serum from weaning pigs fed the experimental diets for three weeks. See TABLE 3.1 for details of the diet formulations. Diet 1 as the negative control (NC); diet 2 as the positive control (PC); diet 3 with retrograded resistant cornstarch (RCS); diet 4 with β-glucan (β-G); diet 5 with Fibersol-2™ (F-2); and diet 6 with inulin (IN). Each point represents mean ± pooled SEM, n = 6 representative pigs from 6 replicate pens.
CHAPTER 4

GENERAL DISCUSSION AND CONCLUSIONS

Weaning is a highly volatile time in the life of a young pig. Separation from the sow, mixing with other animals and the introduction of new dietary ingredients all combine to challenge the animal’s ability to thrive and grow to its full potential. Because of this, antibiotics have long been included in weanling pig diets, which have contributed to the rise of antibiotic resistance and the pursuit of alternatives.

The recognition that prebiotics are able to modulate gut function and the evidence from early studies in pigs presenting positive improvements in performance has led to increased interest in this area. Unfortunately the data in the literature that are currently available do not paint a very clear picture of how prebiotics affect pig gut health and growth performances. The highly diverse nature of the gut microbiota makes predicting the impact given prebiotic additives will have on hindgut bacteria very difficult. This diversity resulting in prediction difficulty has caused in convoluted results in the literature.

Further complicating the discussion of prebiotics is the discussion over the definition of a prebiotic and what can be conclusively labeled as such. Three of the prebiotic additives used in this study, retrograded resistant cornstarch, fibersol-2 and inulin, have accepted prebiotic effects and have been labeled as prebiotics by the scientific community at large. Other soluble fibre additives, such as β-glucan are not yet well studied enough to garner the prebiotic label. Furthermore, sugar supplements such
as lactose have received some attention for their potential prebiotic effect as it relates to the potential influence on gut microbiota.

It is under these considerations that the studies in this thesis were designed and with that we can consider the main findings as follows.

4.1. Main Research Findings and Conclusions

1. No significant changes to growth performance endpoints were noted in weanling pigs fed test diets containing 0.75% of either; retrograded resistant cornstarch, fibersol-2, inulin or β-glucan.

2. Dietary lactose, included in the diet up to 12%, was completely digested by in the weaning pigs as a rapid digestible carbohydrate rather than serving as an effective prebiotic.

3. β-Glucan significantly increased the diarrhea score of weanling pigs fed the test diet containing 0.75% of the carbohydrate, to the nature of enhancing the water-holding capacity of feces because of rather small magnitude of changes.

4. β-Glucan supplementation was associated with changes in serum AP maximal activity that seemed to be independent of the proximal jejunal homogenate AP maximal activity level, which is reflected by the distinctive P enzyme affinity from each of these physiological areas in the weanling pigs. These results support a concept that β-glucan may mediate whole body health status by modulating circulatory blood AP maximal activity for potentially detoxification of lipopolysaccharides.

5. Fibersol-2 feeding was associated with much higher jejunal homogenate AP enzyme affinity than retrograded resistant cornstarch feeding in the weanling pigs.
Fibersol-2 and inulin supplementations at 0.75% was also associated with significantly lower levels of the jejunal homogenate AP maximal activity compared with the NC or the PC diets, suggesting a reduced activation of adaptive immune responses in the gut in weanling pigs fed these prebiotic supplemented diets.

6. Large intestinal and fecal short-chain fatty acids and other volatile compound contents analyzed in samples collected from the weanling pigs fed under group housing conditions similar to swine production practice were more variable and less reliable as endpoints for assessing efficacy of prebiotic effects. Jejunal and serum AP kinetic analyses have the potential to be a valuable biomarker for assessing efficacy of prebiotic effects in weanling pigs.

4.2. General Discussion

The application of prebiotics has a great deal of potential for application in animal feeding. The effect the gut bacterial ecosystem has on whole body health and performance seems to be vast and varied. Unfortunately the potential for prebiotic treatment has yet to be fully realized due to the extremely complex nature of the gut ecosystem and the need for more research. Our current findings indicate that 0.75% supplementation of the test prebiotic and soluble fibre additives was not enough to stimulate changes in the growth performance endpoints. The reason for this is difficult to determine with certainty at this point in time. Roberfroid (2005) suggests that the nature of the gut bacterial ecosystem is too complicated for simple blanket levels of prebiotics to be applied to multiple animals with the expectation that similar results will be seen. This may be the possible reason with no noted effects on the growth performance endpoints.
But it seems more likely that, in this case, lack of growth performance response results may be more related to level of supplementations. Outliers in the data were not common and as a further bolster to this point, other studies that found a positive impact associated with feeding prebiotics had inclusions as high as 10%. Although Roberfroid (2005) suggests that the levels are less important than matching amount and supplement type to the bacterial profile of the animal. Very low levels would ensure that any positive effect that is possible might not be observed.

Finally the consideration of lactose as a rapidly digestible and economical sugar that is digested by animals’ residual lactase in the weanling pig is an important point for reflection. It is well known that growth performance is substantially improved as a result of lactose being included in the weanling pig diet but there has been recent debate over the location of digestion. These data, in conjunction with the data collected by Lackeyram (2012), clearly show that the residual lactase digestive capacity in the weaned pig can sufficiently digest high levels of dietary supplemental lactose. These are useful data as they indicate that prebiotic test diets for weanling pigs can be formulated to contain usual levels of lactose without any concern for convoluted prebiotic trial data. A better understanding of where lactose is digested in the digestive tract and how quickly, will help create of more accurate understanding of the changes associated with weaning and where lactose is broken down along the small intestinal tract and potentially what digestive end-product sugars, including glucose and galactose, may enter the hind gut. This information could be useful when creating an accurate picture of what digestive end product sugar molecules are potentially being absorbed and fermented in the hindgut on weanling pigs.
Retrograded resistant cornstarch, fibersol-2, inulin and β-glucan added at to a corn and SBM-based test diets at 0.75% are not effective at changing growth performance endpoints. These additives also did not cause any noteworthy changes in various volatile compounds in the cecal digesta and feces or blood urea concentrations. There was a small magnitude of significant impact of β-glucan supplementation on fecal diarrhea score likely to the nature of water-holding capacity, but this effect was not easy to link to any other dietary effects noted.

There were changes in AP $V_{\text{max}}$ associated with fibersol-2 and inulin-supplemented test diets. These results were not easily related to any other findings. These changes in AP kinetics seem to indicate that while the ability of the gut to detoxify lipopolysaccharides is decreased, adaptive immune responses in the gut may be less activated in the gut mucosa of the weanling pigs. But no negative impacts on growth performances were noted, indicating these changes were relatively subtle likely due to the fact these pigs were not housed under challenged sanitary environmental conditions.

Perhaps of a greater interest is the change in the serum AP $V_{\text{max}}$ related to β-glucan supplementation in the Chapter 3 study. The findings seem to corroborate the suggestion that β-glucan could have acted as a signaling molecule as has already been shown. This is related to the significant changes noted in serum $V_{\text{max}}$ that are not seen in the jejunal $V_{\text{max}}$ compared with the control diet(s). Results of this nature could indicate that the serum AP kinetics are altered because the β-glucan in the gut is mediating its effects via binding its receptors that have an impact on other parts of the body through cascade type effects.
Another important consideration derived from this research is the ability of the weanling pig to very easily digest lactose. This data suggests there is no prebiotic effect of lactose but further research is needed. This research used enzyme data collected from pigs from a different trial. Differences in diet and experimental conditions make these levels difficult to apply with a high degree of certainty. This information has the potential to change the diet formulations for future weanling pig prebiotic studies. If lactose does not have a prebiotic effect it can be included in diets formulated to observe the impact of prebiotics but follow up research is needed to confirm this.

4.4. Suggestions for Future Study

Additional studies looking at growth performance parameters, fecal scores, plasma urea concentration, AP kinetics and volatile compound concentration should be carried out. Additional studies should evaluate retrograded resistant cornstarch, fibersol-2, inulin and β-glucan at multiple graded levels that reflect effective supplementation effects in the literature. For several of these additives this can be as high as 10%. In conjunction with this, evaluation of the pork industry should be completed to determine what added cost can be tolerated by producers to assess the efficacy of the results gathered.

Looking into the free-water content (i.e., measuring water-holding capacity) in digesta and feces from pigs fed β-glucan containing diets could be an interesting area of consideration. Developing an understanding of the reasons for the increased fecal score is an important area for understanding if β-glucan supplementation is to be considered further. An increase in free-water content would be a more acceptable answer for the
increased diarrhea score than gut irritation and therefore increased gut motility or digesta passage rate.

Beyond this, a more careful consideration of the effects prebiotics have on AP kinetics would be useful. In general, the noted decrease in the jejunal homogenate \( V_{max} \) seems contradictory to the expected positive attributes associated with these two well-studied and commonly described prebiotics. But additional research into how these supplements can impact AP activity kinetics could create more clarity in this area. Future studies should be conducted to partition the jejunal homogenate AP kinetics into apical membrane-associated (i.e., enterocyte-specific) and soluble (i.e., intraepithelial lymphocyte-specific) components. An important methodological consideration is to analyze the AP kinetics at its maximal pH of 10.5 in samples, so the measured AP kinetic parameter estimates will be much less variable and are more reliable for comparisons. Furthermore, the potential for \( \beta \)-glucan to act as a signaling molecule that changes the visceral organs such as liver AP expression and other peripheral organs and tissues such as the primary immune organs as well as the bone should be examined in potential future studies.
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