EVALUATION OF DIRECT-FED MICROBIALS AND XYLANASE SUPPLEMENTATION ON PERFORMANCE AND NUTRIENT DIGESTIBILITY IN PIGS FED HIGH FIBER DIETS

A THESIS
SUBMITTED TO THE FACULTY OF GRADUATE SCHOOL
OF THE UNIVERSITY OF MINNESOTA
BY

Hayford Manu

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF
MASTER OF SCIENCE

Dr. Samuel Kofi Baidoo

April 2015

ACKNOWLEDGMENTS

I would like to thank the almighty God for providing me with strength and wisdom to go through the Master of Science academic program. Many thanks to my supervisor, DR. Samuel K. Baidoo for his unflinching support, patience, respect for humanity, tolerance that never runs out and inculcating into us the habit of appreciating others. Work at graduate school cannot be completed without graduate committee members, Dr. Samuel K. Baidoo, Dr. Sally Noll and Dr. Peter Davies. I appreciate your time for reading through my manuscripts and enormous suggestions as well as guidance that you provided to make my education at graduate school holistic. Mrs. Christie Baidoo was the mother of all. I thank you for inviting us to your home more often and crowning each visit with your delicious meal as well as giving us free family recipes. Mr. Josh Parker was very helpful and responsive during the intestinal morphology measurements. I would also like to thank Dr. Augustine Naazie of University of Ghana Livestock and Poultry Research Center (LIPREC) for the monumental role he played to make my ambition to study in United State of America a reality. My gratitude goes to Prof Kwame Offei (Provost College of Agricultural and Consumer Science), Prof Hanna Barnes and Prof Yangyuoru (Director of Agricultural Research Institute). Without your support graduate school education would not have been possible. I would like to acknowledge the graduate students and staff of the swine unit at Southern Research and Outreach Center, University of Minnesota, Waseca, MN. Last but not the least; I would like to thank my wife, Mrs. Mercy Manu for holding the fort and caring for the children in Ghana when I was studying abroad. Many are those who deserve to be recognized but unfortunately cannot write all the names here. I thank you all tremendously.

Overall Abstract

The main aim of the thesis is to investigate Direct-fed (DFM) Microbial supplementation alone or in combination with xylanase in high fiber diet on performance and nutrient digestibility in pigs. To achieve this main aim, three experiments were conducted.

In study I (I chapter 3), Calsporin® biotechnical feed additive based on viable spores of Bacillus subtilis C-3102 was investigated alone. An experiment was conducted to evaluate the effect of Calsporin® on growth performance; microbial population and carcass characteristics of wean to finish pigs. One hundred and ninety-two (Topigs 20 x Compart's Duroc; 18 d, initial BW (6.7±0.27 kg)) crossbred piglets were allocated two treatment groups in a randomized complete block design. The results showed that supplementation of Calsporin® did not affect ($P > 0.05$) blood urea nitrogen (BUN) concentration. Calsporin® did not influence ($P > 0.05$) the final body weight, average daily gain but numerically reduced average feed intake (ADFI) which resulted in a potential to improve feed conversion efficiency during the overall period of study at an inclusion rate of 1.5 X 10^5/g of diet. The measured carcass characteristics, including hot carcass weight, yield percentage, back fat depth, loin depth, and lean percentage, were not affected ($P > 0.05$) by the dietary treatments. Calsporin® in the diet did not significantly affect ($P > 0.05$) the total counts of Enterobacteriaceae, Bifidobacteria and total anaerobes in the feces but Lactobacillus count was reduced ($P < 0.05$).

Key words: Pigs, Calsporin, growth performance, carcass traits, microbial enumeration

In studies II and III different DFMs were used. Study II (Chapter 3) was conducted to investigate the effect of supplementing xylanase alone or in combination with either *Bacillus* species direct-fed microbials (DFM1) or *lactobacillus* multi-species direct-fed microbials (DFM2) on nutrient digestibility, visceral organ weight and intestinal morphology of nursery pigs fed high fiber diets in a 14 d trial. Thirty-six male pigs (30 d old, 9.0 ± 0.15 kg) of genotype [(Landrace × Yorkshire (Topigs, Winnipeg, Canada)) × Duroc (Compart's, Nicollet, MN) were allocated to 1 of 6 dietary treatments in a randomized complete block design. Six dietary treatments evaluated were T1, basal diet without xylanase or DFM as control; T2, control supplemented with DFM1 at 500g/MT; T3, control supplemented with xylanase 250g/MT; T4, xylanase (250g/MT) and DFM1 (500g/MT); T5, DFM2 (500g/MT) and T6, xylanase 250g/MT and DFM2 (500g/MT). The study reveal that pigs supplemented with DFM1 in combination with xylanase had an improvement ($P < 0.05$) in intestinal length, numerically improved apparent ileal crude protein digestibility by 21 percentage units and reduced water intake compared with the combination of DFM2 with xylanase. Supplementation of DFM1 did not influence DM, CP, fat, NDF and ADF compared to DFM2. Supplementation of xylanase alone significantly improved ATTD of fat, NDF, ADF and numerically improved apparent ileal crude protein digestibility by about 10 percentage units compared to the control. Dietary treatments did not affect pH of ileal digester content ($P > 0.05$) but pH of stomach chyme was reduced ($P < 0.05$) with combination of xylanase and DFM2 and tended to decrease ($P = 0.53$) with combination of xylanase and DFM1 relative to the average effect of xylanase and the DFMs. Addition of xylanase to DFM1 led to a numerical reduction in organ weights of kidney, spleen, cecum and emptied GIT

compared to the control. Dietary treatments did not affect ($P > 0.05$) ATTD of energy, ileal digester viscosity, villus height, crypt depth and villus to crypt ratio compared to the control.

Key words: Xylanase, Direct-fed microbials, fibre diets, nursery pigs, viscosity and digestibility.

In Study III, (Chapter 5) The effect of supplementing xylanase alone or in combination with either *Bacillus* species direct-fed microbials (DFM1) or *lactobacillus* multi-species direct-fed microbials (DFM2) on effect of blood urea nitrogen (BUN) concentration and growth performance of nursery pigs fed high fiber diets was studied in a 28 d trial. One hundred and forty-four (18 d old) [(Landrace × Yorkshire (Topigs, Winnipeg, Canada)) × Duroc (Compart's, Nicollet, MN) pigs were allocated to 1 of 6 dietary treatments with 6 replicates in a randomized complete block design. The treatments were T1, basal diet without xylanase or DFM as control; T2, control supplemented with DFM1 at 500g/MT; T3, control supplemented with xylanase 250g/MT; T4, xylanase (250g/MT) and DFM1 (500g/MT); T5, DFM2 (500g/MT) and T6, xylanase 250g/MT and DFM2 (500g/MT). The study indicated that xylanase, DFM1 or DFM2 individual supplementation did not influence BUN concentrations ($P > 0.05$) but addition of xylanase to either DFM1 or DFM2 numerically reduced BUN concentration ($P > 0.05$) by 26% and 11.5% compared to the control. Comparison between DFM1 and DFM2 did not affect pig performance ($P > 0.05$) during the study. Supplementation of xylanase, DFM1 and DFM2 alone or in combination did not influence ($P > 0.05$) final body weight, average daily feed intake, average daily gain and feed efficiency during the phase I and II of the study. Combination of xylanase with

DFM1 and DFM2 synergizes to numerically reduce feed intake and improve feed efficiency ($P < 0.05$). In conclusion, supplementation of DFMs alone did not impact on production performance but addition of xylanase to either DFM1 or DFM2 numerically reduced BUN concentration and feed intake compared to the control ($P > 0.05$) during the overall period of study. The combination of xylanase with DFMs synergizes to improve feed efficiency ($P < 0.05$) during the overall period of study relative to the individual supplementation.

Key words: Xylanase, Direct-fed microbials, fibre diets, nursery pigs, BUN and performance.

Table of contents **Page**

List of tables

Table No.Chapter 2 **Page No.**

Chapter 5

Chapter 6

List of figures

Figure No. Chapter 2 **Page No.**

Chapter 4

Chapter 5

Chapter 1

INTRODUCTION

The primary goal of food animal production is to provide meat, milk, fiber and eggs that are safe for human consumption taking into consideration the welfare of the animal and the impact on the environment. Pathogens such as Salmonella and Campylobacter are transmitted along the food chain and serve as a source of concern for livestock producers and food processors. Antibiotics have been included in the diet of animals at sub-therapeutic dose to act as growth promoters (Dibner and Richards, 2005) and to ward off bacterial infection. However, consumer concerns about development of antimicrobial resistance and transference of antibiotics resistance genes from animal to human microbiota (Mathur and Singh, 2005; Salyers et al., 2004) resulted in the European Union banning the use of antibiotic growth promoters in animal feed since January 1, 2006 (EC 2001, 2003a). Withdrawal of antibiotics in the feed led to negative consequences on performance and substantial increase in the use of therapeutic antibiotics (Casewell et al., 2003a). Viable alternatives to antibiotics are required to stimulate the natural defense mechanisms of animals and reduce extensive use of antibiotics (Verstegen and Williams, 2002) in order to improve the gut microbiota. A balanced gut microbiota prevents colonization of pathogens, produces metabolic substrates (vitamins and short-chain fatty acids) and stimulates the immune system in a non-inflammatory manner. Direct-fed microbials, prebiotics, synbiotics and enzymes could be possible alternatives to resist colonization by pathogenic organisms to improve the health status of the animals by enhancing nutrient digestibility and performance.

The main objective of this thesis therefore is to investigate the use of Direct-fed Microbials alone or in combination with xylanase on pig performance. To achieve this aim three studies were conducted.

In study I, early weaned pigs received Calsporin® Direct-fed Microbial supplementation for 141 d. The objectives of the study were to evaluate production performance, microbial counts and effect on carcass traits.

Study II was carried out to evaluate the effect of bacillus Direct-fed Microbials (DFM1), lactobacillus multi-species direct-fed microbials (DFM2) and xylanse in nursery pigs. The objective was to evaluate the effect of DFMs or xylanase alone or in combination on water intake, viscosity of ileal digesta, intestinal morphology, organ weights, apparent total tract digestibility (ATTD) of DM, Energy, CP, ADF and NDF, and apparent ileal digestibility (AID) of DM, CP and energy in nursery pigs fed high fiber diets from 23 d post weaning.

Study III was undertaken to assess the effect of bacillus Direct-fed Microbials (DFM1), Lactobacillus multi-species Direct-fed Microbials (DFM2) and xylanse in nursery pigs. The objective was to investigate the effect of DFMs or xylanase alone or in combination on growth performance and blood urea nitrogen concentration (BUN) in nursery pigs fed on high fiber diet 28 d post weaning.

Chapter 2

LITERATURE REVIEW

Historical Perspective and Economic Importance of Probiotics

Bacteria are ubiquitous on the planet and one of the most foremost life forms to appear on Earth. Many bacteria are beneficial to mankind in a variety of ways. The economic importance of bacteria to mankind in the field of agriculture, nutrition and medicine cannot be over-emphasized. Notable examples are decomposition of organic matter for nutrients recycling, fermentation of silage, production of yoghurt, fixation of atmospheric nitrogen and production of antibiotics and vaccines. Much of these landmarks would have gone unnoticed but for the work of Russian biologist Eli Metchinikoff, who worked at Pasteur Institute in Paris at the beginning of the 20th century. In his curiosity to demystify the high life expectancy of over 115 years of Cossacks people of Bulgaria; he observed high consumption of fermented milk product amongst the people at the time. Eli Metchinikoff identified the organism responsible for the fermentation as Lactobacillus bulgaricus and associated human health and longevity with ingestion of these beneficial bacteria (Sonia, 2005). Thereafter the organism was used around 1920's to treat gastrointestinal diseases and diarrhea in humans (Busch et. al., 2004). Interest in probiotics dwindled for about four decades but rekindled between 1960's and 1970's for both livestock and human used.

The first probiotics to satisfy the requirement as feed additives appeared in the European market around the mid-1980s. Besides, the last decades have witnessed the phenomenal boost in the use as probiotic organisms (Kumari et al., 2011; Wells, 2011). Currently, there is a wide range of probiotics that can be used in poultry and livestock

production to improve animal performance. Most of these products are produced from Bacillus spores, Lactic acid bacteria and Yeast. Mono-strain probiotics are probiotics containing one strain of a particular species and multi-strain probiotics contains two or more strains of the same species or closely related species such as *Lactobacillus* casei and *Lactobaccilus acidophilus*. Multi-species probiotics contain species that belong to more than one genus such as *Lactococcus lactis*, *Bifidobacterium longum* and *Enterococcus faecium.* To distinguish between probiotics that are used in food animal production from those used in humans, US-FDA recommends the term Direct-fed microbials for probiotics that are used in livestock production (CDRF, 2011).

What are Probiotics, Prebiotics and Synbiotics?

Probiotics

Probiotics are defined as "live micro-organisms which when administered in adequate amount can confer health benefit to the host by maintaining the intestinal microbial balance" (Chaucheyras-Durand & Durand, 2010; Fuller, 1989). Probiotics are also known as bio regulators, intestinal micro flora stabilizers or direct-fed microbials (DFMs). The United States Food and Drug Administration (US-FDA) recommends that the term Direct-Fed microbial be used for probiotics that are used in livestock production as a distinction from probiotics that are used in humans. Strictly speaking, the term probiotics is limited to products for stabilizing the intestinal microflora that consist of one, or a few, well-defined strains of microorganisms (Holzapfel et al., 2001). The term probiotics is also used in human nutrition and medicine but contrary to animal nutrition, can apply to both inactivated and live microorganisms (Busch et al., 2004). Currently, the

E.U has authorized many different preparations feed additives of DFM's to be included in the diets for livestock (Anadón, 2006). Three different groups of bacteria are mainly used: Lactic acid bacteria (*Lactobacillus species, Enterococcus faecium*), Bacillus (*Bacillus subtilis, Bacillus cereus, and Bacillus licheniformis*) and Saccharomyces yeast (*Saccharomyces cerevisiae*). According to Simon et al., (2003), these DFM products are supplemented at concentrations of 10^8 to 10^9 CFU/kg of diet in a pelleted mixed feed.

Prebiotics

Prebiotics are defined as "non-digestible carbohydrate that beneficially affects the host by preferentially stimulating the growth and /or activity of one or a limited number of bacteria in the digestive tract" (Gibson and Roberfroid, 1995). Increasing prebiotics in the diet is likely to increase and maintain healthy bacterial gut flora in the host (Gibson et al., 2003; Sander, 1998). Diets can be fortified with prebiotics during manufacturing processes to increase probiotic efficacy (Ranadheera et al., 2010). However, for any food ingredient to be classified as a prebiotic it must satisfy these requirements: (1) it should not be hydrolyzed or absorbed in the upper part of the GIT. (2) It must serve as substrate specific to one or few beneficial micro-organisms promoting their growth at the detriment of pathogenic micro-organisms and (3) it must alter the intestinal microbiota to a healthier composition (Collins and Gibson, 1999).

Synbiotics

Synbiotics may be defined as mixtures of probiotics and prebiotics that beneficially affect the host by improving the survival and implantation of live microbial dietary supplements in the gastrointestinal tract (Gibson and Roberfroid, 1995). In human

medicine, synbiotics are also used (Roberfroid, 1998). Effect of synbiotics in swine production is limited although the results are promising. Studies conducted by Nemcova et al., (1999), showed that supplementation of a combination of fructo-oligosaccharide and probiotics to piglets increased lactobacillous, bifidobacterium, total anaerobes and aerobe counts with a decrease in enterobacteria and clostridium counts. Other scientists observed improved growth rate (Kumpretcht and Zobac, 1998) and decreased mortality rate (Nousiainen and Setala, 1993). Similarly, Estrada et al., (2001) fed fructooligosaccharides concurrently with Bifidobacteria longum and found an improvement in feed efficiency.

Criteria for Consideration as Probiotics Micro-organisms

Probiotics micro-organisms incorporated into feed or food products should not lose their functionality and viability or impact negatively on flavors and textures of animal products. They must survive the upper GIT environment to reach their site of action alive. For probiotic organisms to function effectively in the host, they must possess the following characteristics (Parvez et al., 2006, Sonia, 2005; Pal, 1999 and Salminen et al., 1996):

- Ability to colonize the intestine of the host.
- Ability to adhere to the intestinal epithelial lining.
- Ability to survive and resist pH and bile acids.
- Be nonpathogenic and non-toxic.
- Provide benefit to the host.

- Be gram positive.
- Contain a minimum of 3×10^{10} colony forming unit per gram.
- Be stable upon storage.
- Not carry transmissible antibiotic resistance gene

Plasmids are present in the genome of bacterial species and are responsible for transferring antibiotic resistance genes (Deasy, 2009). Plasmids carrying resistant genes degrade antibiotic molecules and become more resistance against the antibiotics. Belletti et al., (2009) provided an example of beta-lactamases as bacterial enzymes that can split the beta-lactam ring of penicillin antibiotics, cephalosporins, carbapenems, and monobactams making the antibiotics ineffective.

Common Probiotics Use in Food Animal Production

Most of the probiotics used in animal nutrition today as feed additives belong to one of three different groups of beneficial microorganisms: lactic acid bacteria, *Bacillus* spores, and yeasts. They differ from one another in their properties, origin and mode of action.

I. Lactic Acid Bacteria

Lactic acid bacteria (*Lactobacilli, Pediococci, Bifidobacteria and Enterococci*) are regarded as natural microbiota of the gastrointestinal tract. They are Gram positive, without cytochromes; anaerobic but aero tolerant; fastidious, acid tolerant, strictly fermentative and produce lactic acid as the main product (Stiles and Holzapfel, 1997).

They exert their influence in the intestinal lumen through the formation of a biofilm to protect the intestinal mucous membrane, secretion of lactic acid, reduction of pH, and eventually reduce *E. coli* and *Enterobacteria* counts (Nousiainen and Setala, 1998).

II. Bacillus species

The soil is the natural habitat for *Bacillus species*, and they are used as probiotics either as single strain or multi-strain preparations (Hong et al., 2005). Bacilli species do not colonize the intestine and are therefore, by definition, are the transient flora. Species commonly used as probiotics are *Bacillus subtilis*, *Bacillus clausii*, *Bacillus coagulan*, *Bacillus licheniformis* and *Bacillus cereus.* In humans, spore probiotics are used as dietary supplements, growth promoters and competitive exclusion agents in livestock production and in aquaculture to enhance the growth and disease resistance of cultured shrimps (Sharma and Devi, 2014). The natural ability of Bacilli probiotics to form spores offers good protection against external influences. The finished product is therefore, stable upon storage in feed. When Bacillus spores are ingested with the feed, they germinate in the digestive tract and develop into vegetative cells but do not proliferate.

III. Saccharomyces Boulardii (Yeast)

Saccharomyces boulardii, are obtained from plant sources and have potential to stimulates intestinal immunity and protect the host against scours (Bust et al., 1990). Probiotic yeast has been successfully used in treating intestinal infections, especially diarrhea (Surawicz et al., 1989; Mombelli; Gismondo, 2000). The spore forming probiotics are heat stable and have some advantages over nonspore formers such as Lactobacillus species. They can be stored in desiccated form without losing viability and

can survive the low pH of the stomach (Spinosa et al., 2000; Barbosa et al., 2005). According to Tuohy et al., (2007), spores can be stored indefinitely without refrigeration and the administered dose will reach the small intestine intact. However, the main activity of probiotics is the maintenance and reconstitution of the equilibrium (eubiosis) of the intestinal micro-flora, which is achieved by various modes of action.

Colonization of Probiotic Micro-organisms in the GIT

At birth, the gastrointestinal tract (G.I.T) is sterile. Immediately after birth, the entire G.I.T. is colonized by microorganisms which may reach concentrations as high as 10^9 CFU/g in 12 hours at the distal colonic content (Swords et al., 1993). The source of the microorganisms could be vaginal secretions, colostrum from the dam or the environment. Initial colonization is mostly by facultative anaerobes such as enterobacteria, coliforms, and lactobacilli (Benno and Mitsuoka, 1986). Competition for nutrients leads to modifications of the microbiota from primary succession to secondary succession until climax is attained where microbiota is stabilized (Ewing and Cole, 1994). The number of micro-organisms in the GIT of an average human is estimated at 10^{14} microbial cells, which are more than 10 times the total cells in human body (Luckey and Floch, 1972). The majority of these micro-organisms are found in the colon at a concentration of 5×10^{11} bacterial cells per gram of digesta, representing more than 400 species. Most of these micro-organisms are Gram-positive, anaerobic genera *Bacteriodes, Eubacterium,* and *Bifidobacterium*. Other microbes such as *Clostridia, Streptococci* and *Lactobacilli* also play important roles in the maintenance of stable gut mucosa and

generation of short chain fatty acids in a beneficial ratio (Holzapfel and Schillinger, 2002).

Diet has major effect on gut microbiota. Early weaning in swine production is recommended to increase the number of times the sow can farrow. However, early-weaned piglets are exposed to several stressors such as nutritional, environmental, social, and microbiological. Disruption in a stabilized microbial equilibrium where beneficial micro-organisms dominate is known as dysbiosis. The consequence is decreased immune response; increase diarrhea and post-weaning growth check (McCracken et al., 1999). To restore the equilibrium (eubiosis), probiotics are administered to enhance the growth of beneficial organism and ultimately colonize the G.I.T. Therefore the basic notion of probiotic supplementation is to restore the disrupted microbial ecology with new and beneficial bacteria.

Direct-fed Microbials Mechanism of Action

Direct-fed microbials are fed to improve the intestinal microbial balance in favor of the indigenous microbes (Havenar and Huis, 1992). In general, the exact mechanisms of Direct-fed microbials are not clearly elucidated. Several mechanisms of action have been proposed to protect the host from intestinal disorders (Rolfe, 2000; Lee et al, 1999). Probiotic effects are strain, species and dose specific. Probiotic micro-organisms are known to produce inhibitory substances such as organic acids, hydrogen peroxide and bacteriocins. All lactic acid bacteria produce organic acid. It is believed that supplementation of probiotics would increase the levels of volatile fatty acids, thereby decreasing the pH of intestinal and creating an unfavorable environment for opportunistic

pathogens. Competitive exclusion at adhesion site is another mechanism proposed for probiotic micro-organisms. E-coli is prevented from adhering to the intestinal epithelial surface so that it colonization in intestinal tract is impeded (Conway et al., 1987). Probiotics are also known to compete favorably with pathogenic organisms to prevent them from acquiring nutrients and consequently the pathogens are expelled from the intestinal tract. It is postulated that probiotics can stimulate specific and nonspecific immunity to protect against intestinal diseases (Fukushina et al., 1998).

Calsporin as Microbial Feed Additives

Calsporin® is the trade name of a microbial feed additive based on viable spores of *Bacillus subtilis* C-3102. The product manufacturer, Calpis is seeking authorization for the use of the product as a zootechnical feed additive for piglets. This product has been approved for meat chickens in the European Union until October 2016 (EFSA, 2010). The European Commission mandated the European Food Safety Authority to prove the efficacy of the Calsporin additive and its safety for the target livestock, the consumer, the user of the product and the environment. According to EFSA, the bacterial species is safe for the targeted animals (poultry and pigs), users of the product, the consumer and the environment. Results of five feeding trials were reported, each with piglets given the additive at the proposed dose of 3×10^8 CFU/kg compared to a control group fed the same diet without Calsporin. A significant improvement in feed to gain ratio and increase in final body weight and average daily gain in animals given the additive at the proposed dose compared to control animals was seen in three trials. Consequently, Calsporin® can

be considered as effective at the proposed dose of 3 x 10^8 CFU/kg in a complete diet (EFSA, 2010).

Characterization of Calsporin

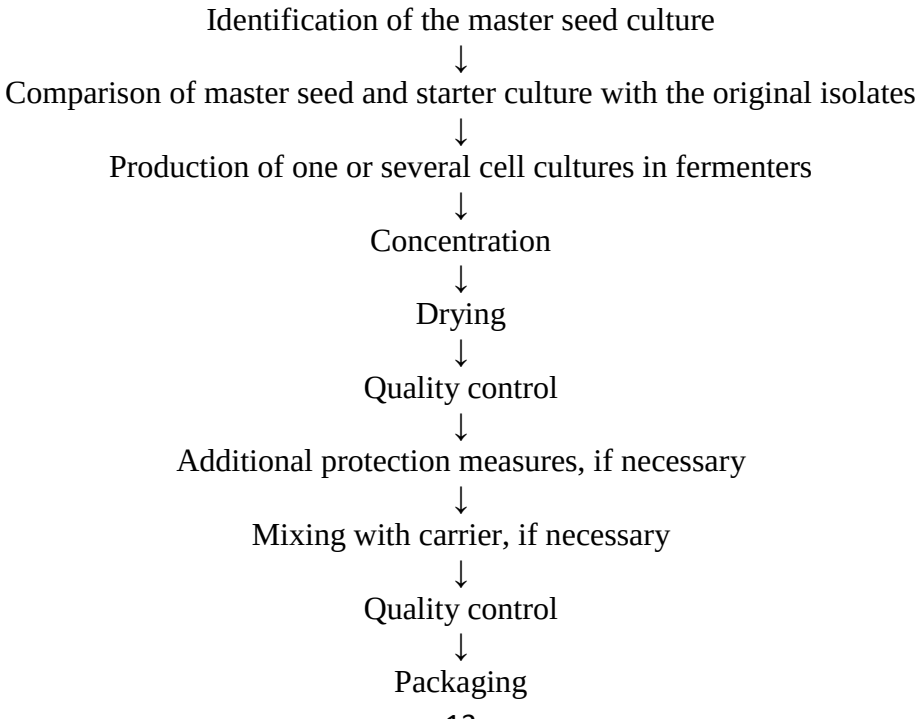
Viable endospores of the single strain of *B. subtilis* were originally isolated from Japanese soil and deposited in the German Collection of Microorganisms and Cell Cultures (DSMZ) with the accession number DSM 15544. *Bacillus subtilis* has not been modified through genetic engineering and do not harbor plasmids. After fermentation the medium and cells are dried and pasteurized to kill vegetative cells and sufficient amounts of calcium carbonate are added to produce a final product meeting the specification of 1.0 x 10^{10} CFU/g of *B. subtilis.* The final product is powdery with an average particle size of about 23 μm (EFSA, 2010).

Production of Direct-fed Microbials

Microbes selected for the production of DFMs are evaluated to establish their suitability for animal nutrition. To achieve this, microbiological tests on fermentation profiles are carried out to evaluate which substrates are fermented to which metabolites (Busch et al., 2004). DNA analysis is also done to provide comprehensive characterization of the microorganisms to show that they do not carry any plasmids. The survival of the microbes in the host animal is also important; the ability to survive the intestinal passage, persistance in the intestine and how it regulates the intestinal ecosystem. In addition, the microorganism should lend itself to large-scale production and should remain genetically stable.

DFMs are produced by fermentation under the controlled supply of nutrients. The sterile fermentation vessel is inoculated with the master seed culture either directly or indirectly after a pre-culture stage with parameters of production being monitored continuously. This is followed by concentration (cell harvesting) through centrifugation. Harvested cells are dried, specific stabilizers added if required, and in some products, the microorganisms are protected by microencapsulation for better quality. Quality control, which is performed both during the production process and on the final product, includes a check for genetic purity, microorganism count and analysis for undesirable substances (for example mycotoxins). Final formulation and standardization are usually achieved by mixing with a carrier to ensure a homogeneous distribution of the DFM in a certain feed type (Busch et al., 2004).

Figure 2.1.Schematic illustration of the manufacturing process of DFM adapted from (Busch et al., 2004)



Effect of Direct-fed Microbials on Pig Performance

Growth rate is one of the determinants of profitability of meat production in the swine industry. Improvement in growth rate and feed efficiency will lead to improved profitability due to greater output and reduction of overhead cost (Campbell, 1997). Many studies have demonstrated that age and weight at weaning closely related to postweaning growth (Quiniou et al., 2002; Mahan et al., 1998). According to Cole and Close (2001), an increase of 1 kg in pig weight at weaning, will result in a pig, which reaches slaughter weight at least 10 days earlier. Average daily gain during the first week post-weaning has a major impact on subsequent growth performance (Tokach et al, 1992). Effects of probiotics on growth performance have not been consistent (Table 2.1). Supplementation of probiotics to piglets and calves improved weight gain and feed conversion ratio (Abe et al., 1995). In the same manner, (Ogle and Inborr, 1987; Kyriakis et al., 1999; Collinder et al., 2000) used either Lactobacillus or Bacillus species to improve weight gain in weanling pigs. On the contrary, no effect on growth was observed when Hale and Newton (1979) and Pollman et al, (1980a) fed Lactobacillus species in grow to finish pigs. Harper et al., (1983) did not find any beneficial effect of lactobacillus probiotics on growth, feed intake and feed efficiency. Many reasons can be assigned for the inconsistent results of probiotic effects in studies. The concentration of the probiotic microorganisms is different in different products.

Microbial strains used as probiotics are very different and are not expected to produce the same effect. Inclusion rate (dose level) of probiotics also varies as well as the conditions under which the probiotics products are manufactured. Probiotics are usually produced by freeze-drying or spray-drying techniques (Holzapfel et al., 2001). All of

these methods result in cell injury (To and Etzel, 1997), suggesting that drying temperature affect the efficacy of probiotic products. The growth of probiotic microorganisms on moist solid substrates in the absence of free flowing water is known as solid substrate fermentation (SSF), (Mitchell and Lonsane, 1992). According to Badu and Satyanarayana, (1995) SSF is better than submerged fermentation due to its requirements for simple culture facilities, relatively low initial cost outlay and production of biomass containing microbial metabolites with reduced waste products. Multi-strain probiotics produced by SSF method were better than probiotics product prepared by submerged liquid fermentation in improving performance, nutrient retention and reducing harmful intestinal bacteria (Choi et al., 2011).

Table 2.1. Effect of probiotics supplementation on pig performance

Animal	Probiotics	Effects	Reference
Growing to Finish	Bacillus species	↑ADG but not G:F	Meng et., al (2010)
Growing to Finish	Bacillus species	No effect on growth performance	Kornegay and Riseley (1996)
Wean to Finish	Lactobacillus acidophilus.	No effect on growth performance	Kornegay et al., (1990)
Weaned piglets	Lactobacillus sp.	↑ ADG and ADFI (d 14)	Huang et al., (2004)
Weanling pigs	Bacillus Subtilis	↑ growth (30/31 studies)	Kremer, (2006)
Weanling pigs	Bacillus probiotics	No effect on growth	Bhandari et al., (2008)

Effect of Calsporin on Performance

The EFSA, (2010) reported on four trials conducted using Calsporin in three different European countries. Two treatments were considered in each experiment; a control group and a treatment group in which animals were fed the control diet supplemented with Calsporin additive at proposed dose of 3×10^8 (CFU/kg feed) with 12

replicates per treatment. In one experiment, a higher dose of 1 x 10^9 (CFU/kg feed) was included in addition to the proposed dose and control treatment. The presence of probiotics microbes was confirmed by analysis of the feed. Equal numbers of male and female large white x Landrace piglets were used in three of the studies while only males (Duroc piglets) were used in one trial for a period of 42 or 43 days in all the trials. Pen was the experimental unit and animals were monitored for zootechnical performance (intake, daily weight gain, body weight and efficiency of feed conversion), general health status and mortality. No significant differences were found in any measured parameter in trial 2 (Table 2.2).

Table 2.2. Summary of performance data of piglets receiving Calsporin®

N	Calsporin®(CFU/kg feed)	Fbw (kg)	ADG (kg/day)	F:G (kg/kg)
112	0	21.4	0.31	1.57
112	3 x 10^8	21.1	0.30	1.59
112	1 x 10^9	21.1	0.30	1.58

(EFSA, 2010)

In contrast, three studies (trials 3, 4 and 5) showed highly significant increases in final body weight and average daily gain compared to control animals and an improvement in feed to gain ratio was seen in the treated group (Table 2.3). There was a numerical reduction in feed intake in the treated group compared to controls. Although this was not statistically significant it was probably a contributory factor in the improved feed to gain ratio.

Table 2.3. Summary of performance data of piglets receiving Calsporin®

	N	Calsporin® (CFU/kg feed)	Fbw (kg)	ADG (kg/day)	F:G (kg/kg)
Trial 2	140	0	28.6b	0.54	1.53
	140	3×10^8	29.8 [2]	0.58 [1]	1.41 [1]
Trial 3	213	0	25.6b	0.43	1.93
	213	3×10^8	27.5 [3]	0.48 [3]	1.72 [2]
Trial 4	210	0	25.3b	0.41	1.88
	210	3×10^8	26.3 [3]	0.44 [3]	1.73 [3]

Treatment means differ significantly from controls 1P<0.05, 2P<0.001, 3P<0.0001 (EFSA, 2010)

Effect of Probiotics on Gut and Fecal Microbiota

Probiotics are included in the diet of livestock to stabilize the gut beneficial micro-biota so that they can compete with pathogenic bacteria to reduce their pathogenicity and numbers (Soccol et al., 2010). Studies have shown that supplementation of lactic acid bacteria has influence on the gastrointestinal tract micro-organism. According to Xuan et al., (2001) inclusion of multi-species probiotics (bifidobacteria and lactobacilli) could protect piglets against potential pathogens. Effects of probiotics on gastro-intestinal tract and fecal micro flora are summarized in Table 2.4. The summary indicates that probiotics are beneficial to some extent in piglet and can influence the gut micro biota. Anti-biotics can negatively affect probiotics micro-organism and as such the two products should not be administered at the same time (Pal, 1999).

Table 2.4. Summary of influence of DFM on microflora in pigs. Adapted from Shim (2005)

Animal	DFM	EFFECT	REFERENCE
Neonate, 2 d old	Milk fermented with L. reuteri	Lactobacillus (↑), E-coli (↓)	Ratcliffe et al., (1986)
Piglet	Ent. Faecum	Fecal and haemolytic E-coli (↓)	Deprez et al., (1986)
Weanling	Ent. Faecalis	Fecal E-coli (↓)	Danek, (1986)
Weanling	Bacillus cereus, Lactobacillus spp	No effect on fecal and haemolytic E-coli	Cupere et al., (1992)
Suckling piglets	Lactobacillus	Fecal lactobacillus (↑)	Jonsson, (1986)
Weanling	Bacillus subtilis	Streptococi and Bifidobacteria (↑), Bacteriodes (↓)	Ozawa et al., (1983)
Finisher pig	Bacillus subtilis	No influence on intestinal microflora	Spriet et al., (1987)
Suckling piglets	Lactobacillus	Coliforms (↓), Lactobacillus (No effect)	Newman, (1990)

(↑) and (↓) shows either significantly increased or decreased

Factors Affecting Direct-fed Microbial (DFM) response

Several claims have been made about the beneficial role of Direct-fed Microbial supplementation in monogastric nutrition including growth stimulation of farm animals, suppression of diarrhea, anti-tumor activity and stimulation of immunity. Results obtained from DFM supplementations however have not been consistent and there is the need to consider the factors responsible for these apparent inconsistencies.

I. The type of organism in the probiotic

Two different strains of the same species may produce different result. Also ability to adhere to the epithelium is host specific. *Lactobacillus acidophilus* strains isolated from the chicken gut will not adhere to the epithelium of the pig to exert any beneficial effect (Fuller, 1992). Other colonization factors such as acid resistance and bile tolerance may vary within species and their variability may provide differences in results.

II. Method of administration and duration of administration

Administration of probiotics could be in the form of powder, liquid, spray, tablets or capsules. Duration of administration can also affect the effectiveness of probiotics. In most cases continual administration is required. Trials in rats, humans and pigs indicate that the effect falls off after administration of the probiotic is withdrawn (Cole and Fuller, 1984; Goldin and Gorbach, 1984). The effect of probiotics will therefore vary by the amount and frequency of dosing.

III. Viability of the preparation

Probiotic preparations cannot always be relied upon to contain the number of viable organisms stated on the label. A survey conducted by Gilliland (1981) on commercial probiotics preparation showed that viable counts vary greatly and three out of fifteen

probiotics preparation had no lactobacillus even though the label indicated its inclusion. Viability of test probiotic product needs to be checked before embarking on any study to verify the presence of stipulated microbes.

IV. Condition of host

Better responses with probiotic supplementation are obtained in starter than growing to finish pigs (Pollman et al., l980a). During the early stages of life the gut micro flora is not in a stable condition and organisms given orally are likely to find a niche, which they can occupy.

V. Condition of gut micro flora

Effectiveness of probiotic supplements depends on the presence of adverse effect which the probiotic is likely to reverse. Probiotics work to stimulates growth by antagonizing the pathogenic microorganisms. Antibiotics are usually not effective in stimulating the growth if chickens are housed in clean environment (Fuller, 1989).

Calsporin Safety and Future for Probiotic Market

European Food Safety Authority (EFSA) recommends that bacterial strains harboring transferable antibiotic resistance genes should not be used in animal feeds, or fermented and probiotic foods for human use. The probiotic species *B. subtilis* is considered by EFSA to have Qualified Presumption of Safety (QPS) status which is synonymous to Generally Recognized as Safe (GRAS) definition in USA and therefore not requiring any specific demonstration of safety for the target species. The safety of Calsporin® for consumers, users of the additive and the wider environment has been established (EFSA, 2010).

In the global market, probiotic products (Dietary supplements, Animal feed, foods and beverages) are expected to grow at a compound annual growth rate (CAGR) of 6.8% from 2013 to 2018 and expected to hit market value of USD 37.9 billion in 2018 Sharma, (2014). The larger share of this market is in China and Japan; with India and other regions showing promising growth. Probiotics of the Lactobacillus genus are having the largest share, representing 61.9% of total sales in 2007 (FAO, 2009).

Regulatory Considerations of Direct-fed Microbial

Despite the significant efforts that are being made by countries such as USA, Canada and Europe, no universal standards for the safety assessment of probiotics exist (EFSA, 2005a, HC, 2006 and FAO/WHO, 2002). However, in the USA, the Food and Drug Administration requires that specific microorganisms for human consumption or utilization should possess "GRAS" status ("Generally Regarded As Safe"). In Europe, the European Food Safety Authority (EFSA) has introduced a similar concept known as Qualified Presumption of Safety (QPS). The EFSA use the QPS concept for evaluation of all requests received for the safety assessments of microorganisms designated to enter into the food chain EFSA (2005b). By comparing the two systems, QPS appears to be more flexible because it takes into account additional criteria to evaluate the safety of bacterial additives such as a history of safe use in the food industry and the acquisition of antibiotic resistance or virulence determinants (Wassenaar and Klein, 2008). QPS system EFSA has published a list of microorganisms, which possess a known historical safety, proposed for QPS status (Table 2.5). *Enterococcus* species are not included due to the

possibility of carrying transmissible resistance to antibiotics (EFSA, 2007a) even though *E. faecium* had shown a long history of apparent safety in food or feed.

The use of some DFM products come with some challenges as studies have shown that organisms cited on the labels of products are not actually contained in the product or microbes that are not listed on the label are found in the product (Wannaprasat et al., (2009), Huff (2004) and Mattarelli et al., (2002)). Also the viability and concentrations of bacteria of the DFM preparations at the point of administration to the animals do not always reflect what is written on the label. Often, either the concentrations were low or absent. It is important to indicate clearly on the product label the exact taxonomic species of DFM utilized so that farmers will know what they are buying. Regulatory bodies must protect the consumer by monitoring and verification of these indications. The DFM manufacturer's claim about the product must reflect the actual composition in the feed until the "best-before" date of the product at the recommended storage conditions with a decrease of one or two logarithmic units at maximum (Czinn and Blanchard, 2009).

Table 2.5. List of DFM microbes proposed for QPS status commonly fed in swine production

Lactobacillus	Bacillus	Yeasts (Saccharomyces)	Bifidobacterium
L. acidophilus	*B. subtilis*	*S. boulardii*	*B. animalis*
L. amylovorus	*B. licheniformis*	*S. carlsbergensis*	*B. lactis*
L. brevis	*B. cereus*		*B. longum*
L. casei			*B. thermophilum*
L. fermentum			
L. plantarum			
L. reuteri			

Adapted from EFSA (2007a).

CONCLUSION

Direct-fed microbials are viable alternatives to antimicrobials in animal nutrition. They are living microorganisms that modulate the digestive microbiota of the host animal in a beneficial way. They develop their activity exclusively in the digestive tract but the exact mode of action is not well known. The most common DFM in feed for animals belong to one of three different groups: lactic acid bacteria, yeasts and Bacillus spores. To reduce or prevent transfer of antibiotic resistance genes, microbes designated as DFMs are carefully selected and tested. DFMs are evaluated based on quality, efficacy and safety for humans, animals and the environment. It is possible that the conditions under which probiotics will have maximum effect are very strictly defined and that only if these conditions are met will it appear positive and consistent. Therefore before any meaningful comparisons between experiments can be done, experimental protocols need to be standardized. The demand for DFM is likely to increase with the increase in human population as well as the growing concern about antibiotic resistance.

Chapter 3

EFFECT OF CALSPORIN™ (BACILLUS-BASED DIRECT-FED MICROBIAL FEED) SUPPLEMENTATION ON GROWTH PERFORMANCE, CARCASS CHARACTERISTICS AND FECAL MICROBIAL ENUMERATION OF WEAN TO FINISH PIGS

Summary

The effects of Calsporin® biotechnical feed additive based on viable spores of Bacillus subtilis C-3102 was evaluated on growth performance; microbial population and carcass characteristics in wean to finish pigs. One hundred and ninety-two crossbred pigs [(Landrace × Yorkshire (Topigs, Winnipeg, Canada)) × Duroc (Compart's, Nicollet, MN); 18 d, initial BW (6.7±0.27 kg)) were selected, weighed and distributed evenly by origin, sex and initial BW into two treatment groups of 12 pens (4 gilts and 4 barrows per pen) each in a randomized complete block design. Pigs were fed a control diet (-ve Calsporin®) and the experimental diet (+ve Calsporin, 1.5 x 10^5/g). Blood and fresh fecal samples were collected on d 84 from 1 pig/pen for determination of blood urea nitrogen (BUN) concentration and microbial population enumeration. Pen was the experimental unit. Data were analyzed by the MIXED procedure of SAS, with treatment and sex as fixed effects and block as a random effect. Data were expressed as means ± SE. Significance level was set at ($P < 0.05$). The results showed that, Calsporin® did not influence ($P > 0.05$) final body weight but numerically reduced feed intake (ADFI) and may have potential to improve feed conversion efficiency. Addition of Calsporin did not elicit any response in terms of average daily gain (ADG) during the entire study period except day d 29 – 57 when pigs on Calsporin diet gained significantly higher body mass ($P < 0.032$) relative to the control group. The measured carcass characteristics, including hot carcass weight, yield percentage, back fat depth, loin depth, and lean percentage, did not differ ($P > 0.05$) between dietary treatments. Supplementation of Calsporin® did not affect ($P > 0.05$) BUN concentration, or counts of total anaerobes, enterobacteriaceae and bifidobacteria in the feces. The lactobacillus count was however reduced with Calsporin

supplementation ($P < 0.05$). In conclusion, at an inclusion rate of 1.5 X 10^5/g of diet, Calsporin® supplementation reduced feed intake and had tendency to improve feed efficiency but did not affect carcass characteristics of wean to finish pigs.

Key words: Pigs, Calsporin, growth performance, carcass traits, microbial enumeration

INTRODUCTION

Public concern about health issues relating to antibiotic resistance has received worldwide attention in medical care delivery over the past decades (Sharma, 2014). Several causes have been identified or proposed. Some of the factors contributing to antibiotic resistance in humans includes antibiotics residues in soil and water bodies (Barton, 2000), overuse of antibiotics to treat animal diseases, and the use of sub-therapeutic dose of antibiotics either to promote growth or ward off bacterial infections in animal agriculture (Newman, 2002; Corpet, 1996 and Cohen, 1992). Therefore, antibiotic resistance can occur indirectly through water, air, soil, and food-chain and directly through injection or oral dose. It has been estimated that over the last 6 decades, 10 million tons of antibiotics have been released into the biosphere (European Commission, 2005). Clinical studies have shown that even short term administration of antibiotics can lead to stabilization of resistant bacterial populations in the human intestine that persist for years (Jakobsson et al., 2010; Jernberg et al., 2007; Lo¨fmark et al, 2006). According to a report submitted by the European Union, medical health care costs due to drug-resistance bacteria are €1.5 ($2.04) billion annually and yet the lives of 25, 000 patients suffering from infections each year are lost globally (Zigglers, 2011). However, Cromwell (2002) states: "Even though antibiotics have been fed for nearly 50 years to literally billions of animals, there is still no convincing evidence of unfavorable health effects in humans that can be directly linked to the feeding of subtherapeutic levels of antibiotics to swine or other animals. Hopefully, policy decisions in the future regarding the use of antimicrobials in animals will be based on science and sound risk assessment, and not on emotion."

Antibiotics are widely used in human and veterinary medicine, and have been essential for ensuring human and animal health. The selective pressure of intensive use of antibiotics has increased bacteria sensitive to antibiotics to become resistant in order to survive (Andersson and Hughes, 2010). Unfortunately, some antibiotics that are used to treat farm animals have the same active ingredients as those used in human medicine. Examples include tetracycline, macrolides, penicillin, and sulfonamides. These drugs are used to treat serious diseases such as pneumonia, scarlet fever, rheumatic fever, venereal disease, skin infections, as well as bioterrorism agents like virus and anthrax.

The emergergence of resistance is considered as a big threat to the advances that have been made in the human medicine in treatment of bacterial diseases. Therefore in 1997, the World Health Organization (W.H.O) called for a ban on the use of human use antibiotics to promote growth in animal production. The European Union instituted a similar ban in livestock production in 2006. South Korea in July 2011, declared a complete ban of antibiotic growth promoters in animal food production (MIFAFF, 2010). Recently, the United State Food and Drug Administration (US FDA) has issued an order to ban usage of some antimicrobial drugs in animal agriculture, effective April 5, 2012 (FDA, 2012; Docket No. FDA-2008-N-0326).

The livestock sector is therefore challenged to find alternative strategies to optimize animal production to meet the demand for animal protein with the increase in world population. Some of the alternatives to antimicrobial agents are organic acids, essential oils such as allylisothiocynate (Mustard), thymol and carvacrol (Oregano),

Piperin (Black pepper); enzymes, antibodies and Probiotics. Probiotics have been known to possess anti-microbial properties such as Batiricin (Sonia, 2005).

Probiotics are defined as live micro-organisms which when administered in adequate amounts can confer health benefits to the host by improving the microbial balance (Chaucheyras-Durand & Durand, 2010; Fuller, 1989). Calsporin is zootechnical feed additive based on viable spores of Bacillus subtilis C-3102, approved in the European Union, United States, Mexico, Japan, and Brazil for use in broilers, weaned piglets, turkeys and minor avian species. Calpis now wish to extend the authorization to fattening of pigs. It is hypothesized that Calsporin could improve performance, carcass traits and beneficial microorganisms. The objective of the study therefore was to evaluate the efficacy of Calsporin in wean to finish pigs. The specific objectives are:

1. To evaluate Calsporin on feed intake, weight gain and feed efficiency in wean to finish pigs.
2. To determine the effect of Calsporin on percent carcass, rib eye area and backfat thickness.
3. Enumeration of fecal microbial counts of total anaerobes, enterobacteriaceae, bifidobacterium species and Lactobacillus species.

MATERIALS AND METHODS

The experiment was reviewed and approved by the Institutional Animal Care and Use Committee of the University of Minnesota (Protocol No. 1104A98947) and animals were cared for according to The Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching (FASS, 1998). The animal nutrition laboratory at the Southern Research and Outreach Center, University of Minnesota at Waseca, MN performed all chemical analyses.

Animals, Housing, Measurements, Diets and Test Product

Animals, Housing and Measurements

One hundred and ninety-two crossbred pigs [(Landrace × Yorkshire (Topigs, Winnipeg, Canada)) × Duroc (Compart's, Nicollet, MN) (age 18±2 d, initial body weight 6.7±0.27 kg) were selected, weighed, tagged and distributed evenly by origin, sex and initial body weight into two treatments of 12 pens (8 pigs/pen) each in a randomized complete block design. Each treatment group was equal to the other in terms of origin, sex and initial body weight. The piglets were raised on completely concrete slatted floor in fattening pens measuring 2.4 × 3.0 meters. Each pen, with 8 pigs, represented the experimental unit. An empty pen to prevent contamination through nose contact separated different groups of piglets. Pigs had unlimited access to water via a single nipple drinker in each pen. Wean to finish rooms were kept between 22˚C and 32˚C for 142 days by artificial ventilation systems. Maximum and minimum daily temperatures were recorded every morning for each room. Pig body weight and feed disappearance from each pen were recorded on d 0, 15, 29, 57, 113, 129 and 141 for determination of

ADFI, ADG and GF ratio. For each weighing, to prevent cross contamination between the two dietary treatments, control pigs were weighed before pigs on the Calsporin diet.

Test Product and Dietary Treatments

The test product was provided to the testing facility in a ready to use form for inclusion into the diet at the recommended rate of 1.5 X 10^5 CFU/kg. Two dietary treatments were investigated in the study with 12 replicates each. Treatment 1 (T1) control pigs were offered diets without Calsporin, while treatment 2 (T2) received the same diet with Calsporin at inclusion rate of 1.5 x 10^5 CFU/kg. The experimental diets were compounded to meet or exceed NRC, 2012 recommendation for Nutrient Requirements of Swine. Water and feed were provided ad-libitum in a 6 stage phase feeding. Nutrients and chemical composition were analyzed using Association of Official Analytical Chemists (AOAC), 2000 official methods of analysis (17^{th} Ed.) Arlington, VA, USA.

Diet Mixing and Sampling

Diets were manufactured using the University of Minnesota feed mill at the Southern Research and Outreach Center, Waseca, MN. Samples were taken in the mill as each diet was being transferred to the grain truck, and again as feed was being administered from the grain bins at the wean to finish barn. The two samples were composited and sub-sampled for analysis. Control feed was prepared and transferred first at each time of feed preparation before the diet containing Calsporin to prevent contamination.

Administration of Test Article and Duration of Treatment

Two diets were prepared. The control diet (T1) without Calsporin and the treatment diet (T2) with Calsporin at an inclusion rate of 1.5 X 10^5 CFU/g. Calsporin was included

in the diet for the entire experimental period of 141 days. Composition and the calculated nutrients analysis of basal diets (as-fed basis) are presented in Table 3.1.

Analysis and Sample Collection

Samples of basal diets were analyzed for dry matter (DM), gross energy (GE), crude protein (CP), crude fat (CF), neutral detergent fiber (NDF), acid detergent fiber (ADF) and ash content. All the analyses were done in duplicate and averaged to assess the co-efficient of variation within samples. Feed samples were dried at 105 ˚C for 4 h in an oven (Thermo Scientific Precision, Thermo Fisher Scientific Inc., Hampton, New Hampshire) to determine DM (AOAC, 2000 method 939.01). The CP in the basal diet was determined using Kjeldahl method (method 976.05, AOAC, 2000; Kjeltec 2300 Analyzer, Foss, Höganäs, Sweden). Gross energy was determined by bomb calorimetry using IKA WERKE c2000 basic bomb calorimeter (IKA Werke GmbH & Co. KG**,** Staufen, Germany). Determination of crude fat was by the ether extract method (AOCS, 2009 method Am 5-04) using an ANKOM XT15 extraction system (ANKOM Technology, Macedon, NY). Analysis for NDF and ADF was carried out using filter bag technique (ANKOM 2000 fiber analyzer, method 12 and 13; ANKOM Technology, Macedon, NY). To determine the total ash content, samples of the basal diet were weighed before and after ashing in a high temperature muffle furnace at 600 ˚C for 6 h (Isotemp Muffle Furnace, Thermo Fisher Scientific Inc. Hampton, New Hampshire).

Blood Sample Collection

Blood samples were collected on day 84 from 1 pig/pen for determination of blood urea nitrogen (BUN) concentration as indirect measure of efficiency of nitrogen utilization. During

sampling, blood samples were collected into 10-mL heparinized tubes via jugular venipuncture and immediately placed in ice. Within 4 h of collection, plasma was centrifuged (Hemle Z300, Labnet Inc, U.S.A.) at 1500g for 15 min and frozen at -20 ˚C for subsequent laboratory analysis of blood urea nitrogen.

Blood Urea Nitrogen (BUN) analysis

Blood urea nitrogen was analyzed in University of Minnesota, Southern Research and Outreach Center, Waseca, using Stanbio Urea Nitrogen kit (BUN) Liqui-UVR (Stanbio laboratory, Boerne, Texas) with the reference method described by Sampson et al. (1980). Samples were thawed at room temperature for 30 min before analysis. Preparation of reagents involved mixing of buffer and enzyme (urease) at a ratio of 5:1 respectively. The resulting solution was allowed to stand for 30 min before use at room temperature of 15 to 25˚C. Working reagent (2.0ml) was added to test tubes and warmed at 37˚C for 3 min. Thermo scientific Genesys 20; spectrophotometer was used to read the absorbance. The spectrophotometer was calibrated at 340 nm with distilled water at zero absorbance. Serum (20µl (0.02ml)) of serum was added to each test tube at a time after spinning the sample for 3 seconds at 1000rpm, mixed gently and absorbance recorded exactly at 30 seconds (A1) and 90 seconds (A2) after mixing. Change in Absorbance was recorded as the difference between A1 and A2 for both serum samples and the standard.

Blood Urea Nitrogen Calculation

Serum BUN (mg/dl) was calculated using the relation: Serum BUN (mg/dl) = ΔAu / ΔAs * 30, where ΔAu and ΔAs are absorbance difference (30 s and 90 s) of serum samples and standard respectively and 30, the concentration of standard (mg/dl).

Fecal Samples

Grab fecal samples of about 20g were collected from two pigs from each pen on d 84 for microbial enumeration. The fresh fecal samples collected for microbial analysis were immediately placed on ice untill analysis was conducted to determine fecal bacteria counts.

Body Weight

Individual body weight of pigs and feed disappearance from each pen was recorded on d: 0, 15, 29, 57, 86,113 and 141 days on trial using an electronic scale (Cardinal, North Star Scale Inc, U.S.A.) to determine weight gain, feed intake and feed efficiency. To prevent contamination, control pigs were weighed before pigs on Calsporin diet each time of weighing.

Microbial Analysis

The microbes in the grab faecal samples were cultured using conventional methods in different growth media for the determination of total anaerobic bacteria (Tryptic soy agar), Bifidobacterium spp. (MRS agar), Lactobacillus spp. (MRS agar+0.02% NaN3+0.05% L-cystine hydrochloride monohydrate), and Enterobacteriacea (violet red bile glucose agar). To measure Lactobacilli count, 1g composite sample was diluted with 9ml (0.9% saline solution) and mixed on a vortex for 5 min. A 10-fold serial dilution in 1% peptone solution on *Lactobacilli* MRS agar plates was carried out. The plates were

incubated for 48 h at 37˚C under anaerobic conditions. Colonies were counted immediately after removal from incubator.

Measurements of Carcass Traits

On d 142, all pigs were tattooed and sent to Tyson Lean Meats (Waterloo, IA) for processing. During processing, the live weight, hot carcass weight, 10th rib back fat thickness and rib eye area (loin depth) were measured by plant staff using (Fat-O-Meater, SFK Technology, Copenhagen, Denmark). Percent carcass was recorded and percent carcass lean was calculated using the following equation: [(58.85 + −0.61 × 10th rib backfat depth, mm) + (0.12 × loin depth, mm)] according to Whitney et al. (2006).

Statistical Analysis

Data generated from the study was analyzed as randomized complete block design using GLM procedure Statistical software (SAS 9.3, Inst. Inc., and Cary, NC). One-way analysis of variance test (ANOVA) was conducted. Significant results were declared at $P \leq 0.05$ while significant trend considered at $0.05 < P \leq 0.1$.

RESULTS

Growth Performance

Effect of Calsporin on body weight, average daily feed intake (ADFI), average daily gain (ADG) and feed conversion efficiency (FCE) are presented in Table 3.2. Across all growth phases, supplementation with Calsporin did not affect body weight compared with the control diet ($P > 0.05$). A similar outcome was observed with the ADFI except

between d 129 – 141 when pigs on Calsporin supplementation consumed less feed (P = 0.03) compared with the control group. Overall, the control pigs consumed numerically more feed than the pigs on Calsporin diet but the difference was not significant (P > 0.05). Addition of Calsporin did not elicit any response in terms of average daily gain (ADG) during the entire study period except day d 29 – 57 when pigs on Calsporin diet gained significantly higher body mass (P < 0.032) relative to the control group. No differences between the two treatments were observed in terms of feed conversion efficiency (FCE) in any phase (P > 0.05). Considering the overall period there was tendency for Calsporin supplementation to improve (P = 0.01) feed efficiency compared to the control group.

Blood Urea Nitrogen (BUN)

Result of BUN is presented in Table 3.3. Two data points (49.64 and 59.14) mg/dl from the Control group was removed before analysis as they were considered as outliers using a scatter plot (Appendix 2). Supplementation with Calsporin DFM did not significantly reduce (P > 0.13) blood urea nitrogen compared with the control.

Fecal Bacterial Population

Results of fecal microbial population are presented in Table 3.4. Dietary treatments with Calsporin supplementation had no effect (P > 0.05) on the fecal total anaerobes, enterobacteriaceae and bifidobacterium spp. population at d 84. Lactobacillus spp. count was reduced with the addition of Bacillus DFM (P < 0.05) compared to the control group.

Carcass Evaluation

The results of carcass evaluation are presented in table 3.5. The final body weight of the pigs on the control and the Calsporin groups were similar. The hot carcass weights were also not different. None of the measured carcass characteristics, including, yield percentage, back fat depth, loin depth, and lean percentage, were affected by the dietary treatments ($P > 0.05$).

Inclusion of Calsporin in the Diet Reduced Feed Intake.

Supplementation of Calsporin numerical reduced feed intake by 355kg for every 100 pigs fed from wean to finish but the difference was not statistically significant (Table 3.6).

DISCUSSION

Dietary supplementation of mono strain Calsporin direct fed microbial (DFM) in the current study did not improve ADFI, ADG, FCR throughout the entire phases, indicating that multi-species or multistrain DFM may be more beneficial than mono strain species. Moreover, the effects of probiotics are genera, species and strain specific, and the use of multi-strain probiotics might be more potent than mono-strain probiotics (Sanders and Huisin't Veld, 1999). In the current study, a mono-strain probiotic product (Calsporin) was investigated. Studies conducted by Meng et al. (2010) and Chen et al. (2006) reported an increased in ADG in growing pigs fed diets supplemented with complex DFM (*Lactobacillus acidophilus*, *Saccharomyces cerevisiae*, and *B. subtilis*) at the amount of 0.2% of diet. Also Davis et al. (2008) fed two strains of bacillus complex in the diet of growing to finish pig and elicited a response in feed efficiency but ADFI and ADG were not affected. However, Kornegay and Risley (1996) reported that dietary

supplementation of a mixture of Bacillus subtilis and Bacillus licheniformis, or a mixture of B. subtilis, B. licheniforms and B. pumilus to finisher pigs did not result in improvement of feed efficiency, ADG and ADFI. It can be inferred from above that the results of DFM supplementation are inconsistent due to variation in species used, age of host and environmental conditions.

The studies reported by the European Food Safety Authority indicated that supplementation with Bacillus subtilis C-3102 led to a reduction in feed intake in pigs. This observation was confirmed in our study (Table 3.6). Supplementation of Calsporin led to a numerical reduction of 355kg of feed for every 100 pigs raised from wean to finish. Consistent with our result, Kornegay and Risely (1996) observed that there was no effect on the growth performance of growing pigs from feeding *Bacillus* products. However, five studies conducted in the European Union and reported by (EFSA, 2010) with supplementation of Bacillus subtilis C-3102 provided contradictory results. Out of the five studies, three of them showed an improvement in performance, one was terminated because of disease outbreak while the other did not show any improvement in ADFI, ADG and feed efficiency even at much higher concentration. According to (Chesson, 1994; Kornegay and Risley, 1996) explained that Bacillus are not considered as natural intestinal inhabitants and do not colonize the host intestine and therefore has limited capacity to improve performance.

More consistent results however, have been obtained with multi-strain DFMs supplementation in nursery pigs (Fialho et al., 1998; Park et al., 2001; Shon et al., 2005; Lee, 2009) than in growing-finishing pigs. As the animals age, gut microbiota become more stable, the immune status is improved, digestibility of feed is higher and the

capacity to resist intestinal disorders develops (Nousiainen and Setälä, 1998 and Jensen, 1998). In the present experiment, ADG was increased with Calsporin supplementation during the early part of study but an increase in ADG was not observed in the finishing phase, which may confirm the idea that older pigs have a better capability to resist intestinal disorders. Direct comparisons between different probiotics studies is difficult since efficacy of probiotic products depend on several factors (Ewing and Cole, 1994) such as species composition, viability, administration level, application method (ie., spraying, feed or water), and frequency of application of probiotics (Chesson, 1994). Also the age of the animal, environmental conditions, health status within the herds and farm hygiene may also influence the efficacy of probiotics. Nevertheless, age could not be a contributing factor, to the lack of effect with Calsporin since the product was administered in feed from wean to finish.

However, environmental conditions, health status within the herds and farm hygiene could explain why the Calsporin product did not impact performance. Additionally, it could also be speculated that the research pens were cleaned more often and the pit drained regularly to an extent that the experimental pigs were not biologically challenged enough to show the effect of Calsporin supplementation in the basal diet. However, the effect of DFM strain differs; different bacterial species, and even strains of the same species, are each unique in their capacity to enhance performance or in nutritional management of disease and as such no extrapolation can be made from one probiotic strain to another (Salim et al., 2013).

Higher BUN values provide metabolic burden on the liver and can be used to access the biological value of protein in feed (Eggum, 1970). During protein synthesis excess

amino acids are metabolized by the liver through transamination and deamination process (Krebs, 1942). One product of the deamination process is ammonia which is toxic and needs to be excreted from the body by liver through the blood in the form of urea. Urea contributes significantly to BUN (Lehninger et al., 2005). It is reported that higher BUN values tend to increase urinary nitrogen excretion and adversely result in poor nitrogen digestibility leading to poor performance (Kohn et al., 2005). However, we did not expect to see any appreciable reduction in BUN values based on the performance result. On the contrary, Meng et al. (2010) observed a tendency for BUN to decrease with DFM supplementation. The differences in BUN results among studies could be attributed to the different products, random error and product concentrations used in experiments. Meng and his colleagues (2010) used DFM mixture of Bacillus subtilis and Clostridium butyricum endospores guaranteed to release at least 1.0×10^{10} viable spores per gram of each microbe in a higher energy diet whereas in the current study mono strain DFM of Bacillus subtilis was fed at much lower concentration of 1.5×10^{5} CFU/g of diet.

To assess the ecological changes associated with dietary supplementation of *Bacillus subtilis C-3102*, total anaerobes, bifidobacteria species, lactobacillus species and enterobacteriaceae were enumerated. Traditionally, the relationship between lactobacillus species and enterobacteriaceae species has been considered as an index of desirable or undesirable bacteria in the pigs. A higher index denotes higher resistance to intestinal disorders (Ewing and Cole, 1994). Direct-fed microbials are preparations with live micro-organisms that have positive impact on the intestinal micro biota (Fuller, 1992). Djouzi et al. (1997) reported that probiotics benefited the host microbiota by improving the intestinal microbial balance. With the addition of the

Bacillus subtilis C-3102, it was expected that the beneficial microbes' population in the treatment diet would increase above that of the control (Mohnl, 2011) and provide a higher lactobacillus and enterobacteriaceae counts. This was not the case in the current study. Rather Calsporin supplementation led to a reduction of the lactobacillus count relative to the control group. Although this finding was not expected, it agrees with previous study conducted by Saier et al. (2002) and Bhandari et al. (2008). A possible explanation for the observed effect is that both *Bacillus* and *Lactobacillus* are gram-positive bacteria and use similar mechanisms to transport nutrients from the intestinal lumen across cell wall. If *Bacillus* transports nutrients more efficiently than *Lactobacillus*, this may lead to a reduction in colonic lactic acid bacteria count (Saier et al., 2002).

CONCLUSION

Calsporin supplementation had no effect on body weight, average daily gain (ADG), average daily feed intake (ADFI), and feed conversion efficiency during the overall period. Calsporin however, led to a numerical reduction of 355kg per 100 pigs raised from wean to finish. In the growth phases however pigs fed diet-containing Calsporin had higher ADG for the d 29-57 period and lower ADFI for the d 129-141 period than those fed the control diet. The measured carcass characteristics, including hot carcass weight, yield percentage, back fat depth, loin depth, and lean percentage, were not affected by the dietary treatments. Calsporin supplementation had no effect on the fecal total anaerobes, *Enterobacteriaceae* and *Bifidobacterium* species. *Lactobacillus* species counts were reduced with the addition of Bacillus DFM compared to the control group.

Table 3.1. Ingredient and composition of basal diets for all phases (% as fed basis)

Phases, kg BW	**7-11**	**11-25**	**25-50**	**50-75**	**75-100**	**100-135**
Ingredients	**Phase 1**	**Phase 2**	**Phase 3**	**Phase 4**	**Phase 5**	**Phase 6**
Corn	44.54	59.65	66.74	69.63	74.19	75.09
Spray-dried porcine plasma	5.00	1.50	0.00	0.00	0.00	0.00
Whey Powder	18.00	8.00	0.00	0.00	0.00	0.00
Soybean meal, (47.5% CP)	20.00	22.80	26.00	25.00	22.00	20.00
Tallow	0.00	0.00	0.00	0.00	1.50	2.00
Choice white grease	3.50	2.00	2.00	2.00	0.00	0.00
Fishmeal	6.00	3.00	1.50	0.00	0.00	0.00
Limestone	0.38	0.66	0.76	0.76	0.76	0.76
Dicalcium Phosphate	0.95	0.60	1.20	1.20	1.20	1.20
Lysine HCL	0.24	0.34	0.40	0.35	0.35	0.10
$_{DL}$ –Methionine	0.18	0.15	0.11	0.09	0.04	0.00
$_{L}$ –Threonine	0.15	0.14	0.12	0.10	0.10	0.00
$_{L}$ –Trytophan	0.01	0.01	0.02	0.02	0.01	0.00
Salt	0.30	0.40	0.40	0.35	0.35	0.35
Vit-TM mix[1]	0.50	0.50	0.50	0.50	0.50	0.50
Zinc Oxide	0.25	0.25	0.25	0.00	0.00	0.00
Total	100.00	100.00	100.00	100.00	100.00	100.00
Calsporin, (15ppm)	+/-	+/-	+/-	+/-	+/-	+/-
Calculated composition						
DM, %	90.88	89.90	89.36	89.27	89.09	89.17
ME, kcal/kg	3441.68	3366.89	3343.89	3354.32	3290.86	3360.20
NDF, %	6.06	7.76	8.72	8.91	9.08	8.99
ADF, %	2.33	2.90	3.27	3.30	3.27	3.18
SID Lys, %	1.44	1.25	1.23	1.10	1.02	0.77
SID Thr, %	0.94	0.79	0.73	0.67	0.63	0.50
SID Met, %	0.51	0.44	0.39	0.35	0.29	0.24
SID Trp, %	0.25	0.21	0.21	0.20	0.18	0.15
Ca/P	1.11	1.12	1.14	1.14	1.11	1.11

[1]The vitamin and trace mineral premix provided the following (per kg of diet): vitamin A, 11,000 IU; vitamin D_3, 2,756 IU; vitamin E, 55 IU; vitamin B_{12}, 55µg; riboflavin, 16,000 mg; pantothenic acid, 44.1 mg; niacin, 82.7 mg; Zn, 150 mg; Fe, 175 mg; Mn, 60 mg; Na, 20mg; Cl, 24mg; K, 30mg; Mg, 45mg; Cu, 17.5 mg; I, 2 mg; and Se, 0.3 mg, choline 495mg, folic acid 1.7mg, thiamine 1.1mg, and biotin 0. 22mg.

Table 3.2. Effect of Calsporin on performance of wean to finish pigs

	Replicates	Control		Calsporin		
Item	**(pens)**	**Mean**	**SEM**	**Mean**	**SEM**	***P* Values**
Body weight, kg						
Day 0	12	6.69	0.270	6.69	0.270	0.809
Day 15	12	11.06	0.310	10.87	0.290	0.289
Day 29	12	19.74	0.530	19.22	0.400	0.160
Day 57	12	42.84	0.660	43.22	0.450	0.479
Day 113	12	106.00	0.900	106.64	0.640	0.493
Day 129	12	123.14	0.950	122.66	0.890	0.593
Day 141	12	132.28	0.770	132.59	1.060	0.781
ADG, kg/d						
Day 0 – 15	12	0.291	0.005	0.279	0.010	0.290
Day 15 – 29	12	0.620	0.020	0.596	0.016	0.313
Day 29 – 57	12	0.825	0.010	0.857	0.009	0.032
Day 57 – 113	12	1.128	0.016	1.133	0.010	0.734
Day 113 – 129	12	1.071	0.041	1.001	0.024	0.119
Day 129 - 141	12	0.762	0.046	0.828	0.058	0.389
Day 0 – 141	12	0.891	0.005	0.893	0.008	0.782
ADFI, kg/d						
Day 0 – 15	12	0.328	0.006	0.338	0.013	0.435
Day 15 – 29	12	0.847	0.027	0.779	0.013	0.052
Day 29 – 57	12	1.672	0.021	1.706	0.022	0.293
Day 57 – 113	12	2.896	0.033	2.893	0.032	0.946
Day 113 – 129	12	3.910	0.042	3.850	0.090	0.530
Day 129 – 141	12	3.817	0.080	3.605	0.074	0.036
Day 0 – 141	12	2.370	0.016	2.340	0.029	0.302
Gain:Feed						
Day 0 – 15	12	0.890	0.018	0.830	0.027	0.084
Day 15 – 29	12	0.733	0.011	0.765	0.016	0.132
Day 29 – 57	12	0.494	0.005	0.504	0.010	0.408
Day 57 – 113	12	0.390	0.004	0.392	0.003	0.669
Day 113 – 129	12	0.274	0.010	0.262	0.009	0.284
Day 129 - 141	12	0.201	0.013	0.228	0.013	0.200
Day 0 – 141	12	0.376	0.002	0.382	0.004	0.099

Table 3.3. Effect of Calsporin supplementation on Blood Urea Nitrogen compared with the control

Item	Control			Calsporin			
	N	MEAN	SEM	N	MEAN	SEM	P-Value
BUN	26	14.2	0.87	24	16.33	1.08	0.128

Table 3.4. Effect of Calsporin supplementation on fecal microbial population in growing pigs

	Control, log_{10} cfu/g	Calsporin, log_{10} cfu/g	SE	P-value
Total anaerobic	9.40	9.38	0.08	0.96
Bifidobacterium spp.	7.22	6.79	0.24	0.51
Lactobacillus spp.	8.59 a	8.15 b	0.15	0.04
Enterobacteriaceae	5.80	5.70	0.42	0.87

Means with different letters within a row are significantly different ($P < 0.05$).

Table 3.5. Result of Calsporin on Carcass evaluation, Experiment 1.

Carcass	N	CONTROL	CALSPORIN	SEM	P-value
IBW, kg	12	6.71	6.70	0.00	0.28
FBW, kg	12	132.56	132.96	0.77	0.72
HCW, kg	12	99.60	99.71	0.45	0.86
Yield, %	12	75.47	75.30	0.38	0.76
Backfat thickness, mm	12	22.57	22.36	0.38	0.71
Loin Depth, mm	12	74.26	74.68	0.66	0.66
Lean, %	12	55.10	55.22	0.17	0.61

Table 3.6. Calsporin supplementation led to a numerical reduction in feed intake.

Control group		Calsporin group	
Phase 1	0.33 X 100 X 15d = 495kg	Phase 1	0.34 X 100 X 15d = 510kg
Phase 2	0.85 X 100 X 14d = 1190kg	Phase 2	0.74 X 100 X 14d = 1036kg
Phase 3	1.68 X 100 X 28d = 4704kg	Phase 3	1.71 X 100 X 28d = 4788kg
Phase 4	2.90 X 100 X 56d = 16240kg	Phase 4	2.91 X 100 X 56d = 16296kg
Phase 5	4.93 X 100 X 16d = 7888kg	Phase 5	4.82 X 100 X 16d = 7712kg
Phase 6	2.48 X 100 X 12d = 2976kg	Phase 6	2.33 X 100 X 12d = 2796kg
Total feed consumed	**= 33493kg**		**= 33138kg**

Total feed saved per 100 pigs with Calsporin supplementation=33,493 - 33,138 = 355kg

Chapter 4

EFFECT OF XYLANASE ALONE OR IN COMBINATION WITH DFM1 OR DFM2 ON PERFORMANCE, ILEAL AND APPARENT TOTAL TRACT DIGESTIBILITY OF NUTRIENTS IN NURSERY PIGS FED HIGH FIBER DIET

LITERATURE REVIEW

INTRODUCTION

The beneficial effect of supplementation of carbohydrases in wheat and barley-based diets is well known since the 1980s (Cowieson, 2010). Since then the global market for feed enzymes has increased rapidly and currently it is worth in excess of $550 million US dollars. The rapid growth is attributed to significant savings of the global feed market, which is estimated at $3 to $5 billion US dollars per year (Adeola and Cowieson, 2011). Among the plethora of enzymes available, the feed enzyme market can be divided into two: 40% nonphytase (xylanase, glucanase, amylase, mannase, lipase, protease, pectinase and galactosidase) and 60% phytase segments. Effective use of xylanase and phytase with careful selection of feed ingredients can be of economic advantage to the farmer as enzyme feed additives come with an additional cost. Approximately, the cost of feed enzymes in finished pig diets is estimated at $1 to $3 USD per tonne (Cowieson, 2010) and this cost should be justified looking at the return on investment.

Enzymes can improve the profitability of swine enterprise by two mechanisms. First and foremost they improve apparent digestibility of nutrients and secondary reduce the nutrient requirement of the animal if their matrix values are appropriately considered. Exogenous enzymes are supplemented to monogastric diets to breakdown plant components which cannot be digested into absorbable nutrients. For instance xylanase supplementation increases the disruption and solubilization of cell wall polysaccharides leading to elimination of the encapsulating effects of the cell wall (Yin et al., 2000). Phytases are also added to diets to release phosphates from phytate phosphorus because six phosphate groups of the inositol ring can bind various cations such as calcium,

magnesium, iron and zinc, starch, free AA and proteins in fixed complexes and thus interfere with their availability (Selle et al., 2000). Oligosaccharides can be also broken down to glucose and galactose by α-galactosidases and then absorbed. In the case of complex NSP of cereal grains, a number of specific enzymes are required to achieve their complete breakdown. When the cell wall structures are broken down, nutrients contained in them are released and digestibility of nutrients as well as the utilization of the energy is improved. According to Li et al. (1994) and Baidoo et al. (1997) increased apparent ileal digestibility of CP and AA with addition of enzymes to barley-based diets for pigs was due to reduction of physical barriers created by the gel-forming property of β-glucans allowing significant interaction between endogenous enzymes and their respective substrates. Limitations imposed by gut capacity, limiting nutrients, as well as negative consequences of fibrous feedstuffs on nursery pigs justify the use of enzymes as an essential dietary intervention.

Enzyme Commission (E.C) Classification of Xylanase

Enzymes are classified into six main classes namely: oxidoreductases, transferases, hydrolases, lyases, isomerases and ligases (synthetases). Enzymes used as additives in animal nutrition are exclusively hydrolases. The hydrolases with the Enzyme Commission number (E.C. No. 3); break down C-O, C-N, and C-C bonds. They are sub-divided into three main groups that play an important role in the use as feed additives: Phosphatases, E.C. 3.1 (e.g. phytase), Glycosidases E.C. 3.2 (e.g. carbohydrases) and Proteases E.C. 3.4. Each molecular group has an inherent series of specific types of bonding. Thus the glycosidases are further sub- divided into: O-glycoside hydrolases degrading (E.C. 3.2.1), N-glycoside hydrolases degrading (E.C. 3.2.2) and S-glycoside

hydrolases degrading (E.C. 3.2.3). Only O-glycoside degrading hydrolases are relevant for use in animal nutrition. The E.C includes a fourth digit that identifies enzyme-specific substrate to be broken down, e.g. xylanase for breaking down the specific carbohydrate xylan (E.C. 3.2.1.8) (Adeola and Cowieson, 2011).

Cost of Cereal Grains in Livestock production

Energy and amino acids are most expensive nutrients in pig diets (de Lange and Birkett, 2005; Patience, 2013 and Woyengo et al., 2014) with energy representing approximately 87% of their total cost (Patience, 2013). Cereals and cereal by-products such as corn, wheat, barley, sorghum, DDGS, wheat middlings and wheat mill run offer a spectrum of ingredients as energy source. However, their use in feed is associated with cost. Profit margins of swine producers have declined in recent years due to feed cost (Schmit et al., 2009) which is the greatest cost of pork production (Niemi et al., 2010). In pig production areas such as North America, Europe, Australia and Africa, corn and / or wheat are mostly used as energy source. Prices of corn and wheat have increased due to the increase in human population with the resulting increase in demand for food and energy. For instance increased demand by the food and ethanol industry (Tyner and Taheripour, 2007), increased crude oil prices (Avalos, 2013), and the 2012 U.S. drought have all contributed to the price increase of corn and wheat (Woyengo et al., 2014). In recent years price of corn in U.S.A. has more than doubled. Patience, (2013) reported that prices of corn, corn DDGS and wheat middlings have increased by 358, 518, and 378% respectively, over the last 7 years soaring up the cost per NE. Currently, prices of corn, barley and oats and sorghum stands at $4.94, $4.70 and $8.74 per bushel respectively whiles DDGS sells at $222.75 per metric ton as at the end of May 2014 in USA (USDA,

2013a). Therefore, any nutritional intervention to maximize the use of cereals and cereal by-product in swine nutrition is paramount.

Cereal and Cereal By-products in Swine Nutrition

Traditionally, cereals and cereal by-products have provided the principal ingredient source (Bach Knudsen, 1997) supplying energy (Mc Alpine, 2012) in swine diets. Cereal grains such as corn, barley, wheat, wheat middlings, wheat mill run, sorghum, triticale and cereal grain co-products are energy sources that are used in animal production depending on their availability.

Corn

In United States and eastern Canada, corn is the major energy source whereas Europe, Australia, and western Canada depend on wheat as their energy source (Woyengo et al., 2014). More temperate regions such as Canada, northern United States, and northern Europe depend on barley and triticale (AAFC, 2005; McGoverin et al., 2011), while sorghum is used in tropical regions such as Mexico.

Barley

Barley is cultivated for the brewing industries for production of malt and animal feed (Fairbairn et al., 1999). Two types of feed barley are hulled and hulless. Hulled barley contains less starch and more fiber than wheat or corn (Table 4.1), whereas hulless barley contains more starch and less fiber than hulled barley. Fiber is negatively correlated with energy value of cereal grains (Fairbairn et al., 1999; Zijlstra et al., 1999) whereas starch is positively correlated (NRC, 2012). Therefore, hulled

barley contains less NE than wheat whereas the NE value of hulless barley and wheat are close (Woyengo, 2014).

Triticale

Cross pollination of wheat and rye produce Triticale as a progenitor with better agronomic attributes than wheat (McGoverin et al., 2011). Triticale is grown mainly for livestock feed even though some may be consumed by humans. Triticale, wheat, and corn have similar NE values since the starch and fiber content are similar to starch and fiber content of wheat and corn (Woyengo et al., 2014).

Sorghum

Sorghum is cultivated for human consumption, livestock feed and ethanol production and it is more drought tolerant than corn (Liu et al., 2013). Sorghum contains similar CP, starch, and fiber than corn (Table 4.1) but also contains more tannins that reduce nutrient utilization (Liu et al., 2013). The content of tannins varies among the cultivars of sorghum and therefore there is wide variation in its nutritive value.

Wheat Millrun

Wheat millrun is a by-product of the dry milling of wheat into flour (Holden and Zimmerman, 1991) and includes the bran, shorts, screening and middlings (Association of America Feed Control officials, 1998). Wheat millrun and middlings contain less starch and more nonstarch components (i.e., fiber, CP, and ether extract) than wheat due to endosperm removal during milling (Table 4.1). The crude fiber content of wheat millrun is about 9.5% (Dale, 1996).

Cereal Grain Co-products

Increase in demand for biofuel has necessitated conversion of cereal grains into ethanol biofuel resulting in co-products that can be fed to livestock. Dried distillers grain with solubles **(**DDGS**)** is the primary co-product from ethanol production. Due to government production subsidies and legislations that stipulated a minimum amount of ethanol in gasoline, ethanol production is on the increase and DDGS is a common by-product used to feed the animal industry in North America and Europe (Tyner and Taheripour, 2007). Processing of wheat into flour also provides co-products available as commodity feedstuffs (FAO, 2009). Wheat millrun is one of the milling co-products that are used to formulate pig diets.

Dried Distillers Grains with Solubles

Corn is mainly used for production of DDGS in United States and eastern Canada whereas that produced in western Canada and Europe is mainly derived from wheat (Nyachoti et al., 2005; Cozannet et al., 2010). However, some ethanol plants fermented wheat and corn, depending on price and availability. Nutritionally, DDGS contain around 3 times more protein, AA, fat, fiber, and minerals than the parent cereal grain (Table 4.1) but potentially also 3 times more mycotoxins (Zhang and Caupert, 2012). Starch in cereal grains is fermented into alcohol and CO_2 that are removed and distiller's grains remain to which the solubles are added and then dried together. The high fiber content in DDGS reduces nutrient utilization. The 10 to 12% of unsaturated fat in corn DDGS reduces pork fat hardness. Even though more oil is been extracted to produce low-oil DDGS with 6 to 9% fat, feeding such DDGS continues to cause problems with softer pork fat (Graham et al., 2013). Also the protein content in DDGS

is damaged during drying. For instance, the SID in pigs was 27% less for CP and 50% less for Lys in corn DDGS than in corn (Almeida et al., 2011). Similarly, the SID in pigs was 16 to 21% less for CP and 36 to 43% for Lys in wheat DDGS (Lan et al., 2008; Cozannet et al., 2010) than in wheat (Hennig et al., 2008).

The major challenge of feeding these cereals apart from cost is their anti-nutritional factors such as nonstarch polysacharides (NSP) and phytin, which serves to limit nutrients that may be utilized by livestock (Adeola and Cowsieson, 2011).

Table 4.1. Nutrient composition, energy value, and standardized ileal digestibility (SID) of Lys for selected feedstuffs for pigs

Feedstuff	Content, % DM					Energy, kcal/kg DM		SID of Lys, %	Source
	CP	EE2	Starch	ADF	Lys	DE	NE		
Cereal grains and co-products									
Sorghum	10.5	3.83	78.4	5.48	0.22	4,023	3,110	82.8	NRC (2012)
Corn	9.33	3.94	70.8	3.2	0.28	3,908	3,026	83.8	NRC (2012)
Triticale	15.4	2	72.7	3.84	0.52	3,752	2,833	88.2	NRC (2012)
Wheat	16.3	2.05	67.1	3.95	0.44	3,736	2,788	92.5	NRC (2012)
Hulless barley	14.3	3.54	60.9	2.42	0.57	3,646	2,751	72.6	NRC (2012)
Hulled barley	12.6	2.35	55.9	6.43	0.44	3,504	2,588	83.4	NRC (2012)
Corn DDGS	30.3	12.8	–	14.6	0.83	3,900	–	54.2	Widyaratne and Zijlstra (2007)
Wheat DDGS	44.5	2.9	–	21.1	0.72	3,548	–	46.4	Widyaratne and Zijlstra (2007)
Wheat middlings	17.7	3.54	24.5	6.65	0.73	3,451	2,371	87.5	NRC (2012)

Adapted from (Woyengo et al., 2014)

Non starch polysaccharides (NSP) in cereal grains

Most of the anti-nutritional factors (ANFs) found in plants provide a natural protection against microbes, insects and birds (Huisman, 1992). Their protective effect is directly linked to their ability to perturb digestive processes in microbes. Similar digestive upset is seen in monogastrics but the ANFs exist where there is no appropriate enzyme in the GIT to render them ineffective (Leons and Walch, 1993). Carbohydrases are added to diets to hydrolyze complex carbohydrates that non-ruminant animals are unable to digest. Some of these compounds such as NSPs are found in the cell wall, thus shielding substrates from contact with the digestive enzymes, or as part of cell content where their presence may interfere with digestion and absorption due to their chemical nature (Adeola and Cowieson, 2011). The main NSP in cereal and cereal coproducts are cellulose, pentosan (arabinoxylans), phytates, and mixed linked β (1–3) (1–4)-d-glucan (i.e., β-glucan) but the proportions, structure, and cross-linkages will differ in cereals (Theander et al., 1989; Bach Knudsen, 1997). The NSP consist of soluble and insoluble polysaccharides mainly present in primary or secondary plant cell walls (Carpita and Gibeaut, 1993; McDougall et al., 1996). The soluble NSP components of the β-glucans and pentosans, glycoproteins as well as pectins, act in the digestive tract by increasing the digesta viscosity, altering the composition of the intestinal flora, increasing absorption of bile, and influence on the intestinal mucosa.

An increase in viscosity of digesta will reduce nutrient availability and absorption especially in young pigs and broilers. Active exogenous enzymes can reduce the viscosity of digesta and increase the nutrient digestibility (Vukic and Wenk, 1993b).

This means that the NSP store large amounts of water (swelling) and the digesta will become more or less viscous and sticky. This increase in viscosity hinders the intestinal absorption of nutrients (Choct et al., 2004) and can result in a negative effect on the consistency of feces. The increased digesta viscosity can in turn lead to a slowing down of the feed passage rate (Morris, 1992) and possibly decrease feed intake. In addition, the thorough mixing of the digesta with endogenous enzymes and bile is also adversely affected.

The insoluble portion of the NSP is attributable to the cage effect. The cage effect refers to some materials which are main components of plant cell wall that entrap other nutrients such as starch, fat and protein which otherwise would have been more digestible. Bach Knudsen (2011) explained that solubilization is not possible in the case of polysaccharides that adopt regular, ordered structures (e.g., cellulose or arabinoxylans) because the linear structure increases the strength of the noncovalent bonds, which stabilize the ordered conformation. Cell wall polysaccharides can adopt a huge number of 3-dimensional shapes and can thereby offer a vast range of functional surfaces (Bach Knudsen, 2011).

Cellulose consists of thousands of glucose molecules linearly linked up by β-glycosidic bonds. β-glucans are also made up of glucose molecule joined together by β-glycosidic bonds in addition to 1, 3-bonds which are responsible for the strong branching of the compound. Branching increases β-glucan's affinity for water and hence swelling of the compound to increase viscosity thus have an anti-nutritive effect. The β-linkages in NSP require microbial enzyme degradation to ultimately yield short-chain fatty acids (SCFA**;** primarily acetate, propionate, and butyrate) and gases (Bach Knudsen, 2005)

through different metabolic pathways. The NSPs reduce effective energy and nutrient utilization by non-ruminant animals since they are not hydrolyzed by porcine endogenous enzymes (Stanogias and Pearce, 1985; Adeola and Cowsieson, 2011), and act as anti-nutrients. Pentosans which are mainly present as arabinoxylans in rye and wheat also show a strong viscosity increase and hence antinutritive effect. Arabinoxylans consist of a main chain of xylopyranose and side chains of arabinofuranose.

Phytates (salt of phytic acid, an ester of the hexaphosphoric acid of inositol) is poorly digested (Bedford, 2001). A maximum of six phosphate groups of the inositol ring can bind various cations such as calcium, magnesium, iron and zinc in fixed complexes and thus interfere with their availability (Selle et al., 2000). In plant based feedstuff approximately 50-80 % of the phosphorus present is bound to phytate. Phosphate from the phytic acid can only be broken down by enzyme phytase, which is not produced by monogastrics (Barrera et al., 2004) and therefore is required as feed additive. The soluble part of NSP is of considerable importance since only soluble β-glucans or pentosans result in the development of viscosity. Concentrations of crude fiber and various NSP-fractions in some cereal grains are presented in Table 4.2.

Table 4.2. Concentrations of crude fibre and various NSP-fractions in some feed ingredients (in g/kg dry matter) adapted from Bühler et al., (2004)

Ingredient	Crude fiber	β-Glucans[1]	Pentosans[1]	Total NSP[1]
Wheat	20-34	2-15	55-95	75-106
Rye	22-32	5-30	75-91	107-128
Triticale	30.00	2-20	54-69	74-103
Barley	42-93	15-107	57-70	135-172
Oats	80-123	30-66	55-69	120-296
Maize	19-30	001-002	40-43	55-117
Wheat bran	106-136	*	150-250	220-337
Soybean extr. Meal	34-99	*	30-45	180-227

*Data not available

[1]Differences in environmental conditions may provide different results.

Classification of Substrates for Enzymatic Action

Among other substrates that are acted upon by either endogenous or exogenous enzymes are starch, proteins and lipids, cellulose, β-glucans, pentosans (arabinoxylans) and phytate. Feed enzymes are substrate specific and substrates to be broken down can be divided into three main groups: 1. Substrates for which monogastric animals synthesize suitable enzymes in their own digestive tract such as starch, proteins and lipids (Moran, 1985 and Gray, 1992). Starch for example is made up of amylose and amylopectin which are glucose molecules linked by α-glycosidic bonds. Enzymes such as α-amylase, glucoamylase, maltase and isomaltase are required for the complete degradation of starch to glucose and subsequently its absorption (Bach Knudsen, 2011). All these enzymes are formed by the monogastric but may not be sufficient in young animals under stress. 2. Substrates for which enzymes are not produced by the animal and have a very low digestibility (e.g. cellulose). Cellulose comprises linear chains of several thousand glucose molecules. These are linked by β-glycosidic bonds which highly indigestible by monogastric and only partly broken down by micro-organisms in the digestive tract (low metabolisable energy). 3. Substrates for which enzymes are not produced by the animal and in addition have antinutritive effects (e.g. 1, 3-1, 4-β-glucans, pentosans and phytate).

Mechanism of Enzyme Action

Enzymes are proteins formed from amino acids. In higher animals proteins are absorbed in a form of free AA and oligopepetides. Therefore enzymes that are added to feed will work exclusively in the digestive tract provided they survive the stomach pH and intestinal conditions (Buhler et al., 2004). Enzymes are highly specific and each enzyme requires a specific substrate to act on. They are not consumed in a reaction and

are available to initiate other reactions when conditions are favorable. For instance, a substrate combines with an enzyme to form substrate- enzyme complex to produce reaction products much faster than would be possible without enzymes. An example of enzymatic breakdown is that of trypsin breaking down a polypeptide (substrate) into several smaller peptides. At the end of the reaction the unchanged enzyme is available to break down yet another polypeptide.

Endogenous enzymes degrade bonds within the molecular strand while the exogenous enzymes only break down the terminal structural building blocks of the molecular strand (Buhler et al., 2004). Besides the specific degradation site in the molecule, enzymes require specific reaction conditions to be effective. The reaction conditions include the pH, temperature, water content and presence of activators or inhibitors as well as the substrate concentration. In animal nutrition enzymes are required to be highly effective around 40°C and maintain their effectiveness after going through pelleting temperatures of 70-80 °C. They must either survive under the acidic pH conditions prevalent in the stomach or must be able to resist both the low pH and the proteolytic action of the pepsin in the stomach in order to be able to act in digestive tract (Simon et al., 1993). Because of the relatively short retention time of feed in the mouth and stomach coupled with low pH value in the stomach, most exogenous enzymes are active in proximal part of the small intestine (Sutton et al., 1992). Enzymatic nutrient degradation is more important than microbial degradation (Wenk, 1993) because enzymatically degradable products of oligo- or monomers such as dipeptides, free amino acids and monosaccharides can be absorbed in the small intestine. Microbial nutrient degradation occurs in the hindgut with volatile fatty acid and lactic acid being the

products. They contribute to the energy supply of the host but the energetic loss is estimated at 5 to 35 %.

Exogenous enzymes are added to monogastric diets to breakdown plant components which cannot be digested into absorbable nutrients by endogenous enzymes. Phytases are added to diets to release phosphates from phytate phosphorus. Oligosaccharides can be also broken down to glucose and galactose by α-galactosidases and then absorbed. In the case of complex NSP a number of specific enzymes are required to achieve their complete breakdown. The relatively short retention time of the digesta and the enzymes contained in the digestive tract are normally not sufficient for completely breaking down NSP. In the case of the pentosans (main group of the NSP in cereal) a complete breakdown is not necessary since the breakdown products xylose and arabinose are absorbed but contribute only marginally to the energy supply of the animal due to the inadequate metabolic utilization. According to Li et al., (1996), partial hydrolysis of NSP compounds in the upper parts of the digestive tract however can result in an increased microbial digestion and the production of short-chain fatty acids in the large intestine. Microbial fermentation in the hindgut is not energy efficient compared to enzymatic hydrolysis in the small intestine (Noblet et al., 1994).

Partial hydrolysis of the NSP's into smaller units, which lose their property of binding water and swelling characteristics. For this purpose, endo-enzymes, i.e. enzymes which attack the internal bonds of long-chain molecules are suitable. Within a short period these enzymes (endo-β-glucanases, endo-xylanases) are able to break down these soluble NSP to the extent that the viscosity increasing property of these fractions is largely reduced. Exo-enzymes which attack the molecule at the endings require a much

longer reaction time to achieve the same effect. Studies have shown that addition of commercial feed enzymes to a wheat based diet significantly improved performance and reduced the incidence of wet and sticky droppings (Annison, 1992 and Choct et al., 1994). When the cell wall structures are broken down, nutrients contained in them are released; digestibilities of nutrients as well as the utilization of the energy contained are improved. Due to the reduced viscosity, a better mixing of the digesta is possible thereby increasing the efficacy of the endogenous enzymes. In addition, the reduced viscosity results in an increased passage rate of the digesta as well as in drier and less sticky feces and a better litter management.

Exogenous Enzymes in Swine Nutrition

At the beginning, enzymes were produced for (food processing, alcohol production, textile industry, detergent preparation etc) other than animal nutrition. Results obtained in animal trials at the time were not encouraging, as the enzymes did not meet the physiological and digestive requirements of the host. Enzymes specially developed and produced for application as feed additives particularly in poultry and piglets, go back as far as 1950 (Adeola and Cowieson, 2011). Improvement in the production processes using genetic engineering together with an application oriented product development has resulted in the use of enzymes becoming a regular component of modern feeding systems. Exogenous enzymes only break down the terminal structural building blocks of the molecular strand whilst endogenous enzymes degrade bonds within the molecular strand. Predominant enzymes in animal nutrition today are Phytase and carbohydrase for breaking down non-starch polysaccharides. According to Adeola and Cowieson (2011), phytase and carbohydrates account for at least 90% of the global feed enzyme market.

Carbohydrase Supplementation Improves Gut Health

Increased digesta viscosity has adverse effects on the gastrointestinal tract. According to (Meyer et al., 1986; Vahjen et al., 1998), increased digesta viscosity slows diffusion rate, encourages greater flow of solids rather than liquid leading to accumulation of particulate matter for microbial adhesion. These factors encourage the proliferation of harmful microorganisms such as enterotoxigenic *E. coli*. McDonald et al. (2001) fed nursery pigs diets that promote increased digesta viscosity. Diets high in NSP diets may cause poor gut health by modifying the morphology of the digestive surfaces in animals receiving a high-NSP diet. Johnson and Gee (1986) reported that in a study where rats were fed gel-forming NSP, adaptive growth of the small intestine occurred, leading to greater mucosal proliferation, broader villi base, and heavier ceca weight. Similar observations were made by McDonald et al. (2001) and Mathlouthi et al. (2002) in swine and poultry. Teirlynck et al. (2009) reported markers of gut damage, increased apoptosis, increased activation of immune responses, and microbial invasion of intestinal tissues in broilers receiving wheat-rye diets compared with those on corn-based diets. Surprisingly, negative effects of NSP were less pronounced in germ-free birds indicative of critical role microorganisms may play in negative effects of NSP (Langhout et al. 2000).

Carbohydrase supplementation alleviates these harmful effects by increasing the proportion of lactic and organic acids (Högberg and Lindberg, 2004; Kiarie et al., 2007), reducing ammonia production (Kiarie et al., 2007), and increasing VFA concentration (Hübener et al., 2002), which is indicative of hydrolytic fragmentation of NSP and supporting growth of beneficial bacteria. High production of lactic acid promotes gut

health by suppressing the growth of pathogens (Pluske et al., 2001). Increased colonization of the gut with lactobacilli (Vahjen et al., 1998) and bifidobacteria (He et al., 2010), have been associated with xylanase supplementation of a wheat diet and reduction in digesta viscosity. Other studies reported reduced relative weight of the digestive tract and associated organs, improved villi length and reduced digesta transit rate with carbohydrase supplementation (Choct et al., 2004; Hopwood et al., 2004; Sieo et al., 2005). Mathlouthi et al. (2002) reported improved gut morphology with xylanase supplementation of a rye-based diet. Reduced relative weight of the intestine is indicative of less cell proliferation and less gut nutrient maintenance requirement.

Effect of Carbohydrates Supplementation on Nitrogen and Amino Acid Digestibility

Dietary fiber can increase the excretion of nitrogen at the terminal ileum of non-ruminant animals (Potkins et al., 1991; Sauer et al., 1991). Part of the increase is related to the secretion of endogenous nitrogenous substances into the gastrointestinal tract (Low, 1989). Loss of pancreatic enzymes and bile, as well as sloughed mucosa, will result in endogenous N and AA losses (Schulze et al., 1995). NDF and ADF have been ascribed to increased loss of endogenous and microbial N due to low availability of N in the fibers themselves, or increased excretion of N trapped in the fibers or the digesta (Stanogias and Pearcet, 1985). Therefore, nutritional intervention to reduce endogenous and exogenous losses and increase hydrolysis of dietary protein such as carbohydrase supplementation to improve N and AA utilization is important. However, observations regarding carbohydrases reducing endogenous protein or AA have been inconsistent. Yin et al. (2000) reported a modest decrease in endogenous AA loss after carbohydrase supplementation, whereas Rutherfurd et al. (2007) did not observe such effects. These

may be diet, ingredient, or enzyme-specific (i.e., presence or absence of certain antinutrients, difference enzymes, and others). Carbohydrases may act to improve N and AA utilization indirectly by increasing the access to protein for digestive proteases. Failure to observe increased N or AA utilization with the use of carbohydrases could be ascribed to inability to break intact cell walls that shield the nutrients from further action by proteases (Adeola and Cowsieson, 2011).

Effect of Xylanase Supplementation on Energy Utilization, Nutrient Digestibility and Pig Performance

Energy digestibility decreases with increased fiber intake (Stanogias and Pearcet, 1985; Nortey et al., 2008) in swine production. Reasons for this phenomenon are increased endogenous energy loss, reduced contact of substrates and digestive enzymes, reduced proportion of energy-yielding fractions in high-fiber feedstuffs and reduction in feed intake because of bulkiness of high-fiber diets combined with stomach capacity of the animal. Studies have shown increased quantities of mono- and oligosaccharides in the ileum after the use of cellulase or xylanase (van der Meulen et al., 2001) and multiactivity carbohydrases (Kiarie et al., 2007). Adeola and Cowieson (2011) suggested that carbohydrases improve energy utilization by shifting production of VFA and absorption of energy-yielding monosaccharaides to foregut. This assertion is supported by Li et al. (1996) who observed decreased net disappearance of nutrients in the large intestine of swine receiving β-glucanase-supplemented diets. Nutrient utilization at the proximal intestine would reduce host-microbe competition for nutrients, ensure availability of nutrients where absorption efficiency is greater, reduce fermentative loss,

and contribute to overall improvement in efficiency of energy utilization (Adeola and Cowieson, 2011).

The production of endogenous enzymes in the tissues of the digestive tract is about 25% of the daily protein turnover and requires energy. These enzymes are able to digest up to 30 times the daily intake of nutrients (Buhler et al., 2004). There is now evidence that the reduction of viscosity also results in decreased secretion of endogenous digestive enzymes. Although the exact mechanism is not known, it appears that due to the reduction in viscosity the contact of the enzyme with the substrate is increased. Hence less endogenous enzymes would be required for breaking down the same amount of substrate. Consequently the energy which, is not needed for endogenous enzyme production, is now available for the deposition of body mass, resulting in conservation of energy and protein.

Varieties of enzymes are used individually or in combination to target antinutritive effects in feeds to release more nutrients, reduce digesta viscosity and enhance performance. The reduction of the digesta viscosity decreases stickiness provides higher dry matter content of the feces with drier litter and hence cleaner animals. Effects of xylanase and multiple carbohydrases on performance are presented in (Table 4.3). It can be inferred from the table that comparison between studies is difficult since the type of enzyme, inclusion rates of the enzymes, the extent of reduction in nutrient density in the control diet and the type of cereal grain(s) used could be different.

Table 4.3. Effect of some exogenous nonstarch polysaccharide enzymes on growth performance of pigs, adapted from Adeola and Cowieson, (2011)

Reference	Stage of growth	Feed stuffs	Major enzyme activity	Observation
Barrera et al., 2004	Growing	Wheat	Xylanase	15% improvement in daily BW gain
Emiola et al., 2009	Finishing	Corn, barley, wheat distillers dried grains with solubles	Multicarbohydrase activities	15% improvement in daily BW gain
He et al., 2010	Weaning	Corn, wheat, wheat bran	Xylanase	20% improvement in daily BW gain
Mavromichalis et al., 2000	Weaning	Wheat	Xylanase	No effect on BW gain
Mavromichalis et al., 2000	Finishing	Wheat	Xylanase	Inconsistent effects on BW gain
Olukosi et al., 2007a	Nursery	Corn, wheat, rye	Xylanase	No effects on BW gain
Olukosi et al., 2007c	Nursery	Corn, wheat middlings	Xylanase, amylase	No effects on BW gain
Olukosi et al., 2007c	Growing-finishing	Wheat, wheat middlings	Xylanase	No effects on BW gain
Vahjen et al., 2007	Weaning	Wheat, wheat bran	Multiple carbohydrase	6% improvement in BW gain
Vahjen et al., 2007	Weaning	Wheat, wheat bran	Xylanase	7% improvement in BW gain
Woyengo et al., 2008	Growing	Wheat	Xylanase	No effect on BW gain

Water Requirement for Growing Pigs

The water content of pigs at any age is relatively constant (NRC, 2012). Therefore amount of water consumed is a reflection of amount of water lost. A neonate of about 1.5kg will have about 82% water content of the empty body weight and declines to about 50% in 110kg body weight of pig (Shields et al., 1983; de Lange et al., 2001). Due to increasing costs of manure management and water limitations, producers are finding cost effective ways to reduce water disappearance without compromising animal well-being (Shaw et al., 2006). Under thermo neutral conditions, water intake depends on body weight, diet composition (Mroz et al., 1995) but at the farm level, water use is influenced by feeder and drinker design (Brumm et al. 2000, Alvarez-Rodriguez et al., 2013). Alvarez-Rodriguez et al. (2013) reported that water disappearance (20 to 100 kg pigs housed at 18 - 22˚C) from bite drinker (9.7 L/d) was highest compare to pig teat drinker (8.2 L/d), nipple square bowl with standard mouthpiece drinker (6.9 L/d) and nipple bowl with short mouthpiece drinker (5.6 L/d).

The voluntary water intake of growing pigs (20 to 90kg) allowed to consume feed ad libitum is approximately 2.5 kg of water for each kilogram of feed; pigs receiving restricted amounts of feed have been reported to consume 3.7 kg of water per kilogram of feed (Cumby, 1986). The difference is due to tendency of pigs to fill themselve's with water if their appetite is not satisfied by their feed allowance (NRC, 2012). Brooks et al. (1984) provided a relationship between daily dry feed intake and water intake in wean pigs where: **Water intake (L/day) = 0.149 + (3.053 X Daily dry feed intake in kg)**. Water wasted (not actually consumed by the pig) from drinkers could range between 25 and 40% of total water used (Li et al. 2005), and it can increase up to 60% when nipple

drinkers are poorly managed (Brooks, 1994). Nipple drinker systems have the advantage of providing a continuous supply of fresh water to pigs when manipulated but the down side of it, is that absence of bowl or tray to catch excess water during drinking, leads to water wastage (Muhlbauer et al., 2010). Research has shown that water wastage is higher when pigs drink from the side of the drinker rather than from the front (Brooks, 1994). He also noted that at a low height, pigs manipulate nipple drinkers more with their noses to waste water. Li et al. (2005) confirmed this observation by demonstrating that adjusting the nipple height at 5 cm above the shoulder height of the smallest pig in the pen compared with an unadjusted nipple height (33 cm) and using the lower flow rate (1L/min) significantly reduced water wasted, in finishing pigs.

Matrix Values for Enzymes

Enzymes release nutrients encapsulated in the cell wall matrix or the cell content and make them available to the host. This additional nutrient released because of the enzyme supplementation is referred to as the matrix value of that enzyme. To maximize enzyme supplementation or to reduce waste in enzyme use, allowance should be made for matrix value in feed formulation. Zhou et al. (2009) demonstrated the need for making allowances for contribution of energy from carbohydrases in diet using decreasing amount of apparent ME (AME) with cocktail mixture of xylanase, amylase and protease supplementation. The highest response to cocktail mixture of xylanase, amylase and protease was observed at the least energy concentration, indicating that enzyme supplementation is more beneficial when ME is suboptimal. Cowieson and Ravindran (2008a; 2008b) reported that mixture of xylanase, amylase and protease had a net effect

of 2.7 g/kg of lipid, 0.1 g/kg of dl-Met, and 0.15 g/kg of lysine-HCl when supplemented in nutritionally marginal diet. It is worthwhile to conduct specific studies to determine matrix values for enzyme since inappropriate use of matrix values can lead to masking of the effect of enzymes. Troche et al. (2007), did not elicit any improvement in performance with carbohydrase supplementation when a wrong matrix value of 140-kcal was used in their feed formulation with corn. If the matrix value used is too small, the enzyme effect is masked, whereas if the matrix value used is too large, the diet remains nutritionally inadequate. Matrix value of enzyme depends on ingredients that are put together since the effect of enzyme supplementation on nutrient or energy availability depends on the structure of the feedstuff (Adeola and Cowieson, 2011)

Formulating Diets with Feed Enzymes

To maximize the value of enzymes in non-ruminant nutrition matrix values for P, Ca, Na, AA and energy must be considered. Cowieson, (2010) suggested matrix values for the new third-generation (engineered *E. coli* as opposed to wild-type *E. coli*) phytases (500 FTU/kg) to be 0.13 to 0.14% P, 0.17 to 0.20% Ca, 0.02 to 0.05% Na, 1 to 6% improvement in digestibility of AA (1% for methionine, 6% for cysteine) and approximately 40 kcal/kg for ME. The use of carbohydrases usually allows the displacement of between 30 and 100 kcal of ME/kg (often achieved via the removal of lipids). Failure to account for these matrix values may lead to high circulating Ca concentrations.

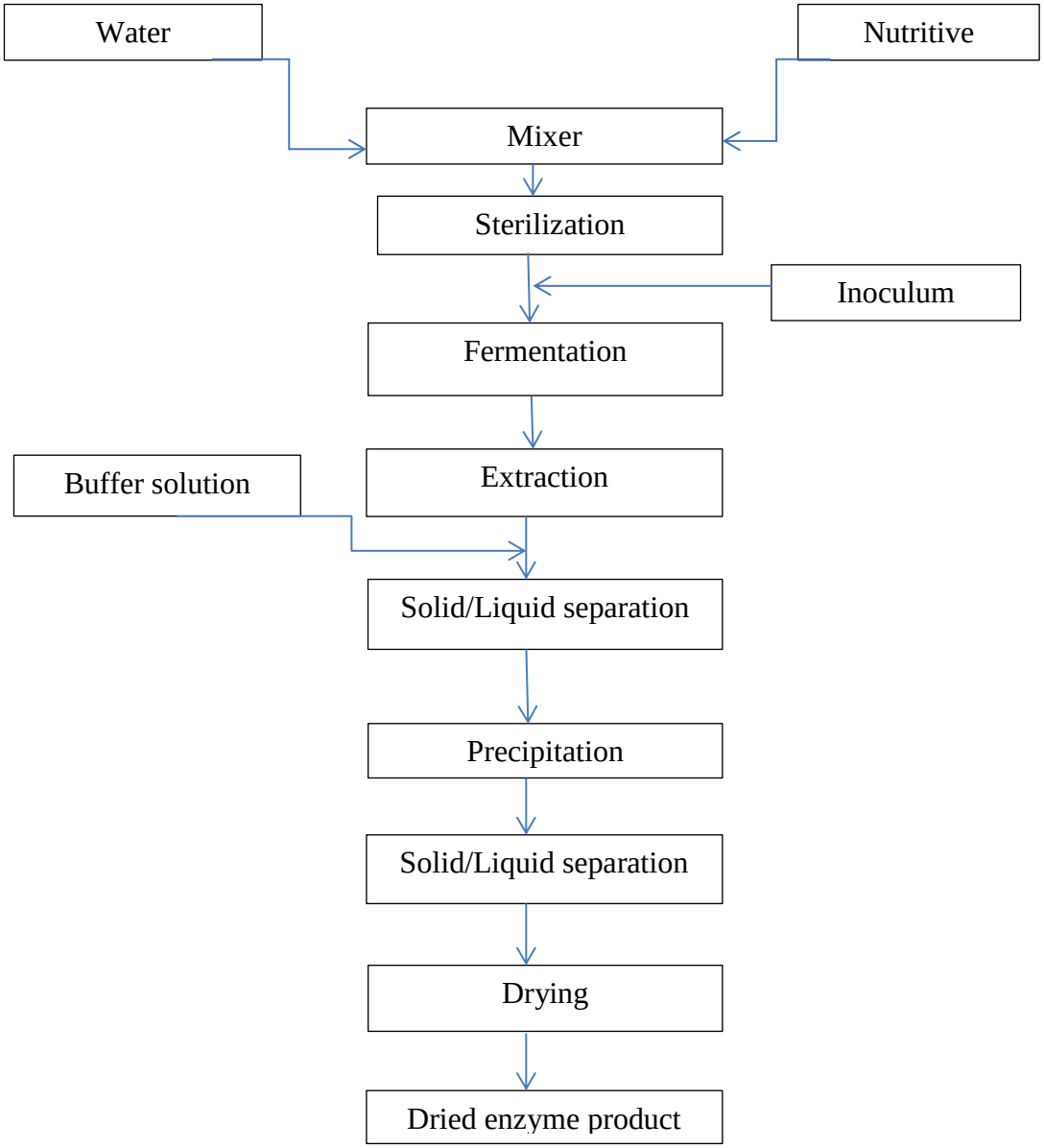
Production of Enzymes

Commercial feed enzymes are produced from optimized fermentation systems using genetically modified bacteria or fungi (Meale et al., 2014). Genetic engineering enables these organisms to overproduce the required protein. For cost-effective production, 50 to 100 g of the active protein per liter of fermentation broth is produced (Schuster and Schmoll, 2010). Production of enzymes from either fungi or bacteria sources is more advantageous than isolating enzymes from plant or animal source materials. Microorganisms from fungi or bacteria source are able to synthesize a very broad spectrum of hydrolytic enzymes which the animal organisms have limited capacity. Many microorganisms are adapted to cope with extreme living conditions such as temperature, pH and osmolarity (Li et al., 2006 and Lin et al., 2007). Microbial enzymes are in this respect often more stable and can be better standardized than enzymes originating from plants and animals. Fungal and bacterial strains that can multiply quickly and show a high biosynthesis performance under industrial production conditions are selected from the wild population and developed or genetic information enzymes transferred by genetic engineering to suitable production strains using specific nutritive media. The composition of the nutritive medium has a decisive role to play. For example, starch in certain strains induces the production of amylases whilst the presence of casein or albumin induces the formation of proteases. In the commercial set up, enzymes are produced either by "surface" (solids or surface fermentation) or "submerged" (liquid nutritive medium) procedures. The surface procedure involves cultivation on solid or pasty nutritive media with surface venting (Figure 4.1). At the end of fermentation process the solids are homogenized, adjusted to a moisture content of 10-12% and then ground to obtain the

final product. In contrast, the submerged process is much more frequently employed than the laborious and cost intensive surface process. In this process the enzyme producing microorganisms are cultivated within a liquid nutritive medium. This method offers a better control of the nutritive medium composition, the pH value, the temperature, venting and reduced risk of foreign infection. The product is marketed in solid and liquid form after purification, standardization and quality control checks. Exposure of enzymes to temperatures greater than 90°C during pelleting of diets leads to about 90% reduction of their activity (Silversides and Bedford, 1999). Currently, enzymes are coated usually with lipid to enhance thermo-stability during feed processing.

Among the microorganisms, fungi are mostly used to produce enzymes and example of such species are *Aspergillus ssp. (e.g. A. niger), Penicillium ssp., Humicola ssp. (e.g. H. insolens)* as well as *Trichoderma ssp. (e.g. T. longibrachiatum)* (Schuster and Schmoll, 2010). All these fungi produce enzymes to breakdown plant cell wall components in the form of high polymer carbohydrates as a common feature. On the other hand, *Bacillus ssp* (*Bacillus licheniformis* and *Bacillus subtilis*) are mostly used to produce enzyme from the bacteria group. For instance proteases, α-amylases, β-glucanases and xylanases can be produced from *Bacillus species.*

Figure 4.1, Schematic diagram of enzyme production by surface process adapted from Buhler et al., (2004).



Enzyme and Environment

Conversion of dietary nutrients such as protein and phosphorus into animal products, may lead to nutrient losses even under optimal conditions in pigs and poultry. Feeding

strategies are required to minimize these losses, which end up in the manure. The most effective measures include a feed which is better adapted to the requirements and the addition of pure amino acids whilst simultaneously reducing the level of crude protein in the feed as well as the addition of feed enzymes. The addition of phytase might be of the greatest value in the reduction of phosphate excretion (Lenis and Jongbloed, 1999). Addition of microbial phytase reduces P-excretion by 30% in manure. Due to the increase digestibility or availability of phosphorus contained in the feed the amount of supplemented phosphorus required to cover the demand can be reduced by the use of enzymes. When adding NSP degrading enzymes the water consumption relative to the feed quantity also drops (Buhler et al., 2004). Hence enzymes make a significant contribution to a better environmental sustainable animal production.

CONCLUSION

The rising cost of cereal grains is due to the demand for ethanol production, increases in human population and recent USA drought; there is need to incorporate cereal by-products to reduce costs of livestock production. The use of these cereal by-products come with a challenge presented by the anti-nutritional factors and the limitation of digestive capacity. Appropriate enzyme supplementation improves nutrients digestibility, availability, absorption and enhance performance. Formulating diets with appropriate matrix value for enzyme reduces the nutrients that are excreted into the environment to minimize environmental pollution while reducing the cost per kilogram of feed fed.

Chapter 5

EFFECT OF XYLANASE AND DIRECT FED MICROBIALS ON ILEAL AND APPARENT TOTAL TRACT DIGESTIBILITY OF NUTRIENTS IN NURSERY PIGS FED HIGH FIBER DIET

Summary

A study was conducted to investigate the effect of supplementing xylanase alone or in combination of either *Bacillus* species Direct-fed Microbials (DFM1) or *Lactobacillus* multi-species Direct-fed Microbials (DFM2) on nutrient digestibility, visceral organ weight and intestinal morphology of nursery pigs fed high fiber diets in a 14 d trial. Thirty-six male pigs (30 d old, 9.0 ± 0.15 kg) of genotype [(Landrace × Yorkshire (Topigs, Winnipeg, Canada)) × Duroc (Compart's, Nicollet, MN), were weighed, ear tagged and allocated to 1 of 6 dietary treatments with 6 replicates in a randomized complete block design. Six dietary treatments evaluated were T1, basal diet without xylanase or DFM as control; T2, control supplemented with DFM1 at 500g/MT; T3, control supplemented with xylanase 250g/MT; T4, xylanase (250g/MT) and DFM1 (500g/MT); T5, DFM2 (500g/MT) and T6, xylanase 250g/MT and DFM2 (500g/MT). All the six diets contained celite as an indigestible marker. Combination of DFM2 with xylanase led to increase villi height, 13% reduction in digesta viscosity, numeric reduction of organ weight and increase ATTD of energy compared to the control. Dietary treatments did not affect pH of ileal digester content ($P > 0.05$) but pH of stomach chyme was reduced ($P < 0.05$) with combination of xylanase and DFM2 and tended to decrease ($P= 0.054$) with the addition of DFM1 to xylanase compared to the average effect of xylanase, and the DFMs. Supplementation of xylanase alone improved ($P < 0.05$) ATTD of fat, NDF, ADF and numerically improved apparent ileal crude protein digestibility by about 10 percentage units compared to the control. Pigs supplemented with DFM2 had improved ($P < 0.05$) ileal crude protein digestibility, decreased water intake, reduced ileal digester viscosity by 13.7% and reduced liver weight compared to pigs fed DFM1.

Similarly, addition of xylanase to DFM2 had a tendency ($P = 0.095$) to reduce intestinal weight, increased ATTD of fat ($P < 0.05$), decreased ileal digester viscosity by 12.6% and numerically improved villi: crypt ratio by 8.9% relative to xylanase and DFM1 combination. Addition of xylanase to DFMs led to a reduction in: stomach pH of chyme, organ weight, ileal viscosity, tended to improve villus height, significantly improved villus to crypt ratio and improved ATTD of energy.

Key words: Xylanase, Direct-fed Microbials, fibre diets, nursery pigs, viscosity and digestibility.

INTRODUCTION

Cereals and cereal by-products are commonly fed in swine production worldwide (United Nations, 2001). The practice however, comes with a challenge which is two folds: feed ingredient factors, which are anti-nutritive in nature, and animal factors due to limits of the capacity of immature digestive tracts after weaning to handle cereals and the cereal by-products. The principal anti-nutritive factors in cereal grains such as wheat and barley are non-starch polysaccharides (NSP) which are in both soluble and insoluble forms (Chesson and Austin, 1998). Soluble NSPs increase digesta viscosity due to their higher molecular weights. The non-starch polysaccharides are present in the endosperm, and the bran is composed of arabinoxylans, β-glucans, cellulose and arabinogalactan-peptides with the dominant component being arabinoxylans.

Cereals contain variable amounts of soluble non-starch polysaccharides. The cell walls of cereal contains up to 15% NSP (Diebold et al., 2004). It is well known that barley, oats, rye, wheat and their by-products contain large amounts of NSP with anti-nutritional activities that impair nutrient utilization in nursery pigs (Hesselman and Aman 1986; Li et al., 1996). The amount of NSP in feed depends on the variety, stage of growth and the growing conditions of the cereal (Scott et al., 1998). According to Annison and Choct (1991), the components of NSP such as arabinoxylan that are not digested in the intestine are of higher molecular weight and result in increased digesta viscosity. In wheat for instance, arabinoxylans are highly concentrated in the aleurone cells of the endosperm (Joyce et al., 2005). The aleurone layer of the kernel is very stable during processing and digestion but contains 15.5% of the proteins, vitamins and minerals.

Significant amounts of nutrients are therefore excreted if the aleurone component is not digested (MacMaster, 1971).

Arabinoxylans of wheat have water absorption characteristics and propensity to gelatinize and increase digesta viscosity. The viscous nature of the arabinoxylans has direct influence on nutrient absorption, interacting with the gut microflora and modifying of the physiological function of the gut. Gelatinization and viscosity reduce passage rate of digesta, increase endogenous nitrogen losses leading to proliferation of gut microbes (Izydorczyk and Biliaderis, 1992). High viscosity leads to decreased digestibility of starch, protein and fat as well as lower apparent metabolizable energy (Austin et al., 1999). Animals on such diets produce sticky droppings, show retarded growth and increased feed to gain ratio. Barrera et al. (2004), explained that monogastrics' digestive enzymes do not hydrolyze cereal structural carbohydrates (NSP) but rather possess microorganisms in the large intestine and the caecum. The NSP and the enclosed nutrients are then acted upon in the large intestine through microbial fermentation (Li et al., 1996). But according to Noblet et al. (1994), hindgut fermentation is less efficient for energy utilization than enzymatic hydrolysis occurring in the small intestine. Feed enzymes can reduce bacterial activity in the ileum by reducing amount of nutrient available for microbial fermentation (Silva and Smithard, 2002). Bedford and Apajalahti (2001) explained that feed enzymes work in two steps described as ileal phase and caecal phase. During the ileal phase, enzymes prevent the formation of viscous content and the caecal phase is characterized by fermentation of degradation of products such as xylose and xylo-oligomers by caecal microbes, thus stimulating the production of short-chain fatty acids.

Many exogenous enzymes are therefore used in swine production to counteract the effect of arabinoxylans and pentosans, allowing higher inclusion rates of cereals and cereal by-products in feed. Xylanase, a glycosidase enxyme, hydrolyzes the arabinoxylan structure to increase the digestion of NSP (Harkonen et al. 1995), release encapsulated protein (Tervila-Wilo et al. 1996), and reduce digesta viscosity (Choct et al., 1999) which culminates in improved animal performance. Supplementation with xylanase in cereal based diets fed to young pigs resulted in improved daily gain and feed intake (Cadogan et al. 2003), increased total tract digestibility of nutrients (Omogbenigun et al. 2004) and improved energy digestibility in a wheat-soybean meal diet (Li et al., 1996).

The response of piglets to dietary intervention such as supplementation with enzymes and / or probiotics is usually assessed using total tract nutrient digestibility and total tract nutrient retention. However, there is dearth of information on possible influences of xylanase in combination with DFMs on nutrient digestibility in nursery pigs. The present study tested the hypothesis that nutrient digestibility can be improved by xylanase and DFMs supplementation alone or in combination. The objective of study 2 was to evaluate the effect of DFM or xylanase combination or alone on viscosity of ileal digesta content, intestinal morphology, organ weights, apparent total tract digestibility (ATTD) of DM, energy, CP, ADF and NDF and apparent ileal digestibility (AID) of DM, energy, CP and energy in nursery pigs fed a high fiber diet.

MATERIALS AND METHODS

The University of Minnesota Institutional Animal Care and Use Committee approved all experimental protocols (Protocol No. 1104A98946). Animals were cared for according to The Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching (FASS, 1998).

Enxymes and Direct-fed Microbials, DFM1 and DFM2

Xylanase, endo-1, 4-β-xylanase (EC 3.2.1.8; Porzyme 9300; Danisco Animal Nutrition, Marlborough, UK), was supplemented in the diet to supply 250g/MT of feed and DFM1 or DFM2 (Danisco Animal Nutrition, Canada) added to supply 500g/MT of feed were used alone or in combination with xylanase in the experiment.

Animal, Diets and Housing

Thirty-six male pigs crossbred pigs [(Landrace × Yorkshire (Topigs, Winnipeg, Canada)) × Duroc (Comparts, Nicollet, MN)], weaned at d 18, were obtained from the University of Minnesota Swine Research Centre, Waseca and fed a common a diet for 9 days before allotment to treatment diets. Pigs were fed experimental diets for 14 days with the first 10 and last 4 days being adaption and collection periods respectively. Pigs were blocked by body weight into six groups and randomly allocated to Metabolic Housing Units (MHU) in a randomized complete block design such that each dietary treatment had approximately equal average body weight (9.0 ± 0.07kg). Each pig in a MHU constituted the experimental unit and was assigned one of six experimental diets, with 6 replicates. Pigs were housed in an environmentally controlled room with ambient temperature set at 28°C ± 2°C. The experimental diets (Table 5.1) were: T1, basal diet without xylanase or DFM as control; T2, control supplemented with DFM1 at 500g/MT;

T3, control supplemented with xylanase 250g/MT; T4, xylanase (250g/MT) and DFM1 (500g/MT); T5, DFM2 (500g/MT) and T6, xylanase 250g/MT and DFM2 (500g/MT).

Xylanase and DFMs were added at the expense of corn. Celite was added to the diets at the rate of 0.4% as an indigestible marker for the determination of total tract nutrient digestibility by the index method. A stainless steel feeder and a bite teat drinker attached to the metabolic unit provided the experimental pigs access to water and feed. Feed was offered at two equal portions at 0800 and 1500 h daily at 4% of body weight. The MHUs had plastic-covered expanded metal floors (1.98m X 0.711m) providing each pig a unit area of 1.4 m^2. The sides of the unit consist of Plexiglas that allows pigs to see each other in adjacent MHUs and provides easy observation by animal attendant during routine checks. The metabolic unit has a receiving stainless steel tray that slopes from the four corners to the middle of the collection tray. A receptacle is placed underneath a spout in the middle of the collection tray for urine collection. Cheese cloth was inserted in the Spout to strain the urine into collection bucket with 5ml of 10% formalin.

Measurement of water disappearance

Water disappearance was recorded daily for each metabolic housing unit using a flow meter connected to the water delivery line.

Fecal and Ileal Digester Samples

Representative grab fecal and urine samples were collected from each pig twice a day during the last 4 d, composited and stored frozen at -20˚C until analysis. Frozen fecal samples were thawed at room temperature for 5 h and dried in an oven at 53 ˚C for 4 d.

The ileal digesta samples were freeze-dried at -51˚C for 5 d using LABCONCO Freeze dryer (LABCONCO Corporation Kansa city, MO). Both fecal and ileal samples were ground to pass through 1mm sieve using an Oster® blender (Sunbeam products, Inc. U.S.A.). The samples were bagged, labeled and stored at room temperature until further analysis.

Chemical Analysis of Basal Diet, Fecal and Ileal Samples

Samples of diets and fecal samples were analyzed for dry matter (DM), gross energy (GE), crude protein (CP), fat, neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid insoluble ash (AIA). All the analyses were done in duplicate. Feed samples were dried at 105 ˚C for 4 h in an oven (Thermo Scientific Precision, Thermo Fisher Scientific Inc., Hampton, New Hampshire) to determine DM (AOAC, 2002 method 934.01). The remaining sample was used to determine GE (AOAC, 1995) by bomb calorimeter using IKA WERKE c2000 basic bomb calorimeter (IKA Werke GmbH & Co. KG**,** Staufen, Germany) with benzoic acid as a calibration standard Samples were analyzed for NDF and ADF using the filter bag technique (ANKOM 2000 fiber analyzer, method 12 and 13; ANKOM Technology, Macedon, NY). Crude protein was determined by digestion of samples in a Tecator Digestion System 20 1015 Digestor and then analyzed in a 2300^{R} Kjeltec Auto Analyzer Unit (Foss North America, Eden Prairie, MN), according to methodology described by AOAC (1995). Fat content (AOAC, 2000) was by extraction with the filter bag technique of $ANKOM^{XT10}$ (ANKOM Technology, Macedon, NY). Briefly, weighed samples were mixed with about 1g of celite and completely encapsulated by heat and sealed. Bags and contents were hydrolyzed using 3N HCL for 60 min at 90 ˚C. Bags were then pressed between absorbent paper to remove

excess acid and dried in ANKOMRD Dryer at 110 ˚C for 2 h 30 min. After cooling in desiccant pouch, samples were extracted with petroleum ether (90 ˚C for 1 h) dried at (110 ˚C for 20 min) and weighed for calculation of acid hydrolysis fat. To determine celite concentration, the samples were weighed before and after ashing in a high temperature muffle furnace at 650 ˚C for 6 h (Isotemp Muffle Furnace, Thermo Fisher Scientific Inc. Hampton, New Hampshire) after 2 h acid digestion at 250 ˚C.

Apparent Total Tract Digestibility Calculation

The apparent total tract digestibility (ATTD) coefficient was calculated by Index method described by Agudelo et al., (2010).

ATTD = [100 – {100 *(% celite in feed / % celite in feces)*(% nutrient in feces / % nutrient in feed)}]. All variables in the equation are expressed as a percentage of DM.

Blood Sampling

Blood samples were taken from each pig for blood urea nitrogen analysis at the end of the experiment. Blood samples were collected from the jugular vein of each pig on d 14 via jugular venipuncture by using heparinized vacuum container tubes (Covidien Inc. USA) before euthanization by intravenous injection of sodium pentobarbital (50 mg/kg, Vedco, Inc., St. Joseph, MO, U.S.A.). The blood samples were centrifuged (Hemle Z300, Labnet Inc, U.S.A.) at 2500 × *g* for 10 min at 4°C to recover the plasma and stored at -20 until analysis for (BUN) concentration using commercial kits (Stanbio laboratory, TX U.S.A.).

Slaughter procedure, Organ harvesting and Digester samples

After 4 d of sample (fecal and urine) collection, pigs were weighed, anesthetized with an intramuscular injection of Telazol (1mg/kg; Tiletamine HCL and Zolezepam

HCL, Fort Dodge Animal Health Inc., IA, U.S.A.) and euthanized by an intravenous injection of sodium pentobarbital 50 mg/kg of BW. After euthanasia, each pig was incised along the midline to harvest the kidney, spleen, liver and heart. Blood on these organs was cleaned with absorbent paper and organs weighed. The total length of the small intestine was measured using a tape measure stretched and fastened onto a horizontal surface. Digesta samples from the distal ileum were collected into sterile plastic containers for pH, viscosity and digestibility determination. Stomach content was obtained for pH measurement and small intestine, stomach, caecum, the entire GIT emptied and weighed. About 15cm from the ileo-cecal junction, a small portion (approximately 10 cm) of ileum was cut, flushed with phosphate buffer, tied at both ends, filled with buffer solution and placed in a plastic container in 10% buffered formalin solution. The sampled portions were sent to the Cancer Center Histopathology Core (University of Minnesota, MN) for intestinal morphology.

Digesta Viscosity and pH measurements

Digesta viscosity was determined as described by Hansen et al., (2010). Briefly, digesta samples were mixed on a vortex, centrifuged at 12,000× *g* for 8 min at 4°C in Hermile z300 (Labnet international, Inc., Edison, NJ, USA) immediately after collection. The supernatant fluid was used in a Brookfield DV-E viscometer (Brookfield Engineering Laboratories Inc., Middleboro MA) to determine the viscosity of intestinal content at a 33 shear rate of 12.25s-1 at 37°C. To prevent contamination, the viscometer was rinsed with deionized water and wiped clean between samples. Viscosity was measured in centipoises (cP). The pH of the ileal digesta and stomach contents were measured immediately after collection using OAKTON pH meter (Eutech Instrument,

Vernon Hills, IL) by inserting the electrode of a calibrated portable pH meter into the digesta samples. The pH probe was rinsed between samples using deionized water.

Blood Urea Nitrogen (BUN) analysis

Plasma samples were analyzed for blood urea nitrogen (BUN) using Stanbio Urea Nitrogen kit Liqui-UVR (Stanbio laboratory, Boerne, Texas) using the method described by Sampson et al. (1980). Samples were thawed at room temperature for 30 min before analysis. Preparation of the reagent involved mixing of buffer and enzyme (urease) in a ratio of 5:1 respectively. The resulting solution was allowed to stand for 30 min before use at room temperature of 15 to 25˚C. Two milliliters of working reagent was added to test tubes and warmed at 37˚C for 3 min. After spinning the sample for 5 seconds at 1000rpm, 20 µl of serum was added to each test tube, mixed gently and absorbance recorded exactly at 30 seconds (A1) and 90 seconds (A2) after mixing using spectrophotometer (Thermo scientific Genesys 20, Vernon Hills, IL) calibrated at 340 nm with distilled water at zero absorbance. Change in absorbance was recorded as the difference between A1 and A2 for both serum samples and the standard.

Calculation: Blood Urea Nitrogen (BUN) concentration

Serum BUN (mg/dl) was calculated using the relation: Serum BUN (mg/dl) = ΔAu / ΔAs * 30, where ΔAu and ΔAs are absorbance difference (30 s and 90 s) of serum samples and standard respectively and 30, the concentration of standard (mg/dl).

Intestinal Morphology

The ileal tissues were aseptically isolated 15cm from the ileo-cecal junction and samples fixed in 10% neutral buffered formalin. Tissues were processed separately.

Paraffin-embedded tissue blocks were made and serially sectioned at 5 microns. The slides were then stained with hematoxylin (Sigma-Aldrich, MO, U.S.A.) and eosin (Sigma-Aldrich, MO, U.S.A.). The stained slides were scanned using an Aperio ScanScope CS digital slide scanner (Aperio Technologies, Inc. Vista, CA). The cross-sections of ileal tissue were examined histologically to determine villus height and crypt depth. Approximately twenty linear measurements of both villus height and crypt depth were taken per pig using the Aperio image analysis algorithm framework software (Aperio Technologies, Inc. Vista, CA). The villus height was measured from the tip of villus to the crypt-villous junction, and the crypt depth was measured from the crypt-villous junction to the base. These measurements were then averaged for each section respectively.

Statistical Analyses and Calculations

Statistical analysis was as appropriate for a randomized complete block design using proc MIXED procedure (SAS version 9.3, Inst. Inc., Cary, NC). Pig was the experimental unit and initial body weight was used as block for all response criteria measured in the performance study. UNIVARIATE procedure was used to verify homogeneity of variance among treatments. Five data points were removed from the water disappearance data due to excessive leakage on the flow meter but was not limited to one treatment. Actual water intake was calculated using an equation developed by Brooks et al., (1984). The final model included the main effect of treatment. Dunnett's test was used for comparisons between the control and other treatments. Regardless of the overall test for the treatment effects, 4 sets of pre-planned contrasts were utilized to evaluate the effect of treatments: (1) average of DFM1 and xylanase vs. (DFM1 +

xylanase), (2) average of DFM2 and xylanase vs. (DFM2 + xylanase), (3) DFM1 vs. DFM2, (4) (DFM1 + xylanase) vs. (DFM2 + xylanase). Contrasts 1 and 2 were used to test the interaction between xylanase and DFM. Linear regression analysis ATTD of NDF and DM were evaluated on ATTD of energy. Least square means are provided with pooled standard error and $P < 0.05$ was considered statistically significant, whereas $P < 0.10$ was considered a tendency.

RESULTS

Apparent total tract digestibility (ATTD) of DM, CP, energy, fat, NDF, and ADF

Effects of xylanase and Direct-fed Microbial supplementation on apparent total tract digestibility of dry matter, energy and crude protein, fat, NDF and ADF of nursery pigs fed high fiber diet in Experiment II are presented in Table 5.2. Supplementation of DFM1 and combination of DFM1 with xylanase reduced ($P < 0.05$) ATTD of fat compared to the control. Inclusion of Xylanase, DFM2 and their combinations in the diet of nursery pigs did not influence ($P > 0.05$) apparent total tract digestibility (ATTD) of DM, energy and crude protein but supplementation of xylanase alone enhanced ATTD of fat ($P < 0.01$), NDF ($P < 0.03$), and ADF ($P < 0.03$), compared to the control. Combination of xylanase with DFM1 did not influence ATTD of DM, energy and crude protein, fat, and NDF but reduced ($P < 0.03$) ATTD of ADF compared to the average effect of xylanase and DFM1 supplementations alone. Supplementation of pigs with DFM2 did not improve ($P > 0.05$) ATTD of DM, energy and crude protein, fat, NDF and ADF relative to DFM1 supplementation. Xylanase in combination with DFM2 combination did not improve ATTD of DM, energy and crude protein, fat, and NDF but reduced ($P < 0.03$) ATTD of ADF compared to the average effect of xylanase and DFM2

supplementations. Addition of DFM2 to xylanase did not improve ($P > 0.05$) ATTD of DM, energy and crude protein, NDF and ADF but increased ATTD of fat ($P < 0.04$) relative to addition of DFM1 to xylanase.

Linear Regression of % ATTD of energy on % ATTD of NDF and DM

A graph of ATTD of energy against ATTD NDF is presented in Figure 5.1. The slope of the regression equation was significant ($P < 0.0001$) with 58.3% variation in ATTD of energy explained by ATTD of NDF. The regression equation relating the two variables is provided as ATTD Energy = 46.84 + 0.44NDF , every 1% improvement in ATTD of NDF results in an average of 0.44% improvement in ATTD of Energy.

A graph of ATTD of energy against ATTD DM is presented in Figure 5.2. The slope of the regression equation was significant ($P < 0.0001$) with 96.1% variation in ATTD of energy explained by ATTD of DM. The regression equation relating the two variables is provided as ATTD Energy = 1.14DM – 11.91 , every 1% improvement in ATTD of DM results in an average of 1.14% improvement in ATTD of Energy.

Apparent ileal digestibility of DM, CP and Energy

Effects of xylanase and direct fed microbial supplementation on apparent ileal digestibility of dry matter, energy and protein of nursery pigs fed high fiber diet in Experiment II is presented in Table 5.3. Xylanase, DFM1, DFM2 and their combinations in the diet of nursery pigs did not enhance ($P > 0.05$) apparent ileal digestibility of DM, energy and crude protein compared to the control. However, xylanase supplementation numerically improved ileal protein digestibility by 11 percentage units compared to the control. Combination of xylanase with DFM1 did not influence ($P > 0.05$) apparent ileal

digestibility of DM, energy and crude protein compared to the average effect of xylanase and DFM1 supplementations. Similarly, xylanase with DFM2 combination did not improve apparent ileal digestibility of DM, and energy but crude protein digestibility was numerically reduced by approximately 15 percentage units compared to the average effect of xylanase and DFM2 supplementations (P > 0.14). Supplementation of pigs with DFM2 improved ileal crude protein digestibility (P<0.024) relative to DFM1 supplementation (68.3 vs. 45.7 %). Addition of DFM2 to xylanase did not improve (P > 0.05) ileal digestibility of DM, energy and crude protein (P > 0.05) relative to addition of DFM1 to xylanase.

Histological measurement

Effect of xylanase and direct fed microbial supplementation on histological measurements of nursery pigs fed high fiber diet in Experiment II is presented in Table 5.4. Xylanase, DFM1, DFM2 and their combination did not influence (P > 0.05) villus height, crypt depth and villus to crypt depth ratio compared to the control. Blending of xylanase with DFM1 numerically improved villus height by 10.5% (P > 0.20), increased (P < 0.04) crypt depth but not villus: crypt ratio compared to the average effect of xylanase and DFM1 supplementations. The combination of xylanase and DFM2 did not increase villus height, crypt depth and villus: crypt ratio compared to the effect of xylanase and DFM1 supplementations. Supplementation of pigs with DFM1 did not show any improvement (P > 0.05) in terms of villus height, crypt depth and villus: crypt ratio compared to DFM2 supplemented pigs. Addition of DFM2 to xylanase did not improve villus height but numerically decreased crypt depth by 10.4%, and improved villus: crypt depth ratio by 8.9% compared to addition of DFM1 to xylanase (P > 0.05)

BUN, ileal digester viscosity, water intake, pH of stomach chyme and ileal digester

Effects of xylanase and Direct-fed Microbial supplementation on ileal pH, stomach pH, BUN, viscosity and water intake of nursery pigs fed high fiber diet in Expt. II is presented in Table 5.5. Addition of DFM1, DFM2, and xylanase alone or in combinations did not affect ($P > 0.05$) water intake, BUN, viscosity and pH of ileal digesta ($P > 0.05$) compared to the control. Supplementation of either xylanase or DFM alone tended to increase ($P = 0.09$) pH of stomach chyme relative to the control. There was tendency for combination of DFM1 with xylanase to reduce the stomach content pH ($P = 0.05$) compared to the average effect of individual supplementation of DFM1 and xylanase. Combination of DFM2 with xylanase however, reduced ($P < 0.05$) the stomach content pH by 1.2 units compared to the average effect of individual supplementation of DFM2 and xylanase. Pigs supplemented with DFM2 had lower water intake ($P = 0.07$) and reduced ileal digesta viscosity by 13.7% compared to pigs supplemented with DFM1. Combination of DFM2 with xylanase did not affect water intake, BUN, pH of ileal and stomach contents but numerically reduced ileal digesta viscosity by 12.6% compared to pigs supplemented with the DFM1 and xylanase combination.

Organ weights

Effects of xylanase and Direct-fed Microbial supplementation on organ development of nursery pigs fed a high fiber diet in Expt. II are presented in Table 5.6. Visceral organ weight is expressed as grams per kg body weight. Supplementation of xylanase, the DFMs, alone or in combination did not affect ($P > 0.05$) visceral organ development of kidney, liver, spleen, emptied stomach, caecum, emptied intestine and the

entire weight of G.I.T compared to the control. Pigs supplemented with DFM2 had increased in mass ($P < 0.02$) of heart compared to the control (6.37 vs 4.89). Relative to the control, pigs supplemented with combined xylanase and DFM1 had longer ($P < 0.01$) intestinal length (67.19 vs 77.52). Specific contrasts between the average effect of individual supplementation of xylanase and DFM1 versus their combination did not affect organ weight ($P > 0.05$) except intestinal length where the combination showed an improvement ($P < 0.01$) over the individual supplementations. Pre-planned contrast between the average effect of individual supplementation of xylanase and DFM2 versus their combination did not affect organ weight ($P > 0.05$) except that the combined supplementation reduced heart weight ($P < 0.01$) and kidney ($P > 0.08$). Supplementation of DFM2 reduced ($P < 0.05$) liver weight but increased weight of heart ($P < 0.05$) and spleen ($P > 0.09$) compared to pigs supplemented with DFM1. Addition of DFM2 to xylanase led to shorter ($P < 0.001$) and lighter ($P < 0.10$) intestine compared to pigs supplemented with DFM1 and Xylanase combined.

Actual water intake as a function of dry feed intake

The effect of dietary treatment on water intake is presented in Table 5.7. Dietary treatments did not affect water intake compared to the control.

DISCUSSION

Direct-fed microbial are preparations that contain live micro-organisms that have positive impact on colonization of gut micro biota (Fuller, 1992). Djouzi et al. (1997) reported that probiotics benefited the host microflora by improving the intestinal microbial balance. According to Collington et al. (1990), the microbiota of the

gastrointestinal tract has a major influence in the modulation of enterocyte activity and the expression of tissue function. Decreased numbers of pathogenic bacteria in the gut may therefore influence proliferation of epithelial cells to form villi and thus enhance absortive capacity (Mourao et al., 2006). Supplementation of either DFM1 or DFM2 led to development of longer villi compared to the Control. Further improvement in villus height was observed when xylanase was added to the DFMs. The longer villi in the ileum of pigs supplemented with DFMs in combination with xylanase fed on high fiber diet might be related to a greater absorption of nutrients and energy in these pigs since increase in villus height provides larger surface area for nutrient absorption. Although not significant, blending of xylanase with DFM1 numerically improved villus height by 10.5% but increased crypt depth compared to the average effect of xylanase and DFM1 supplementations. At the crypt, stem cells divide to allow rejuvenation of the villus, and a large crypt indicates fast tissue turnover, a high demand for new tissue and energy (McBride and Kelly, 1990). Addition of DFM2 to xylanase improved villus: crypt depth ratio by 8.9% compared to addition of DFM1 to xylanase. The villus: crypt ratio expresses the balance between cell loss from the villi and cell production in the crypt. A larger ratio of villus: crypt indicates that the villi are taller or crypt depth is smaller due to less tissue turnover and conservation of energy.

The short villus height and long crypt depth observed in the control compared to the DFMs was in agreement with established literature. According to Kim et al. (2012) a drastic deterioration of intestinal morphology following weaning and switching from consumption of milk to dry diets is normally observed. It is therefore not surprising that the control pigs without any supplementation recorded shorter villi height. In the current

study, pigs supplemented with xylanase and DFM2 had higher villus height and villus height: crypt depth ratio at the ileal mucosa compared with the control, DFM2, or sole supplementation of xylanase. The length of the villi and crypts in this experiment were similar to observations in grower pigs fed on wheat-barley-rye diet supplemented with xylanase and glucanase by Willanil et al. (2012). Mathlouthi et al. (2002) reported improved gut morphology with xylanase supplementation of a rye-based diet. The greater ratio observed in DFM2 with Xylanase supplemented pigs indicate less cell loss, which in turn could have positive implications for the ability of those animals to digest and absorb nutrients (Pluske et al., 1997) and hence potential for better performance.

Fiber content of the diet can impact on GIT development and affect energy metabolism. Feeding high dietary fiber is associated with increase in weight of GIT and visceral organs (Coey and Robinson, 1954; Southgate, 1990; Hansen et al., 1992; Agyekum et al., 2012; Asmus, 2012). Supplementation of DFM2 reduced liver weight but increased heart weight and tended to increase spleen weight compared to pigs supplemented with DFM1. According to McBride and Kelly, (1990), the increased rate of energy expenditure by the visceral organs often results in greater rates of cellular turnover which is manifested by alterations in the intestinal morphology (Jin et al., 1994). It is possible that lowering of dietary fiber content by addition of enzymes as nutritional strategy is worthwhile. In the current study, addition of xylanase to DFM2 led to numeric reduction of organ weights (relative to body weight) of kidney, liver, empty intestinal weight, intestinal length and the entire weight of G.I.T compared to the control. Xylanase supplementation alone led to numeric reduction of the liver and caecum. Visceral organs constitute about 15% or less of body weight and utilize a higher proportion of energy

generated in growing pigs (Pond et al., 1989; Yen, 1997). It can be inferred that relative changes in organ size because of high dietary fiber intake may impact on energy metabolism and the efficiency of growth (Pekas and Wray, 1991). Diets that increase visceral organ mass increase body energy expenditure, leading to less energy retained for body tissue accretion (Ferrell, 1988). Combination of xylanase to DFM1 numerically reduced visceral organ weights of kidney, spleen, entire GIT and cecum relative to the control. This suggests that energy expenditure due to these organs will be reduced and the energy that is conserved will enhance performance.

The NDF content of the basal diet in the present study was 24.2% and dietary supplementation DFMs, xylanase and their combinations had effect on the mass of gastrointestinal tract compared to the control. On the contrary, (Anugwa et al., 1989; Hansen et al., 1992; Jorgensen et al., 1996) reported significant effects of dietary fiber on the hypertrophy of the gastrointestinal tract when fed dietary fiber content ranging between 20 to 40% dietary NDF. The difference in results, even though a similar dietary fiber level was fed was attributed to reduction of entrapped nutrients by addition of enzymes in high fiber diets and different sources of fiber fed in the experiments.

The major site of amino acid degradation in mammals is the liver. The liver metabolizes excess amino acids through transamination and deamination processes (Krebs, 1942) during protein synthesis. Ammonia, a product of deamination, is toxic and needs to be excreted from the body by the liver through the blood in the form of urea. Urea contributes significantly to BUN (Lehninger et al., 2005). Higher BUN values indicate a higher metabolic burden on the liver and can be used to assess the biological value of protein in feed (Eggum, 1970). It is reported that higher BUN values tend to

increase urinary nitrogen excretion and adversely result in poor nitrogen digestibility leading to poor performance (Kohn et al., 2005). Efficiency of nitrogen utilization and lean deposition can be estimated by (BUN) in the serum (Whang et al., 2003). No effect of DFMs, xylanase and their combinations on BUN was detected in the current study compared to the control. Consistent with the current result, Wang et al. (2009) reported that there was no effect of exogenous enzymes on BUN in growing-finishing pigs fed on single, or cocktails of, carbohydrases in low-nutrient-density diets. On the contrary, Jo et al., (2012), fed a corn-soy based diet supplemented with xylanase to growing pigs and observed increase in BUN. It is speculated that the variation in results might be due to differences in diet composition as well as the inclusion rate of enzymes used.

In the current study, corn, barley, wheat, SBM and DDGS represented 71.4 % of the experimental basal diet. Corn contains 10% NSP, which is mainly arabinoxylan and β-galactomannan, and SBM contains 22.7% NSP such as α-galactosides and β-galactomannan (CVB, 1998). Monogastrics cannot hydrolyse these NSP since they lack NSP degrading enzymes to break α-1,6-galactosyl bonds and β-1, 4-mannosyl bonds (Veum and Odle, 2001). However, removal of these NSPs by carbohydrase supplementation is not necessarily of great benefit, especially when oligomers are still indigestible. According to Vahjen et al. (2007) reduction in digesta viscosity may be one of the most important benefits of carbohydrase supplementation. Supplementation of DFM2 numerically reduced ileal digesta viscosity 13.7% compared to DFM1 supplementation. The soluble NSP components of the β-glucans and pentosans, glycoproteins as well as pectins; act in the digestive tract by increasing the viscosity. An increase in viscosity of digesta will reduce nutrient availability, especially to young pigs

and broilers. Active exogenous enzymes can reduce the viscosity of digesta and increase the nutrient digestibility (Vukic and Wenk, 1993b). In the current study, the combination of DFM2 with xylanase numerically reduced ileal digesta viscosity by 12.6% compared to pigs supplemented with DFM1 and xylanase combination. Xylanase attacks the arabinoxylan backbone, causing a decrease in the degree of polymerization (Bengtsson et al., 1992; Courtin and Delcour, 2002; Hu et al., 2008) and thus liberates oligomers. Beneficial effects of addition of amylase (Ritz et al., 1995), proteinase (McNab et al., 1996), or mannanase (Yoon et al., 2010) to diets fed to pigs and poultry to reduce viscosity have been reported. Dietary supplementation of xylanase, DFMs and their combination did not influence ileal digesta viscosity in the present study. However, xylanase supplementation and xylanase in combination with DFM2 numerically reduced ileal digester viscosity by 10.9% and 13.0% compared to the control group respectively. A combination of different enzymes may be required for complete degradation of the substrates in the experimental diets (Kim et al., 2003; Li et al., 2010) hence lack of response to detect significant reduction in viscosity.

Diets with high fiber content are relatively low in digestibility and the use of feed enzymes is highly recommended (Collier and Hardy, 1986; Knudsen and Hansen, 1991). Supplementation of xylanase in the current study improved ATTD of energy, crude protein by 3 and 2.6 percentage points respectively above the control. A possible mechanisms responsible for this improvement is improved access to cellular contents associated with hydrolysis of structural carbohydrates (Bedford, 2002) leading to more substrate available for protease to utilize. To buttress this fact, xylanase supplementation

also enhanced ATTD of NDF and ADF further elucidating the improvement in energy digestibility observed.

Dietary treatment of xylanase, DFMs and their combination did not influence ATTD of DM, energy, and crude protein. Consistent with our result, carbohydrase supplementation did not affect digestibility of DM (Woyengo et al., 2008), CP (Nitrayová et al., 2009) and energy (Olukosi et al., 2007a,c). However, improvements in digestibility of DM (Li et al., 1996; Nortey et al., 2007; Olukosi et al., 2007a) and energy digestibility (Yin et al., 2000; Diebold et al., 2004; Olukosi et al., 2007c) have been reported. Differences in results could be due to the composition of basal diets and inclusion level of the enzymes. For instance, Nortey et al. (2007) fed wheat, wheat millrun and SBM in the basal diets supplemented with 167g per metric tonne of xylanase whereas in the present study diverse fiber sources (corn, wheat, wheat middling's, barley, DDGS and SBM) were fed with the inclusion rate of 250g of xylanase per metric ton of feed. Also multi-enzyme combinations may be required to completely degrade structural carbohydrates in plant cell walls (Morgavi et al., 2012) to enhance nutrient digestibility. Chesson and Forsberg, (1997) and Krause et al. (2003) explained that cellulose and hemicellulose alone requires a number of glycosidic hydrolases.

Dietary supplementation of xylanase, in the present study only marginally improved ATTD of energy compared to the control. Effects of carbohydrase on energy utilization have been contradictory. Carbohydrase supplementation improved energy utilization in corn-soybean meal diets (Meng et al., 2005; Leslie et al., 2007; Rutherfurd et al., 2007; Cowieson and Ravindran, 2008a,b; Yang et al., 2010). Others noted no improvement in energy utilization in response to carbohydrases (Olukosi et al., 2007b).

The differences in the effect of the enzymes on energy of diet may relate to the amount of substrate for the enzyme or availability of energy from the feed, or both. Palander et al. (2005), showed that improvement in energy from cereal grains with carbohydrase supplementation might be masked when the energy value of the cereal grain is large. Similarly, Adeola et al. (2008) reported that, carbohydrases improved ME in diets with reduced ME but not in diets with greater ME. Our experimental diet was formulated to meet or exceed NRC (2012) nutrient requirement. The energy level in diet was therefore not a limiting factor hence lack of response to detect improvement in energy digestibility. McNab (1993) explained that enzymes are often added to formulated diets which provide the necessary nutrients for optimal animal performance and any increase in nutrient availability because of enzyme activity would be excess to the animal's requirements and not necessarily detected in response-type measurements.

The efficiency of energy utilization in fibrous feed ingredients is affected by the digestibility of dietary fiber and the production of VFA (Urriola et al., 2010). Anderson et al. (2012) suggested that GE and total dietary fiber are the significant criteria in estimating energy values of corn coproducts. Therefore any nutritional intervention to improve dietary fiber digestibility in the feed is highly commendable. Xylanase supplementation improved ATTD of NDF and ADF by 6.5 and 9.8 percentage points respectively compared to the control group of pigs.

The ATTD of NDF in the present study was approximately 6.5 percentage units higher in pig supplemented with xylanase compared with pigs fed on the control diet. Assuming a linear relationship (Figure 5.1) between the NDF digestibility and energy

digestibility of the diet, this equates to a 0.44% improvement in energy digestibility for each 1% improvement in NDF digestibility. This is in agreement with Just et al. (1983) who reported a 1.3% increase in energy digestibility for each 1% reduction in dietary fiber content. Noblet and Perez (1993) reported similar results with grower pigs with each 1% decrease in dietary fiber resulting in an improvement in energy digestibility by 1.1%. The differences in values obtained in the current study compare with the previous experiments is attributable to many fiber sources (corn, wheat, barley, DDGS, SBM and wheat middlings) that are insoluble used in the current studies whereas Just et al. (1983) fed low fiber by-products originating from only the corn dry milling process. The source of the insoluble fiber used, inclusion level in diet, and experimental period may account for differences in results among experiments (Agyekum et al., 2012). Also while we analyzed for NDF other studies considered total dietary fiber.

Water supplied through a nipple drinker is not entirely consumed by pigs. A portion of that water is lost due to water flow rate, design of the drinker, type of feed fed, height of the drinker and inclination of the drinker nipple. Teat bite drinkers were used in the current study. Alvarez-Rodriguez et al. (2013) reported that cup drinkers (nipple square bowl and nipple bowl with short mouthpiece) saved on average 29.7% water compared with drinkers without cups (teat and bite types). It is explained that with cup drinkers' water flows into a bowl after pig's manipulation. The bowl or cup catches water from the sides of the pig's mouth during drinking (Muhlbauer et al., 2010) whereas nipple drinkers may be manipulated to dispense water onto pig heads and bodies. A survey within USA growing-finishing facilities where dry feed is fed reveals that total water consumption in

farms with nipple drinkers was 9.1 L/pig per day, while it decreased to 4.4 L/pig per day in farms using cup drinkers (Muhlbauer et al., 2010).

CONCLUSION

The effectiveness of xylanase to improve nutrient digestibility is well established and was confirmed in the present study. Xylanase supplementation improved ATTD of fat, NDF, ADFand numerically improved ATTD of energy and ileal protein digestibility. Supplementation of either DFM1 or DFM2 alone could not improve ATTD of nutrients. Combination of xylanase with DFM1 increased the intestinal length. Addition of the DFM2 to xylanase reduced organ weight, reduced ileal digester viscosity and exhibited potential to improve nutrient digestibility. The current study demonstrated both synergism and antagonistic effects when xylanase is combined with either DFM1 or DFM2 in comparison to feeding of xylanase, DFM1 and DFM2 individually.

Table 5.1. Ingredient and composition of basal diets, on as-fed basis fed to pigs

Indredients, %	Common diet	Phase II
Corn	17.35	18.00
Barley	5.00	5.00
Wheat (Soft red)	22.00	23.20
Spray-dried porcine plasma	5.00	3.50
Wheat middlings	4.50	5.00
Soyabean meal (Solvent extracted)	10.00	10.15
Whey Powder	16.00	15.00
Fishmeal	5.00	5.00
Corn DDGS (<4% oil)	10.00	10.00
Limestone ($CaCO_3$)	0.92	0.92
Dicalcium phosphate	0.40	0.40
Choice White grease	2.00	2.00
$_L$-Lysine HCL	0.30	0.30
$_{DL}$- Methionine	0.20	0.20
$_L$-Threonine	0.05	0.05
$_L$-Tryptophan	0.03	0.03
Salt	0.35	0.35
Vitamin & Mineral premix[1]	0.50	0.50
Celite	0.40	0.40
Total	100.00	100.00

Composition of diet	DM %	NE, kcal/kg	Protein, %	NDF, %	ADF, %	Ca/P
Common diet	89.62	2441	22.87	20.3	5.77	1.14
Treatment diet	89.49	2401	21.48	24.2	5.96	1.16

[1]The vitamin and trace mineral premix provided the following (per kg of diet): vitamin A, 11,000 IU; vitamin D_3, 2,756 IU; vitamin E, 55 IU; vitamin B_{12}, 55µg; riboflavin, 16,000 mg; pantothenic acid, 44.1 mg; niacin, 82.7 mg; Zn, 150 mg; Na, 25mg; Cl, 30mg; K, 45mg; Fe, 175 mg; Mn, 60 mg; Cu, 17.5 mg; I, 2 mg; and Se, 0.3 mg, choline 495mg, folic acid 1.7mg, thiamine 1.1mg, and biotin 0.22mg.

Table 5.2. Apparent total tract digestibility of DM, CP, fat, NDF, and ADF of nursery pigs fed high fiber diets in Exp. II using the index method.

	Xylanase	DFM1	DFM2	Digestibility of nutrient, %							
Diet	**g/kg**	**g/MT**	**g/MT**	**IBW0**	**FBW**	**ENERGY**	**DM**	**CP**	**FAT**	**NDF**	**ADF**
1. Control	0	0	0	9.08	19.98	69.83	71.84	73.41	50.40	53.05	25.87
2. DFM1	0	500	0	9.05	19.90	70.15	71.83	73.51	41.66*	54.64	30.40
3. Xylanase	250	0	0	9.07	19.95	72.82	73.93	75.97	55.12*	59.54*	35.63*
4. Xylanase + DFM1	250	500	0	9.06	19.93	70.68	72.38	73.17	44.33*	55.37	25.10
5. DFM2	0	0	500	9.09	20.00	70.31	71.84	73.63	47.12	51.70	25.87
6. Xylanase + DFM2	250	0	500	8.99	19.77	71.61	72.77	74.97	52.03	53.86	22.83
SEM				0.12	0.96	1.01	0.88	1.19	2.51	1.56	2.71
P-values					0.669	0.311	0.505	0.518	0.007	0.03	0.033
Contrasts											
1 DFM1 + Xylanase vs. (DFM1+Xylanase)					0.132	0.514	0.65	0.292	0.192	0.374	0.025
2 Xylanase + DFM2 vs. (DFM2+Xylanase)					0.884	0.973	0.919	0.912	0.766	0.363	0.025
3 DFM1 vs. DFM2					0.344	0.91	0.992	0.94	0.13	0.193	0.248
4 (DFM1+Xylanase) vs. (DFM2+Xylanase)					0.33	0.515	0.756	0.296	0.037	0.499	0.559

* Indicates significant difference ($P < 0.05$) compared with the control by Dunnett's test.
Note: IBW0 = initial body weight (kg), FBW = final body weight (kg).

Table 5.3. Apparent ileal digestibility of energy, DM and CP of nursery pigs fed high fiber diets in Exp. II.

	Xylanase	DFM1	DFM2	Digestibility of nutrient, %		
Diet	**g/kg**	**g/MT**	**g/MT**	**DM**	**Energy**	**CP**
1. Control	0	0	0	55.4	55.8	61
2. DFM1	0	500	0	50	49.2	45.8
3. Xylanase	250	0	0	53	52.8	70.7
4.Xylanase+DFM1	250	500	0	54.1	54.9	66.6
5.DFM2	0	0	500	55.7	55.2	68.3
6.Xylanase+DFM2	250	0	500	53.7	53.1	55
SEM				4.54	4.75	6.3
P-value				0.963	0.945	0.154
Contrasts						
1	DFM1 + Xylanase vs. (DFM1+Xylanase)			0.6573	0.5197	0.3159
2	Xylanase + DFM2 vs. (DFM2+Xylanase)			0.9193	0.9043	0.1365
3	DFM1 vs. DFM2			0.3926	0.3947	0.0243
4	(DFM1+Xylanase) vs. (DFM2+Xylanase)			0.9609	0.8237	0.2756

Table 5.4. Histological measurement of nursery pigs fed high fiber diet in Exp. II.

	Xylanase	DFM1	DFM2		Histological measurement		
Diet	**g/kg**	**g/MT**	**g/MT**	**FBW**	**Villus height, µm**	**Crypt depth, µm**	**Villi:Crypt**
1. Control	0	0	0	19.5	473.61	192.47	2.47
2. DFM1	0	500	0	19.59	493.54	183.57	2.66
3. Xylanase	250	0	0	19.64	467.76	187.76	2.50
4.Xylanase+DFM1	250	500	0	18.68	531.17	214.02	2.48
5. DFM2	0	0	500	18.92	504.92	196.44	2.57
6.Xylanase+DFM2	250	0	500	19.37	518.2	193.78	2.70
SEM				0.669	34.364	10.835	0.125
P-value				0.96	0.648	0.475	0.653
Contrasts							
1	DFM1 + Xylanase vs. (DFM1+Xylanase)			0.132	0.195	0.044	0.509
2	Xylanase + DFM2 vs. (DFM2+Xylanase)			0.884	0.399	0.895	0.263
3	DFM1 vs. DFM2			0.344	0.809	0.494	0.635
4	(DFM1+Xylanase) vs. (DFM2+Xylanase)			0.33	0.765	0.176	0.196

Table 5.5. Effect of xylanase and Direct fed microbial supplementation on ileal pH, stomach pH, BUN and viscosity of nursery pigs fed high fiber diet in Expt. II

Diet	Xylanase g/kg	DFM1 g/MT	DFM2 g/MT	pH Ileal	pH Stomach	BUN, mg/dl	Viscosity, cP
1. Control	0	0	0	6.3	3.2	1.17	1.92
2. DFM1	0	500	0	6.4	4.4	1.43	2.12
3. Xylanase	250	0	0	6.7	4.7	2.13	1.71
4.Xylanase+DFM1	250	500	0	6.3	3.4	1.75	1.91
5. DFM2	0	0	500	6.7	4.7	2.16	1.83
6.Xylanase+DFM2	250	0	500	6.4	3.5	1.56	1.67
SEM				0.2	0.47	0.26	0.13
P-value				0.466	0.09	0.124	0.164
Contrasts							
1 DFM1 + Xylanase vs. (DFM1+Xylanase)				0.305	0.053	0.936	0.970
2 Xylanase + DFM2 vs. (DFM2+Xylanase)				0.263	0.047	0.122	0.527
3 DFM1 vs. DFM2				0.251	0.603	0.100	0.111
4 (DFM1+Xylanase) vs. (DFM2+Xylanase)				0.619	0.830	0.624	0.189

Table 5.6. Effect of xylanase and Direct fed microbial supplementation on organ development of nursery pigs fed high fiber diet in Expt. II.

	Xylanase	DFM1	DFM2	Intestinal organ, gram per kg BW									
Diet	**g/kg**	**g/ton**	**g/ton**	**FBW**	**Kidney**	**Liver**	**Spleen**	**Heart**	**Stomach**	**GIT[1]**	**Caecum**	**Intesti.[2]**	**Int. L.[3]**
1. Control				19.98	6.04	31.83	2.15	4.89	10.03	87.65	3.76	48.7	67.19
2. DFM1				19.90	5.6	31.58	2.16	4.98	9.81	85.09	2.6	50.31	70.54
3.Xylanase				19.95	6.21	31.39	2.16	5.76	10.13	88.55	3.19	49.99	67.43
4.Xylanase+DFM1				19.93	5.45	32.7	1.9	5.43	9.59	84.75	2.63	53.48	77.52*
5. DFM2				20	6.19	25.59	3.01	6.37*	9.74	84.44	2.75	50.28	68.34
6.Xylanase+DFM2				19.77	5.47	29.81	2.42	5.02	10.62	82.85	2.65	47.24	63.36
SEM				0.96	0.44	2.11	0.34	0.34	0.44	4.23	0.37	2.54	3.24
P value				0.669	0.331	0.203	0.299	0.021	0.617	0.923	0.164	0.65	0.013
Contrasts													
1	DFM1 + Xylanase vs. (DFM1+Xylanase)			0.132	0.268	0.633	0.532	0.882	0.47	0.682	0.547	0.294	0.01
2	Xylanase + DFM2 vs. (DFM2+Xylanase)			0.884	0.08	0.603	0.698	0.013	0.213	0.471	0.464	0.362	0.149
3	DFM1 vs. DFM2			0.344	0.211	0.049	0.089	0.005	0.908	0.912	0.754	0.993	0.535
4	(DFM1+Xylanase) vs. (DFM2+Xylanase)			0.33	0.966	0.329	0.284	0.376	0.105	0.744	0.965	0.095	< 0.001

[1]Emptied weight of entire gastrointestinal tract.
[2]Intestinal organ weight, gram per kilogram of live body weight.
[3]Small intestine length, meters per kg BW.
*Indicates significant difference from the control ($P < 0.05$) by Dunnett's test.

Table 5.7. Effect of xylanase and Direct fed microbial supplementation on water intake of nursery pigs fed high fiber diet in Expt. II.

	T1	T2	T3	T4	T5	T6
Water disappearance, L/d[1]	11	9.99	16.01	13	10.97	9.36
Actual water intake[2]	2.87	3.02	3.00	2.67	2.54	2.73

1. Litres of water measured through flow meter per day.

2. Water intake per d (L) = 0.149 + 3.053 x Dry feed intake (kg), Brooks et al., (1984).

Figure 5.1.A linear regression plot of percentage apparent total tract digestibility of energy against percentage apparent total tract digestibility of neutral detergent fiber

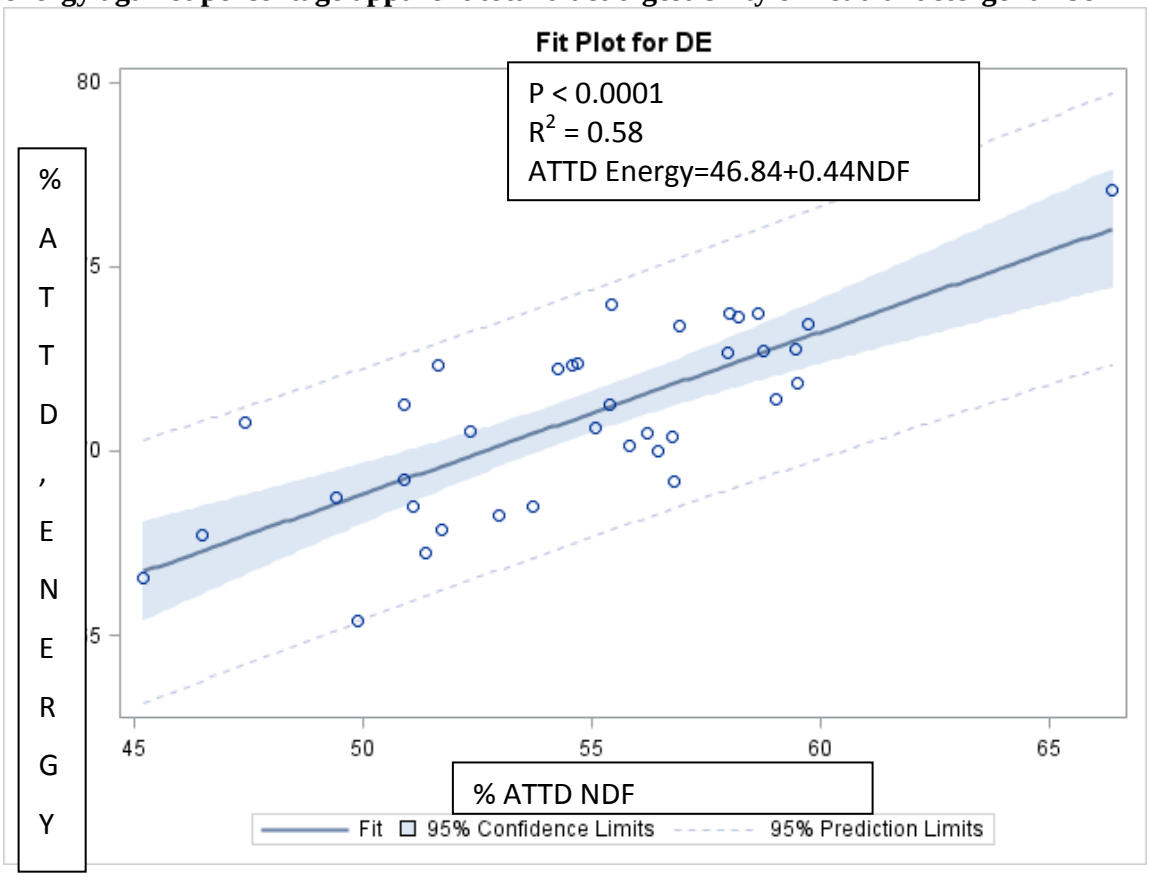
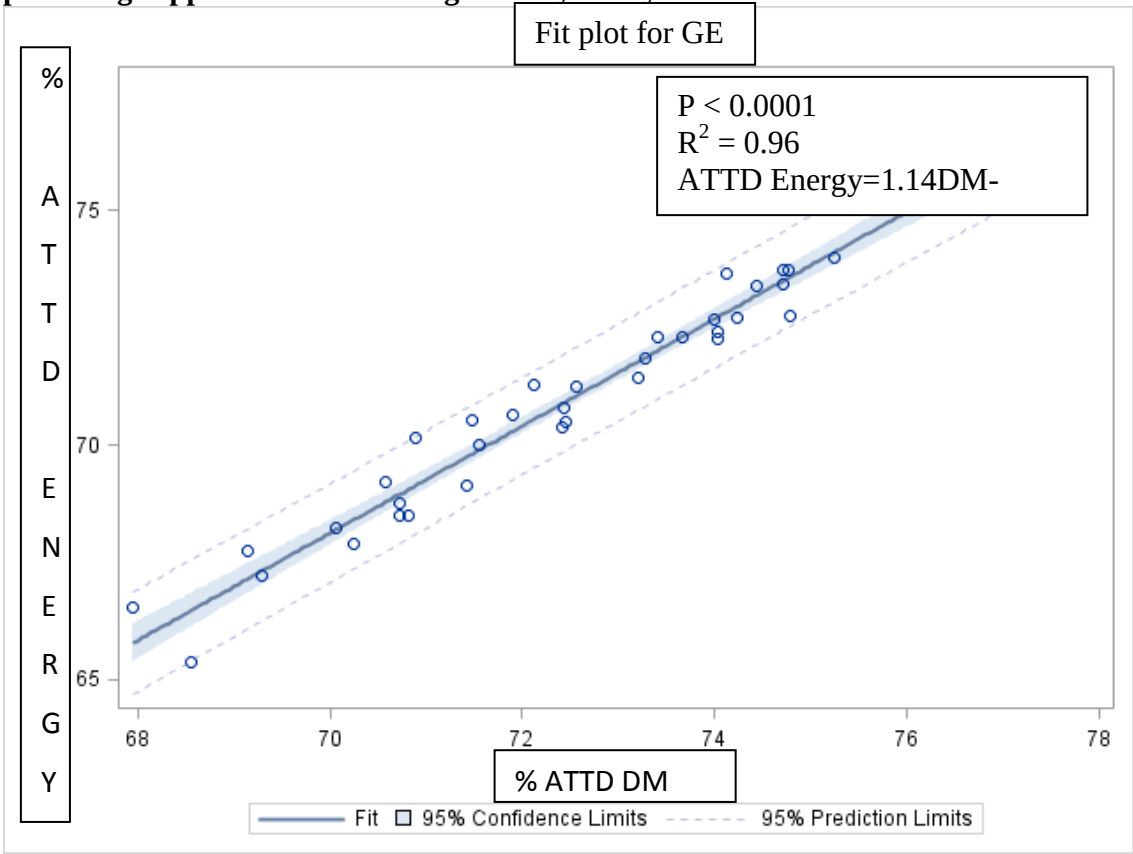


Figure 5.2.A linear plot of percentage apparent total tract digestibility of energy against percentage apparent total tract digestibility of dry matter



Chapter 6

EFFECT OF XYLANASE ALONE OR IN COMBINATION WITH DFM1 OR DFM2 ON PERFORMANCE OF NURSERY PIGS FED ON HIGH FIBER DIET

Summary

In experiment III, effects of supplementing xylanase alone or in combination of either *Bacillus* species Direct-fed microbials (DFM1) or *Lactobacillus* multi-species Direct-fed microbials (DFM2) on blood urea nitrogen concentration and growth performance of nursery pigs fed high fiber diets ware studied in a 28 d trial. One hundred and forty-four (18 d old) [(Landrace × Yorkshire (Topigs, Winnipeg, Canada)) × Duroc (Compart's, Nicollet, MN), of both sexes were weighed and ear tagged. Pigs had an initial mean body weight of (8.60 ± 0.10 kg) and allocated to 1 of 6 dietary treatments with 6 replicates in a randomized complete block design. The treatments were T1, basal diet without xylanase or DFM as control; T2, control supplemented with DFM1 at 500g/MT; T3, control supplemented with xylanase 250g/MT; T4, xylanase (250g/MT) and DFM1 (500g/MT); T5, DFM2 (500g/MT) and T6, xylanase 250g/MT and DFM2 (500g/MT). The study revealed that dietary treatment did not influence BUN concentrations ($P > 0.05$) but addition of xylanase to the DFMs numerically reduced BUN concentration ($P > 0.05$) compared with pigs fed on control diet or xylanase supplemented diet. Supplementation of xylanase, DFM1or DFM2 alone did not influence ($P > 0.05$) final body weight, average daily feed intake, average daily gain and feed efficiency compared to the control. However, xylanase synergizes with DFM to numerically reduce feed intake compared to the control and improve feed efficiency. Combination of either DFM1 or DFM2 with xylanase improved feed efficiency ($P < 0.05$) compared to the average effect of feeding DFM and xylanase alone. Comparison between DFM1 and DFM2 showed no difference in pig performance ($P > 0.05$) during the study. Addition of xylanse to DFM1 in terms of production performance was not

different from addition of xylanase to DFM2 ($P > 0.05$). In conclusion, addition of xylanase to the DFMs numerically reduced BUN concentration, reduced feed intake and improved feed efficiency during the overall period of study ($P < 0.05$).

Key words: Xylanase, Direct-fed microbials, fibre diets, nursery pigs, BUN and performance.

INTRODUCTION

The digestive capacity of piglets at weaning is limited in terms of its physiological function and constitutes a critical period in the developmental stage (Bach Knudsen and Jørgensen, 2001). The situation is further exacerbated by the sudden switch over from highly digestible milk diet to dried mash cereal based feed, which decreases both the digestibility and availability of nutrients for piglets. Low feed intake is therefore observed during this period, which retards the passage rate of the digesta and may contribute to intestinal inflammation (McCracken et al., 1999). Physiologically, villus height is decreased while the crypt depth is increased (Hampson, 1986; Pluske et al., 1997). This phenomena leads to higher availability of substrate and create beneficial conditions for microbial proliferation and establishment of pathogenic strains throughout the gastro-intestinal tract (Hampson et al., 2001). The pathogenic strains adhere to villous enterocytes mainly in the anterior small intestine, where they release enterotoxins that cause fluid and electrolyte loss (Francis, 2002). Unfortunately, the immune system at this stage is immature (Bailey et al., 2001), which renders the piglets highly susceptible to gastro-intestinal disturbances such as diarrhea.

Traditionally, antibiotics are used to treat incidence of diarrhea. With the concern of antibiotic resistance and the use of antibiotics in livestock production, coupled with the need to exclude pathogens, alternatives to antibiotics are required. Some of these alternatives are organic acids, prebiotics and probiotics (Zimmerman and Mosenthin, 2001; Jensen et al., 2003). The response of piglets to dietary intervention with supplementated enzymes and / or probiotics is usually assessed using growth performance responses. Currently, there is a paucity of information on how exogenous

enzymes such as xylanase combined with DFMs affect growth performance in nursery pigs fed high fiber diets. The present study tested the hypothesis that growth performance can be improved by xylanase and DFM alone and in combination. The objective of study 3 was to evaluate the effect of DFMs and xylanase alone or in combination on growth performance and blood urea nitrogen concentration (BUN) in nursery pigs fed for 28 days post weaning on high fiber diets.

MATERIALS AND METHODS

The University of Minnesota Institutional Animal Care and Use Committee approved all experimental protocols (Protocol No. 1104A98932). Animals were cared for according to The Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching (FASS, 1998).

Enzymes and Direct-fed Microbials, (DFM) 1 and 2

Xylanase was endo-1, 4-β-xylanase (EC 3.2.1.8; Porzyme 9300; Danisco Animal Nutrition, Marlborough, UK), supplemented in the diet to supply 250g/MT of feed and DFM1 and DFM2 (Danisco Animal Nutrition, Canada) added to supply 500g/MT of feed were used alone or in combination with xylanase in the study.

Animal, Housing and Diets

The experiment was conducted in environmentally controlled nursery facility at The University of Minnesota, Southern Research Outreach Center, Waseca, MN. One hundred and forty-four 18 d crossbred piglets [(Large white × Yorkshire (Topigs, Winnipeg, Canada)) × Duroc (Compart's, Nicollet, MN)], of both sexes were weighed and ear tagged. Pigs were fed a common a diet without enzymes for 9 days after weaning

before switching on to experimental diets. Pigs were blocked by body weight (mean body weight 8.6 ± 0.10 kg) and sex and randomly allocated to the experimental pens in a randomized complete block design. Each pen housed 4 pigs, which constituted the experimental unit and each pen was assigned one of six experimental diets with 6 replicates. A two-phase feeding program was adopted and each phase lasted for 14 days. The experimental diets were T1, basal diet without xylanase or DFM as control; T2, control supplemented with DFM1at 500g/MT; T3, control supplemented with xylanase 250g/MT; T4, xylanase (250g/MT) and DFM1 (500g/MT); T5, DFM2 (500g/MT) and T6, xylanase 250g/MT and DFM2 (500g/MT).

Xylanase and DFMs were added at the expense of corn. The six experimental nursery diets were formulated to meet or exceed NRC (2012) requirement of nursery pigs (Table 6.1). Diets were fed in mash form and formulated to provide 1.37% SID lysine, 0.8% calcium and 0.65% phosphorus during the first phase, 1.23% total lysine, 0.7% calcium and 0.60% phosphorus during phase the second phase. Feed and water was provided ad-libitum. Each pig had a unit area of 0.46 m^2 in a pen measuring 1.52m X 1.22m. Feed disappearance and live body weight was recorded at the end of each phase for determination of average daily feed intake (ADFI), average daily gain (ADG), and gain to feed ratio (G: F). Blood samples were taken from one pig in each pen for BUN analysis at the end of the second phase.

Chemical Analysis of Basal Diet

Samples of diets and fecal samples were analyzed for dry matter (DM), gross energy (GE), crude protein (CP), fat, neutral detergent fiber (NDF), acid detergent fiber (ADF)

and acid insoluble ash (AIA). All the analyses were done in duplicate. Feed samples were dried at 105 ˚C for 4 h in an oven (Thermo Scientific Precision, Thermo Fisher Scientific Inc., Hampton, New Hampshire) to determine DM (AOAC, 2002 method 934.01). The remaining sample was used to determine GE (AOAC, 1995) by bomb calorimetry using a IKA WERKE c2000 basic bomb calorimeter (IKA Werke GmbH & Co. KG**,** Staufen, Germany) with benzoic acid as a calibration. Samples were analyzed for NDF and ADF using a filter bag technique (ANKOM 2000 fiber analyzer, method 12 and 13; ANKOM Technology, Macedon, NY). Crude protein was determined by digestion of samples in a Tecator Digestion System 20 1015 Digestor and then analyzed in a 2300^{R} Kjeltec Auto Analyzer Unit (Foss North America, Eden Prairie, MN), according to methodology (AOAC 945.18) described by AOAC (2000).

Fat content (AOAC, 2000) was determined by extraction with filter bag technique of $ANKOM^{XT10}$ (ANKOM Technology, Macedon, NY). Briefly, weighed samples were mixed with about 1g of celite and completely encapsulated by heat and sealed. Bags and contents were hydrolyzed using 3N HCL for 60 min at 90 ˚C. Bags were then pressed between absorbent papers to remove excess acid and dried in an $ANKOM^{RD}$ Dryer at 110 ˚C for 2 hr 30 min. After cooling in desiccant pouches, samples were extracted with petroleum ether (90 ˚C for 1 h), dried at (110 ˚C for 20 min) and weighted for calculation of acid hydrolysis fat. To determine celite concentration, the samples were weighed before and after ashing in a high temperature muffle furnace at 650 ˚C for 6 h (Isotherm Muffle Furnace, Thermo Fisher Scientific Inc. Hampton, New Hampshire) after 2 h acid digestion at 250 ˚C.

Blood Sampling

Blood samples were taken from each pig for BUN analysis at the end of the experiment. Blood samples were collected from the jugular vein of each pig on d 14 via jugular venipuncture by using heparinized vacuum container tubes (Covidien Inc. USA) before euthanization by intravenous injection of sodium pentobarbital (50 mg/kg, Vedco, Inc., St. Joseph, MO, U.S.A.). The blood samples were centrifuged (Hemle Z300, Labnet Inc, U.S.A.) at 2500*g* for 10 min at 4°C to recover the plasma and stored at -20°C until analysis for blood urea nitrogen (BUN) concentration using commercial kits (Stanbio laboratory, TX U.S.A.).

Blood Urea Nitrogen (BUN) analysis

Plasma samples were analyzed for BUN using the Stanbio Urea Nitrogen kit Liqui-UVR (Stanbio laboratory, Boerne, Texas) and the method described by Sampson et al. (1980). Samples were thawed at room temperature for 30 min before analysis. Preparation of reagents involved mixing of buffer and enzyme (urease) in a ratio of 5:1 respectively. The resulting solution was allowed to stand for 30 min before use at room temperature (15 to 25˚C). Two milliliters of working reagent was added to test tubes and warmed at 37˚C for 3 minutes. After spinning the sample for 5 seconds at 1000rpm, 20 µl of serum was added to each test tube, mixed gently and absorbance recorded exactly at 30 seconds (A1) and 90 seconds (A2) after mixing using a spectrophotometer (Thermo scientific Genesys 20, Vernon Hills, IL) calibrated at 340 nm with distilled water at zero absorbance. Change in absorbance was recorded as the difference between A1 and A2 for both serum samples and the standard.

Calculation of Blood Urea Nitrogen (BUN) Concentration

Serum BUN (mg/dl) was calculated using the relation: Serum BUN (mg/dl) = ΔAu / ΔAs * 30, where ΔAu and ΔAs are absorbance difference (30 s and 90 s) of serum samples and standard respectively and 30, the concentration of standard (mg/dl).

Statistical Analyses and Calculations

Statistical analysis was as appropriate for a randomized complete block design using the proc MIXED procedure (SAS version 9.3, Inst. Inc., Cary, NC). Pen was the experimental unit and initial body weight was used as block for all response criteria measured in the performance study. The UNIVARIATE procedure was used to verify homogeneity of variance among treatments. The interaction between treatment and sex was not significant and was excluded from the model. The final model included the main effect of treatment. Dunnett's test was used for comparisons between the control and other treatments. Regardless of the overall test for the treatment effects, 4 sets of pre-planned contrasts were utilized to evaluate the effect of treatments: (1) average of DFM1 and xylanase vs. (DFM1 + xylanase), (2) average of DFM2 and xylanase vs. (DFM2 + xylanase), (3) DFM1 vs. DFM2, (4) (DFM1 + xylanase) vs. (DFM2 + xylanase). Contrasts 1 and 2 were used to test the interaction between xylanase and DFM. Least square means are provided with pooled standard error and $P < 0.05$ was considered statistically significant, whereas $P < 0.10$ was considered a tendency.

RESULTS

BUN

Dietary supplementation DFMs, xylanase and their combination had no effect (P > 0.05) on serum BUN concentration compared to pigs fed on the control diet (Table 6.2).

Pigs supplemented on DFM1 and xylanase did not differ (P > 0.65) in BUN concentration from pigs that received DFM2 combined with xylanase. Similarly, DFM1 supplementation did not influence serum BUN concentration compared to DFM2 supplementation. Addition of xylanase to either DFM1 or DFM2 numerically reduced BUN concentration relative to pigs fed on the control diet but the difference was not statistically significant. No interaction between xylanase and DFMs were observed compared to individual supplementation of DFMs and Xylanase.

Performance

Effects of xylanase and direct fed microbial supplementation on growth performance of nursery pigs fed high fiber diets in Experiment III are shown in Table 6.3. Supplementation of xylanase, DFM1 and DFM2 alone or in combination did not influence ($P > 0.05$) final body weight, average daily feed intake, average daily gain or feed efficiency compared to the control in phase I, phase II and the overall phase. Combination of DFM1 and xylanase improved ($P < 0.05$) feed efficiency compared to the DFM1 or xylanase alone. Similarly, combined supplementation of DFM2 and xylanase improved feed efficiency ($P < 0.03$) compared to the DFM2 and xylanase independently. The contrast between DFM1 and DFM2 showed no effect on pig performance (P > 0.44) for all phases as well as the entire phase of study. Addition of xylanase to DFM1 in terms of production performance was not different from addition of xylanase to DFM2 (P > 0.51) during the study.

DISCUSSION

The liver metabolizes excess amino acids through transamination and deamination processes (Krebs, 1942) during protein synthesis. Ammonia, a product of deamination, process is toxic and needs to be excreted from the body by the liver via the blood in the form of urea. Urea contributes significantly to BUN (Lehninger et al., 2005). Higher BUN values indicate higher metabolic burden on the liver and can be used to assess the biological value of proteins in feed (Eggum, 1970). It is reported that higher BUN values tend to increase urinary nitrogen excretion and adversely result in poor nitrogen digestibility leading to poor performance (Kohn et al., 2005). The efficiency of nitrogen utilization and lean deposition can be estimated by blood urea nitrogen (BUN) in the serum (Whang et al., 2003). No effect of DFMs, xylanase and their combinations on BUN was detected in the current study compared to the control. However, addition xylanase to either DFM1 or DFM2 numerically reduced BUN by 20.6% and 10.3% respectively relative to pigs fed the control diet. Consistent with the current study, Wang et al. (2009) reported no effect of exogenous enzymes on BUN in growing-finishing pigs fed single, or cocktails of carbohydrases in low-nutrient-density diets. On the contrary, Jo et al. (2012), fed corn-soy based diet supplemented with xylanase to growing pigs and observed an increase in blood urea nitrogen concentration. It is speculated that the variation in results might be due to differences in diet composition as well as the inclusion rate of enzymes used.

Supplementation with xylanase alone or in combination with direct-fed microbials did not affect final body weight of pigs compared to the control in the performance study. This observation was confirmed in the digestibility study since the same feed was fed,

even though the duration of the two studies varied. Antagonism (Naveed et al., 1999; Saleh et al., 2004), additivity (Zyla et al., 1996; Mulyantini et al., 2005), and synergism (Ravindran et al., 1999) effects of enzyme combinations have been reported. Little is known however, with enzyme and direct-fed microbial combinations. The current study was carried out to determine whether xylanase or combination of xylanase with either DFM1or DFM2 could improve on performance of nursery pigs fed high fiber diet. Neither xylanase nor DFMs supplementation had any positive effects on pig body weight gain compared to the control. Effect of xylanase on pig performance is not universal. Positive responses to xylanase supplementation in diets with high-NSP cereal grains were reported (Cadogan et al., 2003; Barrera et al., 2004; Kiarie et al., 2007), whereas others (Mavromichalis et al., 2000; Olukosi et al., 2007a,c; Woyengo et al., 2008) observed no improvement in BW gain in response to xylanase. Differences in experimental results could be attributed to type and quantity of cereal grains used, stage of growth Omogbenigun et al. (2004), the extent of deficiency of limiting nutrient, and the extent to which the enzyme increased digestible nutrient content (Adeola and Cowieson 2011). Many sources of cereal grains such as corn, barley, wheat, and wheat middlings were used in the current study. Consistent with our result however, Officer (1995) found no improvement in ADG when a barly based diet was supplemented carbohydrase enzyme fed to 20 kg pigs.

Combinations of xylanase with DFM tended to reduce feed intake during the entire period of study. The effective breakdown of the fiber component of diet and microbial fermentation of the resulting products might explain the reduced intake. The extra nutrient released might trigger a feedback mechanism to reduce feed intake through

glucostatic or aminostatic responses or may create a nutrient imbalance within the gastrointestinal tract of the pig. However, the results of this study would suggest that bacterial fermentation might have been enhanced with the combination DFMs with xylanase in high fiber diets to improve feed efficiency. Unexpectedly, supplementation of xylanase alone led to reduced feed efficiency compared to pigs fed on the control diet. However, the improvement in nutrients digestibility observed did not translate into improvement in performance with the group that was supplemented with xylanase. Adeola and Cowieson (2011) explained that improvement in nutrient digestibility does not explain all the effects of carbohydrase supplementation on performance or improvement in nutrient utilization might not be accompanied by increased growth rate. Nortey et al. (2007) reported that xylanase improved energy digestibility but did not translate into improvement ADG. Similarly, Barrera et al. (2004) showed that xylanase supplementation improved AA digestibility by an average of 11% whereas in that same study improvement in performance was only marginal. However, it was observed that pigs supplemented with xylanase recorded heavier weight of the emptied GIT compared to control group. Feeding high dietary fiber is associated with increase in weight of GIT and visceral organs (Agyekum et al., 2012; Asmus, 2012). Diets that increase visceral organ mass increases body energy expenditure, leading to less energy retained for body tissue accretion. Pigs supplemented with xylanase diets had lower villus height relative to control pigs which was not expected. Besides Xylanase supplemented group, the pigs on the control diets had lower villi heights. Reduced villus heights imply a smaller surface area for nutrient absorption per unit of time. Growth is therefore impaired leading to decline in feed efficiency. However, addition of the two DFMs to xylanase improves feed

efficiency suggesting change in microbial composition or microbial fermentation was elevated in the hindgut, although we did not measure VFA.

Although addition of xylanase to either of the DFMs improved feed efficiency, blending of DFM2 with xylanase improved feed efficiency better than addition of xylanase to DFM1. DFM2 was multi-strain of lactobacillus species whereas DFM1 was mono-strain bacillus species. DFMs are added to diets to improve the microbial balance and ensure healthy gut with elimination of pathogenic microorganisms. It is suggested that DFM2 supplementation was more effective in this regard than DFM1. Multi-strain DFMs are found to be more effective than mono-strain (Sanders and Huisin't Veld, 1999) and the effect of probiotics may be genera, species and strain specific.

Meng et al. (2010) and Chen et al. (2006) observed an increase in ADG in growing pigs fed diets supplemented with complex DFM (*Lactobacillus acidophilus*, *Saccharomyces cerevisiae*, and *B. subtilis*). However, Davis et al (2008) fed two strains of bacillus complex in the diet of growing to finish pigs and elicited a response in feed efficiency but not ADFI and ADG. Also, Kornegay and Risley (1996) reported that dietary supplementation of a mixture of *Bacillus subtilis* and *Bacillus licheniformis*, or a mixture of *B. subtilis*, *B. licheniforms* and *B. pumilus* to finisher pigs did not affect ADFI, ADG and feed efficiency. According to (Chesson, 1994; Kornegay and Risley, 1996) Bacillus are not considered as natural intestinal inhabitants that colonize the host intestine and therefore have limited capacity to improve performance.

CONCLUSION

Supplementation of xylanase, DFM1 and DFM2 did not influence final body weight, average daily feed intake, and average daily gain and feed efficiency compared to the control. Synergism between xylanase and the DFMs was observed in the current study. Combination of the DFMs with xylanase led to numerical reduction of BUN and feed intake. This dietary intervention resulted in improvement in overall feed efficiency in pigs fed diets supplemented with xylanase in combination with DFMs. It may be surmised that the improvement in feed efficiency due to the combination of xylanase with DFMs is partly attributable to improvement in microbial balance which promotes gut health and enhance efficient nutrient absorption leading to lowering of voluntary feed intake.

Table 6.1. Ingredient and composition of control basal diets fed during Phase I and Phase II, as fed basis

Indredients, %	Phase I	Phase II
Corn	18.00	23.00
Barley	5.00	10.00
Wheat (Soft red)	23.20	23.50
Spray dried porcine plasma	3.50	0.00
Wheat middlings	5.00	10.00
Soyabean meal (Solvent extracted)	10.15	15.00
Whey Powder	15.00	0.00
Fishmeal	5.00	2.00
Dried distillers grain with solubles (<4% oil)	10.00	10.00
Limestone (CaCO3)	0.92	1.10
Dicalcium phosphate	0.40	0.55
Choice White grease	2.00	2.50
$_{L}$-Lysine HCL	0.30	0.55
$_{DL}$- Methionine	0.20	0.15
$_{L}$-Threonine	0.05	0.30
$_{L}$-Tryptophan	0.03	0.05
Salt	0.35	0.40
Mineral premix[1]	0.50	0.50
Celite	0.40	0.40
Total	100.00	100.00

	DM %	NE, kcal/kg	Protein, %	NDF, %	ADF, %	Ca/P
Phase I	89.49	2401	21.48	24.23	5.96	1.16
Phase II	89.38	2455	20.27	25.88	6.15	1.21

[1]The vitamin and trace mineral premix provided the following (per kg of diet): vitamin A, 11,000 IU; vitamin D_3, 2,756 IU; vitamin E, 55 IU; vitamin B_{12}, 55µg; riboflavin, 16,000 mg; pantothenic acid, 44.1 mg; niacin, 82.7 mg; Zn, 150 mg; Fe, 175 mg; Cl, 30 mg; Na, 25mg; K, 45mg; Mn, 60 mg; Cu, 17.5 mg; I, 2 mg; and Se, 0.3 mg, choline 495mg, folic acid 1.7mg, thiamine 1.1mg, and biotin 0.22mg.

Table 6.2. Effect of xylanase and Direct fed microbial supplementation on BUN of nursery pigs fed high fiber diet, Expt. II

	Xylanase	DFM1	DFM2	
Diet	**g/kg**	**g/MT**	**g/MT**	**BUN, mg/dl**
Control	0	0	0	3.19
DFM1	0	500	0	3.32
Xylanase	250	0	0	2.96
Xylanase + DFM1	250	500	0	2.53
DFM2	0	0	500	3.85
Xylanase + DFM2	250	0	500	2.86
SEM				0.54
P-value				0.586
Contrasts				
1	DFM1 + Xylanase vs. (DFM1+Xylanase)			0.343
2	Xylanase + DFM2 vs. (DFM2+Xylanase)			0.398
3	DFM1 vs. DFM2			0.479
4	(DFM1+Xylanase) vs. (DFM2+Xylanase)			0.654

Table 6.3. Effect of ylanase and Direct fed microbial supplementation on growth performance of nursery pigs fed high fiber diet in Expt. III.

	Xylanase	DFM1	DFM2	Phase I					Phase II				Overall Phase		
Diet	**g/kg**	**g/MT**	**g/MT**	**BW0**	**BW1**	**ADFI1**	**ADG1**	**GF1[1]**	**BW2**	**ADFI2**	**ADG2**	**GF2[1]**	**OADFI**	**OADG**	**OGF[1]**
Control	0	0	0	8.63	13.28	0.527	0.332	0.628	21.76	1.016	0.605	0.601	0.772	0.469	0.607
DFM1	0	500	0	8.62	12.90	0.506	0.305	0.602	21.47	1.023	0.613	0.603	0.764	0.459	0.602
Xylanase	250	0	0	8.56	13.14	0.504	0.309	0.616	21.56	1.029	0.581	0.567	0.766	0.446	0.586
Xyla. +DFM1	250	500	0	8.62	13.17	0.501	0.325	0.650	21.85	1.016	0.619	0.611	0.758	0.472	0.624
DFM2	0	0	500	8.58	13.07	0.514	0.309	0.603	21.73	1.012	0.619	0.611	0.763	0.470	0.615
Xyla.+ DFM2	250	0	500	8.60	12.94	0.482	0.309	0.638	21.03	0.941	0.624	0.610	0.712	0.444	0.635
*S*EM				0.27	0.42	0.02	0.02	0.03	0.64	0.04	0.02	0.02	0.03	0.02	0.01
p-value				0.979	0.899	0.511	0.865	0.71	0.9	0.655	0.62	0.199	0.599	0.794	0.113
Contrasts															
1. DFM1 + Xylanase vs. (DFM1+Xylanase)					0.630	0.851	0.423	0.200	0.620	0.838	0.310	0.129	0.828	0.376	0.050
2. Xylanase + DFM2 vs. (DFM2+Xylanase)					0.614	0.184	0.985	0.370	0.360	0.116	0.360	0.212	0.105	0.543	0.034
3. DFM1 vs. DFM2					0.641	0.728	0.883	0.980	0.740	0.856	0.810	0.656	0.971	0.674	0.439
4. (DFM1+Xylanase) vs. (DFM2+Xylanase)					0.535	0.407	0.524	0.730	0.290	0.197	0.930	0.979	0.210	0.281	0.513

[1] All other values not specified are in kilogram

Chapter 7

OVERALL SUMMARY

Concern about antibiotic resistance in humans and animals has received worldwide attention. Laws have been promulgated to ban usage of in feed antibiotics in some parts of the world such as South Korea and Europe. The range of antibiotics that can be used in food animal production is limited as some antimicrobials are reserved for human use. The FDA is stepping up its regulations regarding the use of antimicrobials in livestock production. The reality is that farmers are challenged to reduce bacterial load in production species to enhance efficiency in order to maximize profit. Alternatives to antibiotics are therefore required. Direct-fed microbials, prebiotics and enzymes have been used in many studies. Calsporin, a Bacillus based direct-fed microbial has improved performance in meat type chickens. Its effect on performance in pigs has been contradictory. However, our studies showed that Calsporin supplementation had no effect on carcass traits or BUN but reduces feed intake and has potential to improve feed efficiency. Total anaerobes, enterobacteriaceae, and bifidobacteria counts did not differ with Calsporin supplementation but lactobacillus count was reduced. Other researchers have explained that bacillus and lactobacillus are both gram positive and have the same mechanisms for transporting nutrients from the intestinal lumen into the cell. It is speculated that bacillus might be more efficient in transporting nutrients than lactobacillus leading to reduction in their numbers.

Enzymes are added to high fiber diets to improve nutrient digestibility. The effects of a combination of Enzymes and Direct-fed Microbials effect on nutrient digestibility

are poorly understood especially in nursery pigs. The current digestibility study revealed that addition of the DFM2 to xylanase improved villus height and villus: crypt depth ratio; reduced organ weight; reduced ileal digester viscosity; and exhibited a potential to improve nutrient digestibility. The current study demonstrated both synergistic and antagonistic effects when xylanase was combined with either DFM1 or DFM2 in comparison to feeding of xylanase, DFM1 or DFM2 individually. Xylanase supplementation improved ATTD of fat, NDF, ADF, energy and numerically improved ileal protein digestibility. Supplementation of either DFM1 or DFM2 alone could not improve ATTD of nutrients. Combination of xylanase with DFM1 increased the intestinal length and improved villus height for nutrient absorption but did not impact nutrient digestibility.

The performance study using xylanase and DFMs in combination also revealed that supplementation of xylanase, DFM1 and DFM2 did not influence final body weight, average daily feed intake, average daily gain, and feed efficiency. Synergism between xylanase and the DFMs was observed in the current study. Combination of the DFMs with xylanase led to reduction of BUN and feed intake which resulted in improvement in overall feed efficiency. The improvement is partly attributable to improvement in microbial balance which promotes gut health and efficient nutrient absorption leading to lowering of voluntary feed intake. In summary, our study show that Calsporin has the potential to improve feed efficiency and it is better combine to xylanase to DFMs rather than individual supplementation of DFMs and xylanase.

LITERATURE CITED

Abe, F., N. Ishibashi, and S. Shimamura.1995. Effect of administration of bifidobacteria and lactic acid bacteria to newborn calves and piglets. J. Dairy Sci. 78:2838-2846.

Adeola, O., and A. J. Cowieson. 2011. BOARD-INVITED REVIEW: Opportunities and challenges in using exogenous enzymes to improve nonruminant animal production. Journal of Animal Science, 89(10), 3189-3218. doi:10.2527/jas.2010-3715 [doi]

Agriculture and Agri-Food Canada (AAFC). 2005. Feed grains in Canada. Bi-weekly Bulletin April 1, 2005 Volume 18 Number 7. AAFC Market Analysis Group, Winnipeg, MB, Canada.

Aguedelo, J. H., Lindermann, M. D., and G. L. Cromwell. 2010. A comparison of two methods to assess nutrient digestibility in pigs. Livestock Science, Volume 133, Issues 1–3, September, Pages 74-77

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

Agyekum, A., Woyengo, T., Slominski, B., Yin, Y., and C. Nyachoti. 2013. Effects of formulating growing pig diet with increasing levels of wheat- corn distillers dried grains with solubles on digestible nutrient basis on growth performance and nutrient digestibility. Journal of Animal Physiology and Animal Nutrition.

Alexopoulos, C., Georgoulakis, I.E., Tzivara, A., Kyriakis, C.S., Govaris, A., and S.C. Kyriakis. 2004. Field evaluation of the effect of a probiotic-containing Bacillus licheniformis andBacillus subtilis spores on the health status, performance, and carcass quality of grower and finisher pigs Journal of Veterinary Medicine Series A, 51, pp. 306–312

Alvarez-Rodriguez, J., Hermida B., Parera J., Morazán H., Balcells J., and D. Babot. 2013. The influence of drinker device on water use and fertiliser value of slurry from growing-finishing pigs. Animal Production Science, 2013, 53, 328–334. CSIRO PUBLISHING.

Anadón, A., Rosa Martínez-Larrañaga, M., and A. M. Martínez. 2006. Probiotics for animal nutrition in the european union. regulation and safety assessment. Regulatory Toxicology and Pharmacology, 45(1), 91-95. doi:http://dx.doi.org/10.1016/j.yrtph.2006.02.004

Anderson, P. V., Kerr B. J., Weber T. E., Ziemer C. J., and G. C. Shurson . 2012. Determination and prediction of digestible and metabolizable energy from chemical analysis of corn coproducts fed to finishing pigs. J. Anim. Sci.90:1242–1254.

Andersson, D. I., and D. Hughes, 2010. Antibiotic resistance and its cost: Is it possible to reverse resistance? Nature Reviews. Microbiology, 8, 260–271.

Angela Busch, Hoffmann-La Roche, Hans-Heinrich Herrmann. 2004. Pioneer Hi-Bred Northern Europe, Imke Kühn, Lohmann Animal Health, Ortwin Simon, Freie

Universität Berlin, Jürgen Struck, Hoechst Roussel Vet , Elvira Süphke, AWT. Probiotics in Animal Nutrition. AgriMedia

Annison, G., and M. Choct. 1991. Anti-nutritive activities of cereal non-starch polysaccharides in brilers iets and strategies minimizing their effects. World's Poult. Sci. J. 47: 232 - 242

Anugwa, F. O. I., V.H. Varel, J. S. Dickinson, W. G. Pond., and L. P. Krook. 1989. Effects of dietary fiber and protein concentrations on growth, feed efficiency, visceral organ weights, and large intestinal microbial populations of swine. J. Nutr. 119:879–886.

AOAC. 1995. AOAC Official methods of analysis (16th ed.) Association of Official Analytical Chemists, Arlington, V.A.

AOAC. 2000. Official Methods of Analysis. 16th ed. Assoc. Off. Anal. Chem., Arlington, VA.

Armuzzi, A, Cremonini F, Bartolozzi F, Canducci F, Candelli M, Ojetti V, Cammarota G, Anti M, De Lorenzo A, Pola P, Gasbarrini G, and A. Gasbarrini. 2001: The effect of oral administration of Lactobacillus GG on antibiotic-associated gastrointestinal side-effects during Helicobacter pylori eradication therapy. Aliment Pharmacol Ther, 15(2): 163-169

Arvola, T, Laiho K, Torkkeli S, Mykkänen H, Salminen S, Maunula L, and E. Isolauri 1999. Prophylactic Lactobacillus GG reduces antibiotic-associated diarrhea in children with respiratory infections: a randomized study. Pediatrics, 104(5):1-4.

Asmus, M. D. 2012. Effects of dietary fiber on the growth performance, carcass characteristics, and carcass fat quality in growing-finishing pigs. MS Thesis, Kansas State Univ., Manhattan.

Association of American feed control officials. 1998. Official publication. Assoc.Am. Feed Control off., Charleston, WV.

Austin, S., Wiseman, C., and J. Chesson, 1999. Influence of non-starch polysaccharides structure on the metabolisable energy of U.K. wheat fed to poultry. Journal of Cereal Science, 29: 77–88.

Avalos, F. 2013. Do oil prices drive food prices? The tale of a structural break. J. Int. Money Financ. doi:10.1016/j.jimonfin.2013.08.014

Bach Knudsen K. E.1997. Carbohydrate and lignin contents of plant materials used in animal feeding. Anim. Feed Sci. Technol. 67:319–338.

Bach Knudsen, K, E. 2001. The nutritional significance of "dietary fibre" analysis. Anim. Feed Sci. Technol. 90, 3-20

Bach Knudsen, K. E. 2011. TRIENNIAL GROWTH SYMPOSIUM: Effects of polymeric carbohydrates on growth and development in pigs.

Bach Knudsen, K. E. 2005. Effect of dietary non-digestible carbohydrates on the rate of SCFA delivery to peripheral tissues. Foods Food Ingred. J. Japan211:1008–1017.

Badu, K. R., and Satyanarayana. 1995. T. α-Amylase production by thermophilic Bacillus coagulans in solid state fermentation. Process Biochem. 30: 305–309

Baidoo, S. K., Liu, Y. G., and R. R. Grandhi. 1997. Exogenous microbial enzymes and hulless barley utilization by pigs. Proceedings Manitoba Swine Seminar, Manitoba University, Winnipeg, 11, 135-140.

Bailey, M., Vega-Lopez, M. A., Rothkötter, H. J., Haverson, K., Bland, P. W., Miller, B. G., and C. R. Stokes. 2001. Enteric immunity and gut health. In: Varley, M. A., Wiseman, J. (Eds.), The Weaner Pig Nutrition Management. CABI Publishing, Wallingford, pp. 207-222

Barbosa, T. M., Serra, C. R., La Ragione, R. M., Woodward, M. J., and A. O. Henriques. 2005. Screening for Bacillus isolates in the broiler gastrointestinal tract. Appl. Environ. Microbiol., 71: 968–978.

Barrera, M., M. Cervantes, W. C. Sauer, A. B. Araiza, N. T orrentera, and M. Cervantes. 2004. Ileal amino acid digestibility and performance of growing pigs fed wheat based diets supplemented with xylanase. J. Anima. Sci. 82:1997-2003.

Barton, M. D., 2000. Antibiotic use in animal feed and its impact on human health.Nutr. Res. Rev. 13:279–299.

Bedford, M. R. 2002. The role of carbohydrases in feedstuff digestion. Pages 319–336 in Poultry Feedstuffs – Supply, Composition and Nutritive Value. J. M. McNab and K. N.Boorman, ed. CABI Publ., England

Bedford, M. R., and J. Apajalahti. 2001. Microbial interactions in the response to exogenous enzyme utilization, in: Bedford, M. R. and Patridge , G. G, (Eds) Enzymes in Farm Animal Nutrition, pp. 299-314 (Wallingford, CABI).

Belletti, N., Gatti, M., Bottari, B., Neviani, E., Tabanelli, G., and F. Gardini. F. 2009. Antibiotic resistance of lactobacilli isolated from two Italian hard cheeses. Journal of Food Protection, 72, 2162–2169.

Bengtsson, S., Åman, P., and R. E. Anderson. 1992. Structural studies on water-soluble arabinoxylans in rye grain using enzymatic hydrolysis. Carbohydr. Polym. 17:277–284.

Bennet, R, Eriksson, M., Nord, C. E., and R. Zetterstrom. 1986. Fecal bacterial microflora ofnewborn infants during intensive care management and treatment with five antibiotic regimens. Pediatr Infect Dis, 5: 533-9.

Benno, Y., and T. Mitsuoka. 1986. Development of intestinal microflora in humans and animals. Bifidobacteria Microflora, 5: 13–25.

Bhandari, S. k., Xu, B., Nyachoti, C. M. Giesting, D. W., and D. O. Krause. 2008. Evaluation of alternatives toantibiotics using an Escherichia coliK88+ model of piglet diarrhea: Effects on gut microbial ecology.

Biller, J. A., Katz A. J., Flores A. F., Buie T. M., and S. L. Gorbach. 1995. Treatment of recurrent Clostridium difficile colitis with Lactobacillus GG. J Pediatr Gastroenterol Nutr, 21: 224-6.

Brooks, P. H. 1994. Water-forgotten nutrient and novel delivery system. In:Biotechnology in the feed industry'. pp. 211–234. (Nottingham Press: Leicestershire).

Brooks, P. H., and J. L. Carpenter. 1993. The water requirement of growing/ finishing pigs: Theoretical and practical considerations.Pp 179-200 in Recent development in Pig nutrition 2, D.J. Cole, W. Haesign, and P. C. Garnsworthy, eds. Loughborough, UK: Nottingham University Press.

Brooks, P.H., S. J. Russel, and J. L. Carpenter. 1984. Water intake of weaned piglets from three to seven weeks old. Veterinary Record 115:513-515.

Brumm, M.C., Dahlquist, J. M, J. M. Heemstra. 2000. Impact of feeders and drinker devices on pig performance, water use and manure volume. Journal of Swine Health and Production 8(2), 51–57.

Buhler, M., Limper, J., Muller, A., Schwarz, G., Simon, O., and W. Spring. 2004. In: Enzyme in Animal Nutrition. AWT. cited on January 20th, 2014.

Busch, A., Roche H., Hermann H., Kuhn I., Ortwin S., Struck J., and Suphke E. 2004. Probiotics in animal nutrition. Agri-Media. http://www.fefana.org/resources/documents/publications/total%20def%20probio.pdf

Bust, J., P. P. Bernasconi, J. P. Vaerman, and C. Dive. 1990. Stimulation of secretory IgA and secretary component of immunoglobulins in small intestine of rats treated with Saccharomyces boulardii. Dig. Dis. Sci. 35:251-256. Antimicrobial era. Science. 257:1050.

Cadogan, D. J., Choct, M., and R. G. Campbell. 2003. Effects of storage time and exogenous xylanase supplementation of new season wheats on the performance of young male pigs. Can. J. Anim. Sci. 83: 105 - 112

Cambel, R. G. 1997. Achieving 700g per day from birth- is it worthwhile? In pig production: The A T Reid Course for veterinarians Proceedings 285. 17-21 February 1997. Published by: Post graduate foundation in veterinary Science University of Sydney. pp 125-131.

Carpita, N. C., and D. M. Gibeaut. 1993. Structural models of primary cell walls in flowering plants: Consistency of molecular structure with the physical properties of the walls during growth. Plant J. 3:1–30.

Casewell, M., Friis, C., Marco, E., McMullin, P., and I. Phillips. 2003a.The European ban on growth-promoting antibiotics and emerging consequences for humans and animals health Journal of Antimicrobial Chemotherapy, 52 (2003), pp. 159–161

CDRF, 2011. Products with probiotics. http://cdrf.org/home/checkoff-investments/usprobiotics/products-with-probiotics/. cited on February 13, 2014.

Chaucheyras-Durand, F., and H. Durand. 2010. Probiotics in animal nutrition and health. Beneficial Microbes. 1(1): 3-9.

Cheeson, A and S. Austin. 1998. Defining the nutritional characteristics of plant carbohydrates- the non-ruminant perspective. In: Proceedings of 19th Western Nutrition Conference, September 16-17, Saskatchoon, Saskatchenwan. 1998.

Chen ,Y. J., Min, B. J., Cho, J. H., Kwon, O. S., Son, K. S., Kim, H. J., and I. H. Kim. 2006. Effects of dietary Bacillus-based probiotic on growth performance, nutrient digestibility, blood characteristics and fecal noxious gas content in finishing pigs. Asian-australas. J. Anim. Sci. 19:587–592.

Chesson, A. 1994. Probiotics and other intestinal mediators. Pages 197–214 in Principles of Pig Science. D. J. A. Cole, J. Wiseman, and M. A. Varley, ed. Nottingham University Press, Nottingham, UK.

Chesson, A., and C. W. Forsberg. 1997. Polysaccharide degradation by rumen microorganisms. In: Hobson P. N., Stewart C. S., editors, The rumen microbial ecosystem. Elsevier Science Publishers Ltd., London, UK. p. 329–381.

Choct, M., Hughes, R. J., and M. R. Bedford. 1999. Effects of a xylanase on individual bird variation, starch digestion throughout the intestine and ileal and caecal volatile fatty acid production in chicken fed wheat. B. Poult. Sci. 40: 419 – 422.

Choct, M., Kocher, A., Waters, D. L. E., Pettersson D., and G. Ross. 2004. A comparison of three xylanases on the nutritive value of two wheats for broiler chickens. Br. J. Nutr. 92:53–61.pmid:15230987

Choi, J. Y., Kim J. S., Ingale, S.L., Kim, K. H., Shinde, P. L., Kwon, I. K., and B. J. Chae. 2011. Effect of potential multimicrobe probiotic product processed by high drying temperature and antibiotic on performance of weanling pigs

Chot, M., and G. Annsion. 1990. Anti-nutritive activity of wheat pentosans in poultry diets. Br. Poult. Sci. 31: 809-819.

Chot, M., Hughes, R. J., Trimble, R. P., and G. Annsion. 1994. The use of enzyme in low-AME wheat broiler diets; effect of birds performance and gut viscosity. Aust Poultry Sci Symp 6, 83-87.

Coey, W. E., and K. L. Robinson. 1954. Some effects of dietary crude fiber on live weight and carcass conformation of pigs. J. Agric. Sci. 45:41–47.

Cohen, M.L. 1992. Epidemiology of drug resistance: implications for a post-antimicrobial era. Science: 257(5073):1050-5. Review.

Cole, C.B., and R. Fuller. 1984. A note on the effect of host specific fermented milk on the coliform population of the neonatal rat gut.J. Appl. Bact. 56, 495-498.

Cole, D.J.A., and W.H. Close. 2001. The mordern pig setting performance targets. Animal talk 8, 3.

Collier, B., and B. Hardy. 1986. The use of enzymes in pig and poultry feed. Feed compounder 6, 28-30.

Collinder, E., G. N. Berge, M. E. Cardona, E. Norin, S. Stein, and T. Midtvedt. 2000. Feed additive to piglets, probiotics or antibiotics.In: Proceedings of the 16th interantional Pig Veterinary Society Congress, Melbourne, Australia. 17-20 Sept. pp 257.

Collington, G. K., Parker, D. S., and D. G. Armstrong 1990. The influence of inclusion of either an antibiotic or a probiotic in the diet on the development of digestive enzyme activity in the pig. Br. J. Nutr. 64:59–70.

Collins, M. D., and G. R.Gibson. 1999. Probiotics, prebiotics, and synbiotics: Approaches for modulating the microbial ecology of the gut. Am. J. Clin. Nutr., 69: 1052S–1057S.

Conway, P. L., L. Gorbach, S.L., and B. R. Goldin. 1987. Survival of lactic acid bacteria in the human stomach and adhesion to intestinal cells. J. Dairy Sci. 70: 1-12.

Corpet, D. E. 1996. Microbiological hazards for humans of antimicrobial growth promoter use in animal production. Rev. Med. Vet 147:851.

Courtin, C. M., and Delcour J. A. 2002. Arabinoxylans and endoxylanases in wheat flour bread-making. J. Cereal Sci. 35:225–243.

Cowieson, A. J. 2010. Strategic selection of exogenous enzymes for corn/soy-based poultry diets. Japan. Poult. Sci. 47:1–7.

Cowieson, A. J., and O. Adeola. 2005. Carbohydrases, protease, and phytase have an additive beneficial effect in nutritionally marginal diets for broiler chicks. Poult. Sci. 84:1860–1867.

Cowieson, A. J., Bedford, M. R., and V. Ravindran. 2010. Interactions between xylanase and glucanase in corn/soy-based diets for broilers. Br. Poult. Sci. 51:246–257.

Cowieson, A. J., V. Ravindran. 2008a. Effect of exogenous enzymes in maize-based diets varying in nutrient density for young broilers: Growth performance and digestibility of energy, minerals and amino acids. Br. Poult. Sci. 49:37–44.

Cowieson, A. J., V. Ravindran. 2008b. Sensitivity of broiler starters to three doses of an enzyme cocktail in maize-based diets. Br. Poult. Sci. 49:340–346.

Cozannet, P., Primot, Y., Gady, C., Metayer, J. P., Lessire, M., Skiba, F., and J. Noblet .2010. Energy value of wheat distillers grains with solubles for growing pigs and adult sows. J. Anim. Sci. 88:2382–2392.

Cromwell, G. L. 2002. Why and how antibiotics are used in swine production. Anim. Biotechnol. 13:7–27.

Crowder, S.A., Meyer, T.A., Weeden, T., Otomo, N., and T. Lorhmann. 2013. Effects of grow-finish diets supplemented with Calsporin® (Bacillus subtilis C-3102 spores) or Stafac® on pig performance. Mid-west animal nutritional conference presentation, 2013.

Cumby, T. R. 1986. Design requirement of liquid feeding systems for pigs: A review. Journal of Agricultural Engineering and Resources 34; 153-172.

Cupere, F. D, P. Deprez, D. Demeulenaere, and E. Muylle. 1992. Evaluation of the effect of 3 probiotics on experimental E. coli enterotoxaemia in weaned piglets. J. Vet. Med. B39:277-284.

CVB. 1998. Centraal Veevoeder Bureau. Veevoedertalel (Feeding value of feed ingredients). Centraal Veevoeder Bureau, Runderweg 6, Lelystad, the Netherlands.

Czinn, S. J. and S.S. Blanchard. 2009. Probiotics in foods and supplements S. Michail, P.M. Sherman (Eds.), Nutrition and Health: Probiotics in Pediatric Medicine, Humana Press, Totowa, NJ (2009), pp. 299–306

Dale, N. 1996. The metabolizable energy of wheat by-products. J. Appl. Poult. Res. %:105-108.

Danek, 1986. Zmeny Fekalni milkroflory selat behem aplikace Streptococcus faecium M-74 (Lactiferm) v krmne smesi. Zivocisna Vyroba. 31:71-86.

Davis, M. E., T. Parrot, D. C. Brown, B. Z. de Rodas, Z. B. Johnson, C. V. Maxwell, and T. Rehberger. 2008. Effect of Bacillus-based direct-fed microbial feed supplementation on growth performance and pen cleaning characteristics of growing-finishing pigs. J. Anim Sci. 86:1459-1467.

de Lange, C. F. M., Birkett, S. H., and P. C. H. More 2001. Protein, fat and bone tissue growth in swine. Pp 65-81 in swine nutrition, 2nd Ed., A. J. Lewis and L.L. Southern eds. Boca Ration, FL:CRC Press.

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

Deasy, 2009. Antibiotic resistant. The ongoing challenge for effective drug therapy-JAAPA. Official Journal for the America Academy for the Physian Assistant,22, 18-22.

Deprez, P., D. H. C. Van, E. Muylle, and W. Oyaert. 1986. The influence of the administration of sow's milk on postweaning excretion of hemolytic E. coli in pigs. Vet Res. Comm. 10:468-478.

Dibner, J. J., and J. D. Richards. 2005. Antibiotic growth promoters in agriculture: history and mode of action Poultry Science, 84, pp. 634–643

Diebold, G., R. Mosenthin, H. P. Piepho, and W. C. Sauer. 2004. Effect of supplementation of phospholipase to a wheat-based diet for weanling pigs on nutrient digestibility and concentrations of microbial metabolites in ileal digesta and feces.

Djouzi, Z., Andrieux, C., Degivry, M. C., Bouley, C., and O. Szylit. 1997. The association of yogurt starters with Lactobacillus casei DN 114.001 in fermented milk

alters the composition and metabolism of intestinal microflora in germ-free rats and in human flora-associated rats. J. Nutr. 127:2260–2266.

EC, 2001. Commission of the European Communities, Commission Recommendation, 2001/459/EC Official Journal of European Union L, 161. pp. 42–44

EC, 2003a. Commission of the European Communities, Commission Regulation (EC) No. 1831/2003. Official Journal of European Union L, 268, pp. 29–43

EFSA, 2005a. European Food Safety Authority. Opinion of the scientific committee on a request from EFSA related to a generic approach to the safety assessment by EFSA of microorganisms used in food/feed and the production of food/feed additives EFSA Journal, 226. pp. 1–12

EFSA, 2005b. Opinion of the scientific panel on additives and products or substances used in animal feed on the updating of the criteria used in the assessment of bacteria for resistance to antibiotics of human or veterinary importance EFSA Journal, 223 (2005), pp. 1–12

EFSA, 2007a. European Food Safety Authority. Introduction of a Qualified Presumption of Safety (QPS) approach for assessment of selected microorganisms referred to EFSA Opinion of the Scientific Committee (Question No EFSA-Q-2005-293) Adopted on 19 November 2007 EFSA Journal, 587 (2007), pp. 1–16

EFSA, 2010. Scientific opinion on the safety and efficacy of calsporin® (bacillus subtilis) for turkeys for fattening, ducks, geese, pigeons and other game birds for

meat production, ducks, geese, pigeons, game birds, ornamental and sporting birds for rearing to point of lay, turkeys reared for breeding and chickens reared for laying. EFSAJournal, 8(10), http://www.efsa.europa.eu/en/efsajournal/doc/1867.pdf

Eggum, B. O. 1970. Blood urea measurement as a technique for assessing protein quality. Br. J. Nutr. 24:983-988.

Emiola, I. A., Opapeju, F. O., Slominski, B. A., and O. Nyachoti. 2009. Growth performance and nutrient digestibility in pigs fed wheat distillers dried grains with solubles-based diets supplemented with a multicarbohydrase enzyme. Journal of animal science, 87(7), 2315-2322.

Estrada, A., Drew. M. D., Kessel, and A. Van. 2001. Effect of dietary supplementation of fructooligosaccharides and Bifidobacteria longum to early-weaned pigs on performance and fecal bacteria populations. Canadian Journal of Animal Science 81:141-148.

European Commission, 2005. Opinion of the scientific panel on additives and products or substances uesd in animal feed on the updating the criteria used in the assessment of bacteria for resistance to antibiotic o human or veterinary importance. EFSA Journal 223, 1-12

Ewing, W. N., and D. J. A. Cole. 1994. The Living Gut: An Introduction to Micro-Organisms in Nutrition. Context Graphics, Dungannon, UK.

Fairbairn, S. L., Patience, J. F., Classen, H. L., and R. T. Zijlstra. 1999. The energy content of barley fed to growing pigs: Characterizing the nature of its variability and developing prediction equations for its estimation. J. Anim. Sci. 77:1502–1512.

FAO/WHO, 2001. Expert Consultation on Evaluation of Health and Nutritional Properties of Probiotics in Food Including Powder Milk with Live Lactic Acid Bacteria, October 2001

FAO/WHO, 2002. Joint FAO/WHO (Food and Agriculture Organization/World Health Organization) working group report on drafting guidelines for the evaluation of probiotics in food. London, Ontario, Canada.guidelines for the evaluation of probiotics in food. Joint working group report on drafting. London, Ontario, 2002: 1–11.

FDA. 2012. The Food and Drug Administration. U.S. Department of Health and Human Services. New Animal Drugs; Cephalosporin Drugs; Extra label Animal Drug Use; Order of Prohibition. Docket No. FDA-2008-N-0326.

Ferrell, C. L. 1988. Contribution of visceral organs to animal energy expenditures. J. Anim. Sci. 66:23–34.

Fialho, E. T., M. Vassalo, J. A. F. Lima, and A. G. Bertechine. 1998. Probiotic utilization for piglets from 10 to 30 kg (performance and metabolism assay). Pages 622–623 in Proc. 8th World Conf. Anim. Prod. Seoul Natl. Univ., Seoul, Korea.

Food and Agriculture Organization of the United Nations (FAO). 2009.Wheat flour. Agribusiness Handbook. FAO, Rome, Italy.

Francis, D. H . 2002. Enterotoxigenic Esherichia coli infections in pigs and its diagnosis. J Swine Health Prod 10, 171-175

Fukushima, Y., Y. Kawata, H. Hara, A. Terada, and T. Mitsuoka. 1998. Effect of probiotic formula on intestinal immunoglobulin A production in healthy children. Int. J. Food Microbiol. 42: 39-44.

Fuller, R. 1989. Probiotics in man and animals. The Journal of Applied Bacteriology, 66(5), 365.

Fuller, R. 1992. Ed:. Probiotics. The scientific bases. Chapman and Hall, London.

Gibson, G. R., Rastall, R. A., and R. Fuller. 2003. "The health benefits of probiotics and prebiotics". In Gut Flora, Nutrition, Immunity and Health, , 1st Ed., Edited by: Fuller, R. and Perdigon, G. 52–76. Oxford: Blackwell Publishing.

Gibson, G.R., and M.B. Roberfroid. 1995. Dietary modulation of the human colonicmicrobiota: introducing the concept of prebiotics. J. Nutr. 125: 1401- 1412.

Gilliland, S.E. 1981. Enumeration and identification of lactobacili in feed supplements marketed as sources of Lactobacillus acidophilus. Okla. Agric. Exp. Sta., Misc. Publ. 108, 61-63.

Goldin, B. R., and S. L. Gorbach. 1984. The effect of milk and lactobacillus feeding on human intestinal bacterial enzyme activity. Am. J. Clin. Nutr. 39, 756-761

Gorbach, SL, Chang, TW, B. Goldin. 1987. Successful treatment of relapsing Clostridiumdifficile colitis with Lactobacillus GG. Lancet, 26: 1519.

Graham, A. B., Goodband R. D., Tokach M. D., Dritz S. S., DeRouchey J. M., and S. Nitikanchana. 2013. The effects of medium-oil dried distillers grains with solubles on growth performance, carcass traits, and nutrient digestibility in growing-finishing pigs. J. Anim. Sci. (in press). doi10.2527/jas.2013-6798

Gray, G. M. 1992. Starch digestion and absorption in nonruminants. J. Nutr. 122:172–177.

Hale, O. M., and G. L. Newton, (1979). Effects of nonviable Lactobacillus species fermentation product on performance of pigs. J. Anim. Sci. Vol. 48 No. 4 pp 770-775.

Hampson, D. J., 1986. Alternations in piglet small intestinal structure at weaning. Res. Vet Sci. 40, 32-40.

Hampson, D. J., Pluske, J. R., and D. E. Pethick. 2001. Dietary manipulation of enteric disease. In: Lindberg, J. E., Ogle, B. (Eds.), Digestive Physiology of pigs. CABI Publishing, Wallingford, pp. 247-258.

Hansen, C. F., Phillips, N. D., La, T., Hernandez, A., Mansfield, J., Kim, J. C., and J. R. Pluske. 2010. Diets containing inulin but not lupins help to prevent swine dysentery

in experimentally challenged pigs. Journal of Animal Science, 88(10), 3327-3336. doi:10.2527/jas.2009-2719.

Hansen, I. K., E. B. Knudsen, and B. O. Eggum. 1992. Gastrointestinal implications in the rat of wheat bran, oat bran, and pea fiber. Br. J. Nutr. 68:451–456.

Härkönen, H., Lehtinen, P., Suortti, T., Parkkonen, T., Siikaaho M., and K. Poutanen, 1995.The effects of xylanase and a β-Glucanase from trichoderma reesei on the non-starch polysaccharides of whole meal rye slurry. J. Cereal Sci. 21: 173-183.

Harper, A. F., E. T. Kornegay., K. L. Bryant, and H. R. Thoman. 1983. Efficacy of

virginiamycin and a commercially available lactobacillus probiotics in swine diets. Anim. Feed Sci. Technol. 8:69-79.

Havenaar, R., and Huisb In't Veld. 1992. Probiotics a general view. In:Lactic acid Bacteria in health and disease. Vol. 1. Elsevier Applied Science publishers, Amsterdam.

HC, 2006. Health Canada. Evidence for Safety and Efficacy of Finished Natural Health Products. [online]. Ottawa (ON): Natural Health Products Directorate, Health Canada. [Accessed 2008 May 28].

He, J., Yin J., Wang, L., Yu B., and D. Chen. 2010. Functional characterisation of a recombinant xylanase from Pichia pastoris and effect of the enzyme on nutrient digestibility in weaned swine. Br. J. Nutr. 103:1507–1513.

Hennig, U., Hack, W., Priepke, A., Tuchscherer A., and Souffrant W. B., C. C. Metges .2008. Comparison of ileal apparent, standardized and true digestibilities of amino acids in pigs fed wheat and lupine seeds. Livest. Sci.118:61–71.

Hesselman, k., and P. Aman. 1986. The effect of β-glucanase on the utilization of starch and nitrogen by broiler chicks fed on barley of low or high-viscosity. Anim. Feed Sci. Technol. 14:83-93

Högberg, A., and J. E. Lindberg. 2004. Influence of cereal non-starch polysaccharides and enzyme supplementation on digestion site and gut environment in weaned piglets. Anim. Feed Sci. Technol. 116:113–128.

Holden, P. J., and D. R. Zimmerman. 1991. Utilization of cereal grain by-products in feeding swine. Pages 585-593 in Swine Nutrition. E. R. Miller, D. E. Ullrey, and A. J. Lewis, ed. Butterworth-Heineman, Boston, MA.

Holzapfel, W. H. and Schillinger, U. 2002. Introduction to pre- and probiotics. Food Res. Int., 35: 109–116.

Holzapfel, W. H., Haberer, P., Geisen, R., Bjorkroth, J., and U. Schillinger. 2001. Taxonomy and important features of probiotic microorganisms in food and nutrition. Am. J. Clin. Nutr. 73:365S–373S.

Hong, H. A., L. H. Duc, and S. M. Cutting. 2005. The use of bacterial spore formers as probiotics. FEM Microbial. Rev. 29:813-835.

Hopwood, D. E., Pethick, D. W., Pluske, J. R., and D. J.Hampson 2004. Addition of pearl barley to a rice-based diet for newly weaned piglets increases the viscosity of the intestinal contents, reduces starch digestibility and exacerbates post-weaning colibacillosis. Br. J. Nutr. 92:419–427.

Hu, Y., Wang Z., and S . Xu. 2008. Treatment of corn bran dietary fiber with xylanase increases its ability to bind bile salts, in vitro. Food Chem. 106:113–121.

Huang, M. K., Choi Y. J., Houde R., Lee J. W., Lee B., and X. Zhao. 2004. Effects oflactobacilli and an acidophilic fungus on the production performance and immune responses in broiler chickens. Poult. Sci. 83:788–795.

Hübener, K., Vahjen W., and O. Simon. 2002. Bacterial responses to different dietary cereal types and xylanase supplementation in the intestine of broiler chicken. Arch. Anim. Nutr. 56:167–187.

Huff, B. A. 2004. Caveat emptor. "Probiotics" might not be what they seem Canadian Family Physician, 50 (2004), pp. 583–587.

Huisman, J., and G. Tolman. 1992. In: Recent Advances in Animal Nutrition, 3-31. Garnsworthy, P., Haresign, W. and Cole, D., eds Butterworth Heinemann.

Izydorcyk, M. S, and C. G. Billiaderis. 1992. Effect of molecular size on physical propertities of wheat arbinoxylan. J. Agric. Food Chem.40:561-568.

Jakobsson, H. E., Jernberg, C., Andersson, A. F., Sjo¨lund-Karlsson, M., Jansson, J. K. and L. Engstrand. 2010. Short-term antibiotic treatment has differing long-term impacts on the human throat and gut microbiome. PLoS ONE 5, e9836.

Jensen, B. B., Hojberg, O., Mikkelsen, L, L., Skou Hedemann M., and N. Canibe. 2003. Enhancing intestinal function to treat and prevent intestinal disease. In: Ball, R. O. (Ed.), DPP. 9th International symposium on Digestive Physiology in pigs. Banff, Alberta, Canada. Unversity of Alberta, Edmonton, May 14-17, pp. 103-119.

Jessen, B. B. 1998. The impact of feed additives on the microbial ecology of the gut in young pigs. J.Anim. Feed Sci. 7:45-64.

Jin, L., L. P. Reynolds, D. A. Redmer, J. S. Caton, and J. D. Crenshaw. 1994. Effects of dietary fiber on intestinal growth, cell proliferation, and morphology in growing pigs. J. Anim. Sci. 72:2270–2278.

Jo, J. K., Ingale, S. L., Kim, J. S., Kim, Y. W., Kim, K. H., Lohakare, J. D., and B. J. Chae. 2012. Effect s of exogenous enzyme supplementation to corn-soybean based or complex diets on growth performance, nutrient digestibility and blood metabolites in growing pigs. J. Anim. Sci, 89, 1795-1804.

Johnson, I. T., and J. M. Gee. 1986. Gastrointestinal adaptation in respone to soluble non-available polysaccharides in the rat. Br. J. Nutr. 55:497–505.

Jørgensen, H., X. Q. Zhao, and B. O. Eggum. 1996. The influence of dietary fibre and environmental temperature on the development of the gastrointestinal tract,

digestibility, degree of fermentation in the hind-gut and energy metabolism in pigs. Br. J. Nutr. 75:365–378.

Joyce, C., A. Deneau, K. Peterson, I Ockenden, V. Raboy, and J. N. A. Lott. 2005. The concentrations and the distributions of phytic acid phosphorus and other mineral nutrients in wild-type and low phytic acid Js-12-LPA wheat (Triticum eastivum) Grain parts. Can. J. Bot. 83:1599-1607.

Just, A., J. A. Fernández, and H. Jørgensen. 1983. The net energy of diets for growth in pigs in relation to the fermentative processes in the digestive tract and site of absorption of nutrients. Livest. Prod. Sci. 10:171–186.

Kiarie, E., Nyachoti, C. M., Slominski, B. A., and G. Blank. 2007. Growth performance, gastrointestinal microbial activity, and nutrient digestibility in early-weaned swine fed diets containing flaxseed and carbohydrase enzyme. J. Anim. Sci. 85:2982–2993.

Kim, J. C., Hansen, C. F., Mullan, B. P., and J. R. Pluske. 2012. Nutrition and pathology of weaner pigs: nutritional strategies to support barrier function in the gastrointestinal tract. Animal Feed Science and Technology, 173(1), 3-16.

Kim, M. 2002. The water-soluble extract of chicory affects rat intestinal morphology similarly to other non-starch polysaccharides. Nutrition Research, 22(11), 1299-1307.

Knudsen, K. E. B., and I. Hansen. 1991. Gastrointestinal implications in pigs of wheat and oat fractions. 1. Digestibility and bulking properties of polysaccharides and other major constituents. Br. J. Nutr. 65, 217-232.

Kohn, R. A., Dinneen, M. M., and E. Russek-Cohen. 2005. Using blood urea nitrogen to predict nitrogen excretion and efficiency of nitrogen utilization in cattle, sheep, goats, horses, pigs, and rats. J. Anim. Sci. 83:879–889.

Kornegay, E. T., C. R. Risley 1996. Nutrient digestibilities of a corn-soybean meal diet as influenced by Bacillus products fed to finishing swine. J. Anim. Sci. 74:799–805.

Kornegay, E. T., Z. Zhang, and D. M. Denbow. 1999. Influence of microbial phytase supplementation of a low protein/amino acid diet on performance, ileal digestibility of protein and amino acids, and carcass measurements of finishing broilers. Pages 557–572 in Phytase in Animal Nutrition and Waste Management, 2nd ed. BASF Corporation, Mount Olive, NJ.

Krause, D. O., Denman, S. E., Roderick, I. M., Marrison, M., Rae A. L., Attwood, G. T., and S . McSweeney. 2003. Opportunities to improve fibre degradation in the rumen: Microbiology, ecology, and genomics. FEMS Microbiol. Rev. 27:663–693.

Krebs, H. A. 1942. Urea formation in mammalian liver. Biochem. J. 36:758-767.

Kremer, B., 2006. DFM products provide consistent outcomes. Feedstuffs 24, 14–15.

Kumari, A., Catanzaro, R., F. Marotta. 2011. Clinical importance of lactic acid bacteria: a short review. Acta Biomedica 82, 177-180.

Kumpretecht, I., and P. Zobac. 1998. Study of the effect of combined preparation containing enterococcus faecium M-74 and mannan-oligosaccharidesz in diets for weaningly piglets. Czech J. Anim. Sci. 43: 477-481.

Kyriakis, S. C., V. K. Tsiloyiannis, J. Vlemmas, K. Sarris, A. C. Tsinas, C. Alexopoulos, and L. Jansegers 1999. The effect of probiotics LSP 122 on the control of post-weaning diarrhea syndrome of piglets. Res. Vet. Sci. 67: 223-228.

Lan, Y., Opapeju, F. O., and C. M. Nyachoti. 2008. True ileal protein and amino acid digestibilities in wheat dried distillers' grains with solubles fed to finishing pigs. Anim. Feed Sci. Technol. 140:155–163.

Langhout, D. J., Schutte, J. B., de Jong J., Sloetjes H., Verstegen W. A., and S. Tamminga. 2000. Effect of viscosity on digestion of nutrients in conventional and germ-free chicks. Br. J. Nutr. 83:533–540.

Laulaund, S. 1994. Commercial aspect of formulation, distribution and marketing aspect of probiotics products. In: Human health: the contribution of microorganisms (Ed.: Gibson, S.A.W.) Springer-Verlag, New York, 119-144.

Lee, S. J., Lee S. S., Shim N. H., Ok J. U., Jung H. S., Chu G. M., Kim I. H., and J. D. Kim. 2009. Effects of dietary synbiotics from anaerobic microflora on growth performance, noxious gas emission and fecal pathogenic bacteria population in weaning pigs. Asian-australas. J. Anim. Sci. 22:1202–1208.

Lee, Y. K., K. Nomoto, S. Salminen, and S. L. Gorbach. 1999. Mechanisms of probiotics. In Handbook of Probiotics. A. Wiley-Interscience Publication. John Wiley & Son Inc. pp 147-193.

Lehninger, A. L., D. L. Nelson, and M. M. Cox. 2005. Lehninger principles of biochemistry. 4th ed. WH Freeman and Co. NY.

Lenis, and Jongbloed. 1999. New technologies in low polution swine diets: diet manipulation and the use of synthetic amino acids, phytase and phase feeding for reduction of nitrogen and phosphorus excretion and ammonia emmission-A Review. Asia-Australasian journal of Animal Science 12, 305-327.

Leons, and Walch. 1993. In Enzymes in Animal nutrition. Proceedings of the first symposium Kartause Ittingen, Switzerland October 13 - 16, 1993.

Leslie, M. A., Moran E. T. Jr., and J. D. Bedford. 2007. The effect of phytase and glucanase on the ileal digestible energy of corn and soybean meal fed to broilers. Poult. Sci. 86:2350–2357.

Li, S., Sauer, W. C., Huang, S. X., and R. Mosenthin. 1994. Effect of cellulase supplementation to barley-based diets on digestibilities of energy, beta-glucans, crude protein and amino acids in early-weaned pigs. In: Souffrant, W. B., Hagemeister, H. (Eds.), Proceedings of the sixth international symposium on Digestive physiology in pigs, Bad Doberan, germany, pp. 357- 359.

Li, S., Sauer, W. C., Huang, S. X., and V. M. Gabert. 1996. Effect of beta-glucanase supplementation to hulless barley or wheat-soybean meal diets on digestibilities of energy, protein, beta-glucans, and amino acids in young pigs. J. Anim. Sci. 74: 1649 – 1656.

Li, Y. N., Meng, K., Wangm, Y. R., and B. Yao. 2006. β-mannanase from B. subtilis B36: purification, properties sequencing, gene cloning and expression in E. coli. Z. Naturforsch (C) 61: 840–846.

Li, Y. Z., Chénard, L., Lemay, S. P, and H. W. Gonyou. 2005. Water intake and wastage at nipple drinkers by growing-finishing pigs. Journal of Animal Science 83, 1413–1422.

Li, Y., Fang, Z., Dai, J., Partridge, G., Ru Y., and J. Peng. 2010. Corn extrusion and enzyme addition improves digestibility of corn/soy based diets by pigs: In vitro and in vivo studies. Anim. Feed Sci. Technol. 158:146–154.

Lin, S. S., Dou, W. F., Xu H., Li H. Z., Xu Z. H., and Y. Ma. 2007. Optimization of medium composition for the production of alkaline β-mannanase by alkaliphilic Bacillus sp. N16-5 using response surface methodology. Appl. Microbiol. Biotechnol. 75(5)1015–1022.

Liu, S. Y., Selle, P. H., and A. J. Cowieson. 2013. Strategies to enhance the performance of pigs and poultry on sorghum-based diets. Anim. Feed Sci. Technol. 181:1–14.

Lo¨fmark, S., Jernberg, C., Jansson, J. K., and C. Edlund. 2006.Clindamycin-induced enrichment and long-term persistence of resistant Bacteroides spp. and resistance genes. J Antimicrob Chemother 58, 1160–1167.

Low, A. G. 1989. Secretory response of the pig gut to non-starch polysaccharides. Anim. Feed. Sci. Technol. 23:55.

MacMaster, M. M., Hinton, J. J. C., and D. Bradbury. 1971. In 'Wheat: Chemistry and Technology, (Y. Pomeranz, ed.), American Association of Cereal Chemists, St. Paul ,MN51- 113.

Mahan, D. C., G. L. Cromwell, R. C. Ewan, C. R. Hamilton, and J. T. Yen 1998. Evaluation of the feed duration of a phase 1 nursery diet to three-week-old pigs of two weaning weights. J. Anim. Sci. (1998). 76: 578-583.

Mathlouthi, N., Lalles, J. P., Lepercq, P., Juste, C., and M. Larbier. 2002. Xylanase and {beta}-glucanase supplementation improve conjugated bile acid fraction in intestinal contents and increase villus size of small intestine wall in broiler chickens fed a rye-based diet. J. Anim. Sci. 80:2773–2779.

Mathur, S. and R. Singh. 2005. Antibiotic resistance in food lactic acid bacteria. A review. International Journal of Food Microbiology, 105 (2005), pp. 281–295.

Mattarelli, P., Brandi, G., Modesto, M., and B. Biavati, 2002.Discrepancy between declared and recovered bifidobacteria in a human probiotic. Annals of Microbiology, 52 (2002), pp. 283–286.

Mavromichalis, I., Hancock, J. D., Senne, B. W., Gugle, T. L., Kennedy, G. A., Hines, R. H., and C. L. Wyatt. 2000. Enzyme supplementation and particle size of wheat in diets for nursery and finishing swine. J. Anim. Sci. 78:3086–3095.

Mc Alpine, P. O., O'Shea, C. J., Varley, P. F., and J. V. O'Doherty. 2012. The effect of protease and xylanase enzymes on growth performance and nutrient digestibility in finisher pigs. Journal of animal science, 90(Supplement 4), 375-377.

McBride, B. W., and J. M. Kelly. 1990. Energy cost of absorption and metabolism in the ruminant gastrointestinal tract and liver: a review. J. Anim. Sci. 68:2997–3010.

McCracken, B., Spurlock, M. E., Roos, M. A., Zuckermann, F. A., and H. R. Gsakin. 1999. Weaning anorexia may contribute to local inflammation in the piglet intestine. J. Nutr. 129, 613-619.

McDonald, D. E., Pethick, D. W., Mullan, B. P., and D. J. Hampson. 2001. Increasing viscosity of the intestinal contents alters small intestinal structure and intestinal growth, and stimulates proliferation of enterotoxigenic Escherichia coli in newly-weaned swine. Br. J. Nutr. 86:487–498.

McDougall, G. J., Morrison, I. M., Stewart, D., and J. R. Hillman. 1996. Plant cell walls as dietary fibre: Range, structure, processing and function. J. Sci. Food Agric. 70:133–150.

McGoverin, C. M., Snyders, F., Muller, N., Botes, W., Fox G., and M. Manley. 2011. A review of triticale uses and the effect of growth environment on grain quality. J. Sci. Food Agric. 91:1155–1165.

McNab, J. M. 1993. Optimal use of Enzymes for special ingredients. In: Enzymes in Animal nutrition. Proceedings of the 1st Sysposium Kartause lttingen, Switzerland October 13 – 16, 1993.

McNab, J. M., K. Bernard, A. I. Knox, L. McNeill, and M. R. Bedford. 1996. Effect of dietary enzyme addition on digestibility of nitrogen and energy metabolism in young turkeys, broiler chickens, and adult cockerels. Br. Poult. Sci. 37(Suppl. 1):65–66. (Abstr.).

Meale, S. J., Beauchemin, K. A., Hristov, A. N., Chaves, A. V., and T. A. McAllister. 2014. BOARD-INVITED REVIEW: Opportunities and challenges in using exogenous enzymes to improve ruminant production. Journal of animal science, 92(2), 427-442.

Meng, Q. W., Yan, L., Ao, X., Zhou, T. X., Wang, J. P., Lee, J. H., and I. H. Kim. 2010. Influence of probiotics in different energy and nutrient density diets on growth performance, nutrient digestibility, meat quality, and blood characteristics in growing-finishing pigs. Journal of Animal Science, 88(10), 3320-3326. doi:10.2527/jas.2009-2308.

Meng, X., Slominski, B. A., Nyachoti, C. M., Campbell, L. D., and W. Guenter. 2005. Degradation of cell wall polysaccharides by combinations of carbohydrase enzymes

and their effect on nutrient utilization and broiler chicken performance. Poult. Sci. 84:37–47.

Meyer, J. H., Gu Y., Elashoff, J., Reedy T., Dressman J., and G. Amidon. 1986. Effects of viscosity and fluid outflow on postcibal gastric emptying of solids. Am. J. Physiol. 250:G161–G164.

MIFAFF. 2010. Classification and Specification of Hazardous Feeds (Regulation No. 2010–30). Ministry for Food, Agriculture, Forestry and Fisheries, Republic of Korea.

Mitchell, D. A., and B. K. Lonsane. 1992. Definition characteristics and potential. Pages 1–16 in Solid Substrate Cultivation. H. W. Doelle, D. A. Mitchell, and C. E. Rolz, ed. Elsevier, London, UK.

Mohn, M. 2011. Poultry production. How probiotics can play a role. Poultry Health Featured Articles, The poultry site. Accessed Dec. 3, 2011. http://www.thepoultrysite.com/articles/2256/poultry-production-hoe-probiotics-can-play-a-role.

Mombelli, B., and M. R. Gismondo. 2000. The use of probiotics in medical practice. Int. J. Ant. Agents., 16: 531–536.

Moran, E. T. Jr. 1985. Digestion and absorption of carbohydrates in fowl and events through perinatal development. J. Nutr. 115:665–674.

Morgavi, D. P., Kelly, W. J., Janssen, P. H., and G. T. Attwood. 2012. Rumen microbial (meta)genomics and its application to ruminant production. Animal 7:184–201.

Morris, E. R. 1992. Physico-chemical properties of food polysaccharides. Pages 41–55 in Dietary Fibre: A Component of Food: Nutritional Function in Health and Disease. T. F. Schweizer and C. A. Edwards, ed. Springer-Verlag, London, UK.

Mourão, J. L., Pinheiro, V., Alves, A., Guedes, C. M., Pinto, L., Saavedra, M. J., and A. . Kocher (2006). Effect of mannan oligosaccharides on the performance, intestinal morphology and cecal fermentation of fattening rabbits.Animal feed science and technology, 126(1), 107-120.

Mroz, Z, Jongbloed, AW, Lenis, NP, and K. Vreman. 1995. Water in pig nutrition:physiology, allowances and environmental implications. Nutrition Research Reviews 8, 137–164. doi:10.1079/NRR19950010.

Mulyantini, N. G. A., M. Choct, X. Li, and U. R. Lole. 2005. The effect of xylanase, phytase and lipase supplementation on the performance of broiler chickens fed a diet with a high level of rice bran. Proc. Aust. Poult. Sci. Symp. 17:305–307.

Naveed, A., T. Acamovic, and M. R. Bedford. 1999. The influence of carbohydrase and protease supplementation on amino acid digestibility of lupin-based diets for broiler chicks. Proc. Aust. Poult. Sci. Symp. 11:93–96.

Nemcova, R., A. Bomba, S. Gancarcikova, R. Herich, and P. Guba. 1999. Study of the effect of lactobacillus paracasei and fructo-oligosaccharides on fecal microflora in weanling piglets. Berl. Munich. Tierarztl Wschr. 122:225-228.

Newman, K. E., K. A. Dawson, and M. C. Morehead. 1990. Antagonistic activities of bacteria isolates from probiotic feed supplements upon pathogenic and rumen bacteria. J. anim. Sci. 68 (Suppl.), 505.

Newman, M.G. 2002. Antibiotics resistance is a reality: novel techniques forovercoming antibiotics resistance when using new growth promoters. Pp98-106. Nutritional Biotechnology in the feed and food industries. Proceedings of Alltech's 18th annual symposium. Nottingham University Press.

Niemi, J. K., Sevón-Aimonen, M.-L., Pietola, K., and K. J. Stalder. 2010. The value of precision feeding technologies for grow-finish swine. Livest. Sci.129:13–23.

Nitrayová, S., Heger, J., Patráš, P., Kluge, H., J. Brož. 2009. Effect of xylanase on apparent ileal and total tract digestibility of nutrients and energy of rye in young swine. Arch. Anim. Nutr. 63:281–291.

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

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

Nortey, T. N., Patience, J. F., Sands, J. S., Trottier N. L., and R. T. Zijlstra. 2008. Effects of xylanase supplementation on the apparent digestibility and digestible content of energy, amino acids, phosphorus, and calcium in wheat and wheat by-products from dry milling fed to grower swine. J. Anim. Sci. 86:3450–3464.

Nortey, T. N., Patience, J. F., Simmins, P. H., Trottier, N. L., and R. T. Zijlstra. 2007. Effects of individual or combined xylanase and phytase supplementation on energy, amino acid, and phosphorus digestibility and growth performance of grower swine fed wheat-based diets containing wheat millrun. J. Anim. Sci. 85:1432–1443.

Nousiainen, J., and J. Setala. 1993. Lactic acid bacteria as animal probiotics. In: Lactic Acid Bacteria (Edu. Salminen. S and A. Von Wright). Marcel Dekker, New York, USA. pp315-356.

Nousiainen, J., and J. Setala. 1998. Lactic acid bacteria as animal probiotics. In: lactic acid.

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

Nyachoti, C. M., House, J. D., Slominski, B. A., and I. R. Seddon. 2005.Energy and nutrient digestibilities in wheat dried distillers' grains with solubles fed to growing pigs. J. Sci. Food Agric. 85:2581–2586.

Officer, D. I. 1995. Effect of multienzyme supplements on the growth performance of piglets during the pre- and post-weaning periods. Anim. Feed Sci. Technol. 56:55–65.

Ogle, R. B. and J. Inborr. 1987. Alternatives to low does antibiotics in piglet feeds in Sweden. 38th Meeting of the European Association for Animal Production, Portugal, Commission on Pig production, Session 4. pp 1-6.

Olukosi, O. A., Bedford, M. R., and O. Adeola. 2007a. Xylanase in diets for growing swine and broiler chicks. Can. J. Anim. Sci. 87:227–235.

Olukosi, O. A., Cowieson, A. J., and O. Adeola. 2007b. Age-related influence of a cocktail of xylanase, amylase, and protease or phytase individually or in combination in broilers. Poult. Sci. 86:77–86.

Olukosi, O. A., Sands, J. S., and O. Adeola. 2007c. Supplementation of carbohydrases or phytase individually or in combination to diets for weanling and growing-finishing swine. J. Anim. Sci. 85:1702–1711.

Omogbenigun, F. O., Nyachoti, C. M., and B. A.Slominski. 2004. Dietary supplementation with multi-enzyme preparations improved nutrient utilization and growth performance in weaned pigs. J. Anim. Sci. 82: 1053-1061.

Pal, P. U. C. 1999. Probiotics benefits. Poultry International. October. pp 40-44. Park D. Y., Namkung H., Paik I. K. 2001. Effects of supplementary enzymes or probiotics on

the performance and ammonia gas production in weanling pigs. Kor. J. Anim. Sci. Technol. 43:485–496.

Palander, S., Näsi, M., and S. Järvinen. 2005. Effect of age of growing turkeys on digesta viscosity and nutrient digestibility of maize, wheat, barley and oats fed as such or with enzyme supplementation. Arch. Anim. Nutr. 59:191–203.

Park, D. Y., Namkung, H., and I. K. Paik. 2001. Effects of supplementary enzymes or probiotics on the performance and ammonia gas production in weanling pigs. Kor. J. Anim. Sci. Technol. 43:485–496.

Parvez, S., Malik, K.M., Ah Kang, S., and H.Y. Kim. 2006. Probiotics and their fermented food products are beneficial for health. J. Appl. Microbiol., 100, 1171–1185.

Patience, J. F. 2013. Managing energy intake and costs of grow-finish pigs. Advances in Pork Production. In: Proc. 2013. Banff Pork Seminar, Banff, AB, Canada. p. 29–35.

Pekas, J. C., and J. E. Wray. 1991. Principle gastrointestinal variables associated with metabolic heat production in pigs: statistical cluster analyses. J. Nutr. 121:231–239.

Pluske, J. R., Hampson, D. J., and I. H. Williams. 1997. Factors influencing the structure and function of the small intestine in the weaned pigs: a review. Livest. Prod. Sci. 51 215- 236.

Pluske, J. R., J. C. Kim, D. E. McDonald, D. W. Pethick, and D. J. Hampson. 2001. Non-starch polysaccharides in the diets of young weaned piglets. Pages 81–112 in The

Weaner Pig: Nutrition and Management. M. A. Varley and J. Wiseman, ed. CABI Publishing, Wallingford, UK.

Pollman, D. S., D. M. Danielson, and E. R. Peo, Jr, 1980. Effect of microbial feed additives on performance of starter and growing-finishing pigs. J. Anim. Sci. Vol No.3 pp 577-581.

Pond, W. G., V. H. Varel, J. S. Dickson, and W. M Haschek. 1989. Comparative response of swine and rats to high-fiber or high-protein diets. J. Anim. Sci. 687:716–723.

Quiniou, N., J. Dargon, and D. Gaudre. 2002. Variation of piglets' birth weight and consequences on subsequent performance. Livestock Production Science. 78:63-70.

Ranadheera, R. D. C. S., Baines, S. K., and M. C. Adams. 2010. Importance of food in probiotic efficacy. Food Res. Int, 43: 1–7.

Ratcliffe, B., C. B. Cole., R. Fuller, and M. J. Newport. 1986. The effect of yoghurt and milk fermented with a porcine intestinal strain of lactobacillus reuteri on the reuteri on the performance and gastrointestinal flora of pigs weaned at two days of age. Food Microbiology., 3:203-211.

Ravindran, V., P. H. Selle, and W. L. Bryden. 1999. Effects of phytase supplementation, individually and in combination with glycanase, on the nutritive value of wheat and barley. Poult. Sci. 78:1588–1595.

Ritz, C. W., R. M. Hulet, B. B. Self, and D. M. Denbow. 1995. Growth and intestinal morphology of male turkey as influenced by dietary supplementation of amylase and xylanase. Poult. Sci. 74:1329–133

Roberfroid, M. B. 1998. Prebiotics and synbiotics: concepts and nutritional properties. Br. J. Nutr. 80(Suppl.2). S197-S202.

Roberfroid, M.B., J.A. Van Loo, and G.R. Gibson. 1998. The bifidogenic nature of chicory inulin and its hydrolysis product. J. Nutr.128(1):11-9. Review.

Rolfe, R. D. 2000. The role of probiotic culture in the control of gastrointestinal health. J Nutr. 130: 396S-402S.

Rutherfurd, S. M., Chung T. K., and P. J. Moughan. 2007. The effect of a commercial enzyme preparation on apparent metabolizable energy, the true ileal amino acid digestibility, and endogenous ileal lysine losses in broiler chickens. Poult. Sci. 86:665–672.

Saier, M. H., Jr., S. R. Goldman, R. R. Maile, M. S. Moreno, W. Weyler, N. Yang, and I. T. Paulsen. 2002. Transport capabilities encoded within theBacillus subtilis genome. J. Mol. Microbiol. Biotechnol. 4:37–67.

Salminen, S., E. Isolauri, and E. Salminen. 1996. Clinical uses of probiotics for stabilizing the gut mucosal barrier: successful strain and future challenges. Antonie van Leeuwenhoek. 70: 347-358.

Salyers, A.A. Salyers, A. Gupta, and Y. Wang. 2004. Human intestinal bacteria as reservoirs for antibiotic resistance genes Trends in Microbiology, 12 (2004), pp. 412–416.

Sampson, E. J., M. A. Baird, C. Burtis, E. M. Smith, D. Witte, and D. D. Bayse. 1980. A coupled-enzyme equilibrium method for measuring urea in serum: Optimization and evaluation of the AACC study group on urea candidate reference method. Clin. Chem. 26:816-826.

Sanders, M. A. 1998. Overview of functional foods: Emphasis on probiotic bacteria. Int. Dairy J., 8: 341–347.

Sanders, M. E., and J. Huis in't Veld. 1999. Bringing a probiotic-containing functional food to the market: Microbiological, product, regulatory and labeling issues. Antonie van Leeuwenhoek., 76: 293–315.

Sauer, W. C., Mosenthin, R., Ahrens, F., and L. A. Den Hartog. 1991. The effect of source of fiber on ileal and fecal amino acid digestibility and bacterial nitrogen excretion in growing pigs. Journal of Animal Science, 69(10), 4070-4077.

Schmit, T. M., Verteramo, L., and W. G. Tomek. 2009. Implications of growing biofuel demands on northeast livestock feed costs. Agric. Resour. Econ. Rev.38:200–212.

Schulze, H., van Leeuwen, P., Verstegen M. W., and J. W. van den Berg. 1995. Dietary level and source of neutral detergent fiber and ileal endogenous nitrogen flow in swine. J. Anim. Sci. 73:441–448.

Schuster, A., and M. Schmoll. 2010. Biology and biotechnology of Trichoderma. Appl Microbiol Biotechnol, Vol. 87, No. 3, pp. 787-799, ISSN 1432-0614.

Scott, T. A., F. G. Silversides, H. L. Classen, M. L. Swift, and M.R. Bedford. 1998. Effect of cultivar and environment on the feeding value of Western Canadian wheat and barley samples with and without enzyme supplementation. Can.J. Anim. Sci. 78:649–656.

Selle, P. H., Ravindra, V., Caldwell, R. A., and W. L. Bryden. 2000. Phytate and phytase: Consequences for protein utilization. Nutr. Res. Rev. 13:255-278.

Sharma, P., Tomar, S. K., Goswami, P., Sangwan, V., and R. Singh. 2014. Antibiotic resistance among commercially available probiotics. Food research International. Vol. 57 pages 176-195.

Shaw, M. I, Beaulieu, A. D., and J. F. Patience. 2006. Effect of diet composition onwater consumption in growing pigs. Journal of Animal Science 84,3123–3132.

Shields, R. G., Mahan, D. C. Jr., and P. L. Graham. 1983. Changes in swine body composition from birth to 145 kg. Journal of Animal Science 57:43-54.

Shim, S. B. 2005. Effects of prebiotics, probiotics and synbiotics in the diet of young pigs.

Shon, K. S., Hong, J. W., Kwon, O. S., Min, B. J., Lee, W. B., Kim, I. H., Park, Y. H., and I. S. Lee. 2005. Effects of Lactobacillus reuteri-based direct-fed microbial

supplementation for growing-finishing pigs. Asian-australas. J. Anim. Sci. 18:370–374.

Sieo, C. C., Abdullah, N., Tan W. S., and Y. W. Ho. 2005. Influence of beta-glucanase-producing Lactobacillus strains on intestinal characteristics and feed passage rate of broiler chickens. Poult. Sci. 84:734–741.

Silver, S. S. P., and R. R. Smithard. 2002. Effect of enzyme supplementation of a rye-based diet on xylanase activity in the small intestine of broilers, on intestinal crypt proliferation and nutrient digestibility and growth performance of birds. British poultry Science, 43: 274-282.

Silversides, F. G., and M. R. Bedford. 1999. Effect of pelleting temperature on the recovery and efficacy of a xylanase enzyme in wheat-based diets. Poult. Sci. 78:1184–1190.

Simon, O., Politz, O. and R. Borris. 1993. In: Enzymes in Animal nutrition: Procedeedings of the 1st Symposium Kartause Ittingen, Switzerland October 13 - 16, 1993.

Simon, O., Vahjen, W., and Sharek, l., (2003).Micro-organisms as feed additives-probiotics. Pages 295 to 318 in 9th International Symposium on digestive physiology in pigs, Canada.

Soccol, C.R. 2010. The Potential of Probiotics, Food Technol. Biotechnol. 48 (4) 413–434.

Sonia, M. 2005. Probiotics: The hope, the hype, and the reality: In Series #2. Practical Gastroenterology. The Mechanism of Action of Probiotics. May 2005.

Southgate, D. A. T. 1990. Dietary Fiber and Health In: D. A. T. Southgate (ed.) Dietary Fiber: Chemical and Biochemical Aspects. R. Soc. Chem. Spec. Publ., Cambridge, UK.

Spinosa, M. R., Braccini, T., Ricca, E., De Felice, M., Morelli, L., Pozzi, G., and M. R. Oggioni. 2000. On the fate of ingested Bacillus spores. Res. Microbiol., 151: 361–368.

Spriet, S. M., J. A. Decuypere, and H. K. Henderickx. 1987. Effect of bacillus toyoi (Toyocerin) on the gastrointestinal microflora, concentration of some bacterial metabolites, digestibility of the nutrients and small intestinal mean retention time in pigs. Med. Fac. Landbouww. Rijksuniv. Gent. 52: 1673-1683.

Stanogias, G., and G. R. Pearce. 1985. The digestion of fibre by pigs. The effects of amount and type of fibre on apparent digestibility, nitrogen balance and rate of passage. Br. J. Nutr. 53:513–530.

Stanogias, G., and G. R. Pearcet. 1985. The digestion of fibre by swine. Br. J. Nutr. 53:513–530.

Stiles, M. E., and W. H. Holzapfel. 1997. Lactic acid bacteria of foods and their current taxonomy. Int. J. Food Microbiol., 36: 1–29.

Stutton, E. 1992. In Enzymes in Animal nutrition. Proceedings of the first symposium Kartause Ittingen, Switzerland October 13 - 16, 1993.

Surawicz, C. M., Elmer, G. W., Speelman, P., McFarland, L., Chinn, J., and G. van Belle. 1989. Prevention of antibiotic-associated diarrhea by Saccharomyces boulardii. A prospective study. Gastroenterology, 84: 981–988.

Swords, W. E., Wu, C. C., Champlin, F. C., and R. K. Buddington. 1993. Postnatal challenges in selected bacterial groups of the pig colonic microflora. Biol. Neonate 63:191-200.

Teirlynck, E., Bjerrum, L., Eeckhaut V., Huygebaert, G., Pasmans, F., Haesebrouck, F., Dewulf, J., Ducatelle, R., and F. Van Immerseel. 2009. The cereal type in feed influences gut wall morphology and intestinal immune cell infiltration in broiler chickens. Br. J. Nutr. 102:1453–1461.

Tervilä-Wilo, A., Parkkonen, T. Morgan, A. Hopeakoski-Nurminen, M. Poutanen, K.Heikinen P., and K. Autio. 1996. In vitro digestion of wheat microstructure with xylanase and cellulose from Trichoderma reesei. J. Cereal Sci. 24: 215-225.

Theander, O., Westerlund, E., Åman, P., and H. Graham. 1989. Plant cell walls and monogastric diets. Anim. Feed Sci. Technol. 23:205–225.

To, B. C. S. and M. R. Etzel 1997. Survival of Brevibacterium linens (ATCC 9174) after spray-drying, freeze drying, or freezing. J. Food Sci. 62:167–170.

Tokach, M. D., R. D. Good, J. L. Nelssen, and L. J. Kats. 1992. Influence of weaning weight and growth during the first week post-weaning on subsequent pig performance. Swine Day, Kansas State University. p 15- 17.

Troche, C., Sun X., McElroy A. P., Remus J., and C. L. Novak. 2007. Supplementation of Avizyme 1502 to corn-soybean meal-wheat diets fed to turkey tom poults: The first fifty-six days of age. Poult. Sci. 86:496–502.

Tuohy, K. M., Pinart-Gilberga, M., Jones, M., Hoyles, L., McCartney, A. L., and G. R. Gibson. 2007. Survivability of a probiotic Lactobacillus casei in the gastrointestinal tract of healthy human volunteers and its impact on the faecal microflora. J. Appl. Microbiol., 102: 1026–1032.

Tyner, W. E. and F. Taheripour. 2007. Renewable energy policy alternatives for the future. Am. J. Agric. Econ. 89:1303–1310.

Unite Nations, 2001. Statistical Yearbook. Forty-fifth issue. United Nations Reproduction Section, New York, pp. 312-319.

Urriola, P. E., Shurson, G. C., and H. H. Stein. 2010. Digestibility of dietary fiber in distillers coproducts fed to growing pigs. Journal of animal science, 88(7), 2373-2381.

USDA. 2013a. Feed grains: Yearbook tables. www.ers.usda.gov/data-products/feed-grains-database/feed-grains-yearbook-tables.aspx#.U6C5yvldUz4 (cited on 6/16/2014).

Ushe, T.C., and B. Nagy. 1985. Inhibition of small intestinal colonization of enterotoxigenic Escherichia coli by Streptococcus faecium M74 in pigs. Zbl. Bakt. Hyg., I.Abt. Orig. B 181, 374-382

Vahjen, W., Gläser, K., Schäfer, K., and O. Simon. 1998. Influence of xylanase-supplemented feed on the development of selected bacterial groups in the intestinal tract of broiler chicks. J. Agric. Sci. 130:489–500.

Vahjen, W., Osswald, T., Schäfer, K., and O. Simon. 2007. Comparison of a xylanase and a complex of non starch polysaccharide-degrading enzymes with regard to performance and bacterial metabolism in weaned piglets. Arch. Anim. Nutr. 61:90–102.

van der Meulen, J., Inborr, J., and J. G. M. Barker. 2001. Effects of cell wall degrading enzymes on carbohydrate fractions and metabolites in stomach and ileum of swine fed wheat bran based diets. Arch. Anim. Nutr. 54:101–115.

Vanderhoof, J. A., Whitney, D. B., Antonson, D. L., Hanner, T. L. , Lupo, J. V., and R. J. Young. 1999. Lactobacillus GG in the prevention of antibiotic-associated diarrhoea in children. JPediatr, 135: 564-568.

Veum, T. L., and J. Odle. 2001. Feeding neonatal pigs. Pages 671−690 in Swine Nutrition. A. J. Lewis and L. L. Southern, ed. CRC Press, New York.

Verstegen, M. W., and B. A. Williams. 2002. Alternatives to the use ofantibiotics as growth promoters for monogastric animals. Anim. Biotechnol.13:113–127.

Vukic-Vranjes, M., and C. Wenk. 1993b. Einfluss der thermischen Behandlung auf die Wirksamkeit von polysaccharidspaltenden Enzymen in Legehennenfutter. Proc. Soc. Nutr. Physiol.

Wang, J.P., Hong, S. M., Yan, L., Yoo, J. S., Lee, J. H., Jang, H. D., Kim, H. J., and I. H. Kim. 2009. Effects of single carbohydrases cocktail in low-nutrient-density diets on growth performance, nutrient digestibility, blood characteristics, and carcass traits in growing-finishing pigs. livest. Sci. 126:215-220.

Wang, J.P., Lee, J. H. ,Yoo, J. S., Cho J. H. , Kim, H. J., and I.H. Kim 2010. Effects of phenyllactic acid on growth performance, intestinal microbiota , relative organ weigth , blood characteristics and meat quality of broiler chicks. Poult. Sci. 89;1549-1555.

Wannaprasat, W., Koowatananukul, C., Ekkapobyotin, C., and R. Chuanchuen. 2009. Quality analysis of commercial probiotic products for food animals Southeast Asian Journal of Tropical Medicine and Public Health, 40 (2009), pp. 1103–1112

Wassenaar, T.M., G. Klein. 2008. Safety aspects and implications of regulation of probiotic bacteria in food and food supplements Journal of Food Protection, 71 (2008), pp. 1734–1741.

Wells, M. J. 2011. Microbial Cell Factories Volume 10 Supplement 1, 2011: Proceedings of the 10th Symposium on Lactic Acid Bacterium.

Whang, K. Y., Kim, S. W., Donovan, S. M., McKeith, F. K., and R. A. Easter. 2003. Effects of protein deprivation on subsequent growth performance, gain of body components, and protein requirements in growing pigs. Journal of animal science, 81(3), 705-716.

Whitney, M. H., G. C. Shurson, L. J. Johnston, D. M. Wulf, and B. C. Shanks. 2006. Growth performance and carcass characteristics of grower-finisher pigs fed high-quality corn distillers dried grain with solubles originating from a modern Midwestern ethanol plant. J. Anim. Sci. 84:3356–3363.

Willamil, J., Badiola, I., Devillard, E., Geraert, P. A., and D. Torrallardona. 2012. Wheat-barley-rye-or corn-fed growing pigs respond differently to dietary supplementation with a carbohydrase complex. Journal of animal science,90 (3), 824-832.

Woyengo, T. A., Sands, J. S., Guenter, W., and C. M. Nyachoti. 2008. Nutrient digestibility and performance responses of growing pigs fed phytase- and xylanase-supplemented wheat-based diets. J. Anim. Sci. 86:848–857.

Woyengo, T. A., Beltranena, E., and R. T. Zijlstra. 2014. NONRUMINANT NUTRITION SYMPOSIUM: Controlling feed cost by including alternative ingredients into pig diets: A review.

Xuan, Z. N., J. D. KIM, K. N. Heo, H. J. Jung, J. H. Lee, Y. K. Han, Y. Y. Kim, and In K. Han 2001. Study on the development of probiotics complex for weaned pigs. Asian- Aust. J. Anim. Sci. 14:1425-1428.

Yang, T.S., B. Howard, and W. V. McFarlane. 1981. Effect of food on drinking behavior of growing pigs. Applied Animal Ethology 7:259 - 270.

Yang, Z. B., Yang, W. R., Jiang, S. Z., Zhang, G. G., Zhang, Q. Q., and K. C. Siow. 2010. Effects of a thermotolerant multi-enzyme product on nutrient and energy utilization of broilers fed mash or crumbled corn-soybean meal diets. J. Appl. Poult. Res. 19:38–45.

Yen, J. T. 1997. Oxygen consumption and energy flux of porcine splanchnic tissues. Pages 260–269 in Digestive Physiology in Pigs. J. P. Laplace, C. Fevrier, and A. Barbeau, ed. Proc. 7th 439 Int. Symp. Digest. Physiol. in Pigs. EAAP Publication No. 88, Saint Malo, France.

Yen, J. T., J. A. Nienaber, D. A. Hill, and W. G. Pond. 1989. Oxygen consumption by portal vein-drained organs and by whole animal in conscious growing swine. Proc. Soc. Exp. Biol. Med. 190:393–398.

Yin, F., Zhang Z., Huang J., and Y. Yin. 2010. digestion rate of dietary starch affects systemic circulation of amino acids in weaned swine. Br. J. Nutr. 103:1404–1412.

Yin, Y. L., McEvoy, J. D. G., Schulze, H., Hennig, U., Souffrant, W. B., and K. J. MacCracken 2000. Apparent digestibility (ileal and overall) of nutrients and endogenous nitrogen losses in growing swine fed wheat (var. Soissons) or its by-products without or with xylanase supplementation. Livest. Prod. Sci. 62:119-132.

Yoon, S. Y., Y. X. Yang, P. L. Shinde, J. Y. Choi, J. S. Kim, Y. W. Kim, K. Yun, K. Jo, J. H. Lee, S. J. Ohh, I. K. Kwon, and B. J. Chae. 2010. Effects of mannanase and distillers dried grain with solubles on growth performance, nutrient digestibility, and carcass characteristics of grower-finisher pigs. J. Anim. Sci. 88:181–191.

Zhang, Y., and J. Caupert. 2012. Survey of mycotoxins in U.S. distiller's dried grains with solubles from 2009 to 2011. J. Agric. Food Chem. 60:539–543.

Zhou, Y., Jiang, Z., Lv D., and T. Wang. 2009. Improved energy-utilizing efficiency by enzyme preparation supplement in broiler diets with different metabolizable energy levels. Poult. Sci. 88:316–322.

Ziggers, D. 2011. Animal Feed News. EU 12-point antibiotic action plan released, 18 November, 2011. http://www.allaboutfeed.net/news/eu-12-point-antibiotic-action-plan-released-12443.html.

Zijlstra, R. T., de Lange, C. F. M., and J. F. Patience. 1999. Nutritional value of wheat for growing pigs: Chemical composition and digestible energy content. Can. J. Anim. Sci. 79:187–194.

Zimmermann, E. B., and R. Mosenthin. 2001. Pro- and prebiotics in pig nutrition-potential modulators of gut health? J. Anim. Feed Sci. 10, 47-56.

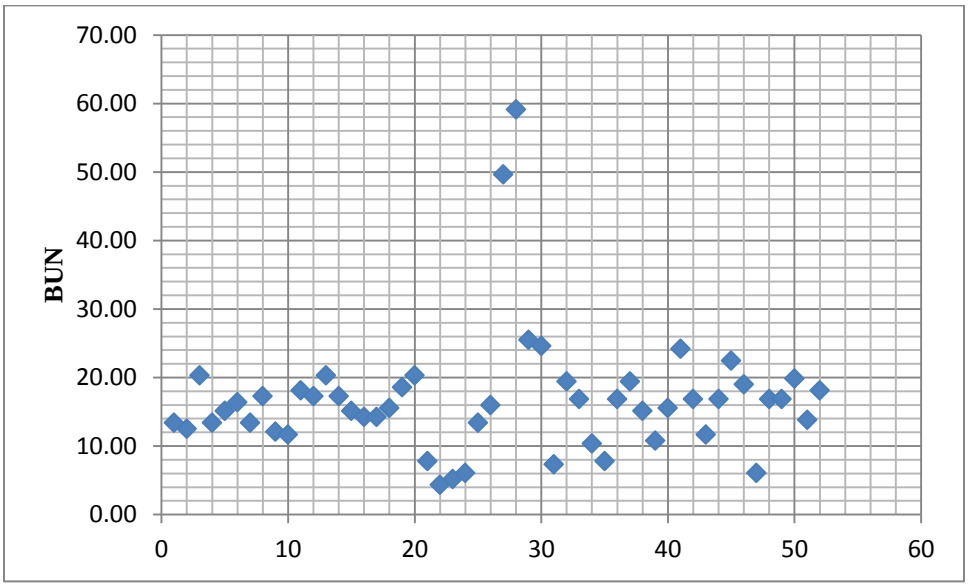
Zyla, K., D. R. Ledoux, M. Kujawski, and T. L. Veum. 1996. The efficacy of an enzymic cocktail and a fungal mycelium indephosphorylating corn-soybean meal-based feeds fed to growing turkeys. Poulty Sci. 75:381–387.

APPENDICES

Appendix 1.Experimental design Calsporin study

Room 4			Room 3		
Pen 408 **T2**		Pen 409 **T1**	Pen 308 **T1**		Pen 309 **T2**
Pen 407 **T2**		Pen 410 **T1**	Pen 307 **T1**		Pen 310 **T2**
Pen 406 **T2**		Pen 411 **T1**	Pen 306 **T1**		Pen 311 **T2**
Pen 404 **T1**		Pen 413 **T2**	Pen 304 **T2**		Pen 313 **T1**
Pen 403 **T1**		Pen 414 **T2**	Pen 303 **T2**		Pen 314 **T1**
Pen 402 **T1**		Pen 415 **T2**	Pen 302 **T2**		Pen 315 **T1**
CORRIDOR					

Appendix 2. Scatter plot of BUN values of both the Control and Calsporin groups



Appendix 3. Telazol and Euthasol dosing based on body weight

Pen	Pig ID	Treatment	Weight, kg	Telazol, ml	Euthasol, ml
1	149	1	21.18	1	5
2	163	2	20.5	1	3
3	156	6	23.09	1	3
4	169	3	21.45	1	3
5	166	5	22.68	1	3
6	172	1	22	1	3
7	159	2	20.73	1	3
8	157	1	20.64	1	3
9	151	3	19.6	1	5
10	171	4	21.68	1	3
11	178	2	21.36	1	3
12	145	4	17.6	1	3
13	160	4	21.09	1	3
14	161	6	21.45	1	5
15	176	6	21.05	2.5	3
16	162	3	20.87	1	3
17	175	3	18.82	1	3
18	179	2	20.5	1	3
19	150	5	18.82	1	3
20	152	5	17.15	1	3
21	146	1	20.73	1	3
22	170	6	19.46	1	3
23	174	3	17.69	1	3
24	173	4	17.69	1	3
25	165	3	17.92	1	3
26	180	1	20.09	3	5
27	167	4	18.69	1	3
28	158	3	17.46	1	3
29	164	6	16.47	1	5
30	155	5	17.46	1	3
31	153	2	15.51	1	3
32	148	4	15.78	1	3
33	177	1	16.74	1	3
34	147	6	15.29	1	3
35	154	5	15.88	3	3
36	168	5	17.46	1	5

Appendix 4.Summary of Animal Allotment, Digestibility study

Summary of Animal Allotment							
Rep / Trt	1	2	3	4	5	6	Mean
1	23.10	21.80	20.30	20.10	18.50	16.00	**19.97**
2	22.90	21.10	20.30	19.60	18.70	16.80	**19.90**
3	22.80	22.00	20.30	20.20	17.60	16.80	**19.95**
4	23.10	21.90	20.60	19.40	17.40	17.20	**19.93**
5	23.20	22.70	20.60	20.10	17.50	15.90	**20.00**
6	23.20	21.30	21.00	19.40	19.10	14.60	**19.77**
Mean	**23.05**	**21.80**	**20.52**	**19.80**	**18.13**	**16.22**	**19.92**
CV	*0.71*	*2.59*	*1.36*	*1.89*	*3.99*	*5.79*	*0.41*
Analysis of Variance							
Source	DF	SS	MS	F value	Pr > F		
Rep	5	183.6514	36.7303	95.22	0.0000		
Trt	5	0.2014	0.0403	0.10	0.9903		
Error	25	9.6436	0.3857				
Total	35	193.4964					

Appendix 5. Daily Maximum and Minimum Room Temperatures during performance study I