2014

Effect Of Feeding Sows Diets Supplemented With Oat And Yeast Culture On The Incidence Of Post Weaning Diarrhea In Their Offspring

Torel Daniels
North Carolina Agricultural and Technical State University

Effect of Feeding Sows Diets Supplemented with Oat and Yeast Culture on the Incidence of Post Weaning Diarrhea in Their Offspring

Torel Daniels

North Carolina A&T State University

A thesis submitted to the graduate faculty

in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Department: Agribusiness, Applied Economics and Agriscience Education

Major: Agriculture Education

Major Professor: Dr. Radiah C. Minor

Greensboro, North Carolina

2014

The Graduate School
North Carolina Agricultural and Technical State University
This is to certify that the Master's Thesis of

Torel Daniels

has met the thesis requirements of
North Carolina Agricultural and Technical State University

Greensboro, North Carolina
2014

Approved by:

Dr. Radiah C. Minor Major Professor	Dr. Antoine J. Alston Committee Member
Dr. Leonard L. Williams Committee Member	Dr. Salam A. Ibrahim Committee Member
Dr. Anthony K. Yeboah Department Chair	Dr. Sanjiv Sarin Dean, The Graduate School

Biographical Sketch

Torel Daniels was born on February 27, 1988 in Greenville, North Carolina. He graduated from North Carolina Agricultural and Technical State University in the Spring of 2011 with a Bachelor of Science in Animal Science and Laboratory Animal Science. During graduate school he attended the North Carolina Louis Stokes Alliance for Minority Participation Conference, the Annual North Carolina Alliance to Create Opportunity through Education Conference, the Thurgood Marshall College Fund Leadership Institute and the Association of 1890 Research Directors, Inc. Conference in which he received 2nd place in the Graduate Oral Presentation. Torel is a part of the Dean's Student Council, the School of Agriculture Web Design Team, the Student Ambassador Team and was inducted in to the Gamma Sigma Delta Honor Society of Agriculture. He is also a mentor in his community volunteering at Lindley Park Elementary School. He is a candidate for the Master of Science degree in Agriculture Education.

Dedication

This publication is dedicated to the four most important people in my life; without their uplift, kind words, inspiration and wisdom throughout my life this journey would not be feasible. I want to dedicate this publication to my father Leroy Daniels, my brother Antony Daniels, my Grandmother Inetta Fleming and most importantly the love of my life, my mother Yvonne Daniels.

Acknowledgements

I am extremely thankful for my mentor and advisor Dr. Minor whose encouragement, guidance and knowledge from day one to the final population enabled me to develop an understanding of the subject. I would like to thank Teo Barrios, Brad Pope and the entire North Carolina A&T farm staff for countless hours helping me and assisting with the manual labor of my project. I would like to show my gratitude to my committee members Drs. Williams, Ibrahim and Alston for their skillful work expertise and advice. I would also like to thank Dr. Noble for his expertise and clarification. Next I would like to thank Mrs. Shurrita Davis and all of the lab assistants (North Carolina Research Campus in Kannapolis, NC) that had a hand at helping me with the tedious lab preparation. I am honored to have worked with such a talented bunch. I am grateful for the help from my colleagues Dawn Conklin, Barry Donavon, Mykea McKnight and Kristen Locke. Without them the sample collection process would have been more difficult. I am also grateful for Dr. Waterman and the students in the Waterman Lab for their assistance and help. In particular I thank Carresse Gerald for being a magnificent colleague and mentor. It is a pleasure to thank the two people who without them this thesis would have not been possible, my wonderful parents for daily support and love. Lastly, I offer my regards and blessings to all of those who supported me in any respect or way during the completion of my Master's education. This work was made possible by Grant # NCX-254-5-11-120-1 from the National Institute of Food and Agriculture. Its contents are solely the responsibility of the authors and do not necessarily represent the official views of the National Institute of Food and Agriculture.

Table of Contents

List of Figures

List of Tables

Abstract

Post Weaning Diarrhea (PWD) is a leading cause of mortality in piglets during the first two weeks of weaning. According to the Fairbrother, Nadeau et al. (2005) report, PWD is a major gastrointestinal disease caused by stress, pathogenic intestinal bacteria and immature immune responses. Antibiotic growth promoters (AGP) are used to combat PWD but there is public concern surrounding their use (Casewell, Friis, Marco, McMullin, & Phillips, 2003). The purpose of this study is to investigate natural alternatives to AGP that prevent PWD. Therefore, we designed a study whereby yeast culture and oat were fed to sows during the last 30 days of gestation and during lactation in hopes to enhance the intestinal microbiota and overall post wean performance of their offspring. Piglet feed intake was monitored from birth until 14 days post weaning. The total feed consumed in week 1 and 2 post weaning showed no significant difference between all the piglets. This data suggests that feeding a CON+YC, CON+O, or CON+YC+O diet to gestational sows 30 days prior to farrowing may not affect feed intake. Piglet weight gains were evaluated on day of farrowing, day of weaning, and days 1, 4, 7, and 14 post weaning. The piglets whose mothers consumed oats weighed the most at birth and lost the least amount of weight during the post-weaning period. This suggests that feeding sows a diet containing oat may be better for sustaining growth of her piglets. The fecal scores were recorded on day 0, 7 and 14. On day seven, fecal scores of piglets born of sows on oat supplemented diet were significantly less than those born of sows that consumed control and yeast+ oat diets. This observation suggests that feeding sows a diet consisting of 15% oat may prevent post wean diarrhea in their offspring during weaning. Fecal samples were collected from all 16 of the sows on day 84 of gestation, farrowing day, and weaning day. Samples were collected from the colon of the sows. During farrowing day sows from the CON+YC+O diet had an *Escherichia coli*

bacteria count that was significantly lower than CON+YC and CON+O and highly significantly lower than the sows on CON diets alone. Further, by weaning day sows feed CON +YC+O had significantly lower than *Escherichia coli* than those fed CON or CON+YC. This suggests that feeding sows 5g/kg Diamond V Mills Yeast Culture together with 15% oat during the last 30 days of gestation may have an impact on *Escherichia coli* population in the gut. In addition, it was observed that the *Staphylococcus aureus* population in the colon of sows fed CON+O had decreased from the introduction of the diet on day 84 of gestation until farrowing day, and was undetectable by day of weaning. This data suggests that feeding sows a diet consisting of CON+O during gestation and lactation may help protect against colonization of the gut with *Staphylococcus aureus* in sows. Genotyping by polymerase chain reaction was also performed to identify and compare the presence of shiga toxin-producing (Stx) *Escherichia coli*. By qualitative analysis we detected fewer colonies of toxigenic *E.coli* in all of the diet groups as compared to control. This suggest that feeding sows a diet supplemented with either 15% oat, Diamond V. Mills Yeast Culture separate or in combination as a synbiotic may help in preventing gut colonization with high population of shiga toxin producing *Escherichia coli*. Together these data suggest that feeding sows a diet containing oat alone or together with YC during gestation and lactation may help promote healthy gut colonization in the sows and help protect their offspring from developing post weaning diarrhea.

CHAPTER 1

Introduction

Hog production is an important industry to the US and worldwide market. America is second in the world behind China in pork production. According to the American Meat Institute, in 2012 the US processed 113.2 million hogs. Within the US market, North Carolina ranks second in pork production raising 15.2% of the U.S pork population (NC Department of Agriculture and Consumer Services, 2013). Each year, the pork industry contributes $27 billion dollars to the economy and every year the pork industry faces production challenges, such as high feed prices, new pest and diseases, a greater demand for meat, and offspring early wean stressors (Hurt, 1994).

One of the leading causes of production and financial losses to the pork industry is deaths of weaned piglets because of poor gut health (Fairbrother et al., 2005). A common disease of weaned piglets associated with gut health is post weaning diarrhea (PWD). PWD is a major gastrointestinal disease and is the leading cause of losses in young pigs during the first two weeks after weaning. To combat this condition, antibiotic growth promoters (AGP) are used. But, unfortunately there is controversy surrounding the use of AGP, as several studies suggest that AGP are linked to the rise in antibiotic resistant bacteria in livestock and that these bacteria could be transmitted to humans (Casewell et al., 2003). This is a great public health concern. Because of this concern, the European Union banned the use of AGP in livestock production and the US has begun to phase out its use. Therefore alternatives to AGP that could be used during weaning that promote gut health are being investigated.

Probiotics, for example lactobacillus, bifidobacterium, enterococcus and yeast such as *Saccharomyces cerevisiae* are live microbial food ingredients that are beneficial to the gut.

Prebiotics are non-digestible food elements that also have beneficial effects on the gut. Probiotics and prebiotics benefit the host by selectively stimulating the growth of beneficial bacteria in the colon which improves host health (Stein & Kil, 2006). Use of prebiotics and probiotics as a food additive are being investigated for their effectiveness against a range of gastrointestinal diseases and disorders including PWD (Tuohy, Probert, Smejkal, & Gibson, 2003).

Neonate and weaned piglets experience major changes in intestinal microbiota composition that is heavily influenced by the host, diet, and environmental factors. For this reason a mother's diet has the opportunity to affect the health of her offspring (Lalles, Bosi, Smidt, & Stokes, 2007). The best time for protecting the offspring and help to reduce diseases through consumption of nutrients is during the mothers lactation period (Le Huërou-Luron, Blat, & Boudry, 2010). Through the mother's diet, probiotics and prebiotics have been proven to improve litter health such as, decrease piglet diarrhea score, decrease pre- weaning mortality rate and aid to increase piglet body weight at weaning (Alexopoulos et al., 2004).

Many studies show that probiotics as a dietary supplement have been practiced in ruminants to improve overall milk production and feed intake; however, effects of probiotics in the offspring through lactation of the mother's diet have not been carefully investigated (Kim et al., 2008). To our knowledge there are not many studies that focus on the impact that inclusion of oat and yeast culture into a mother's diet has on the incidence of PWD in her offspring.

Our lab is interested in identifying an alternative to AGP that helps prevent the onset of PWD in weaned piglets. The focus of this study was to evaluate the effect that inclusion of oat (prebiotic source), yeast culture (probiotic) and oat+ yeast (synbiotic) in gestation and lactation

diets had on gut bacteria colonization on sows and piglets as well as weight gain, incidence of PWD in piglets. The specific aims of the project were as follows:

Specific Aim 1 Evaluate the effect that inclusion of oat and YC feed given during gestation and lactation had on gut-bacterial colonization of sows.

Specific Aim 2 Evaluate the effect that inclusion of oat and YC feed given during gestation and lactation had on birth weight and growth (birth- weaning).

Specific Aim 3 Evaluate the effect that inclusion of oat and YC feed given during gestation and lactation to sows had on incidence of PWD and gut-bacterial colonization of piglets.

CHAPTER 2

Literature Review

Pork production is an essential part of the US economy. In 2011 there were nearly 35,000 full time jobs in pork production which helped to create more than 515,000 indirect jobs (National Pork Producers Council, 2012). America is ranked number two in pork production imports and exports in the world but they are dealing with a worldwide emerging issue.

2.1 Swine Industry Challenges

Pork is an enormous sector within the agriculture economy. Retail sales total around $27 billion dollars per year (Hurt, 1994). Around the late 1990s the average consumer in the United States consumed 53 pounds of pork per year. This means that the emphasis is on the U.S. swine industry to produce a consistent quality of safe meat to consumers (Pan & Kinsey, 2002).

According to Pan and Kinsey, the demographics of America after 1990 rapidly changed. This demographic climb helped to increase overall population by 13.2%. This amplification in American population has increased the variety of pork production that is demanded. In response to consumer demand for leaner and overall more meat, the United States swine industry is forced to produce leaner but heaver hogs (Pan & Kinsey, 2002). Through this demand model change the number of hog operations has decreased by 70% while hog sales rose around 23% from 1969 to 1992 (McBride, 1997). This means that swine production in the United States is changing to fewer and larger production units (Honeyman, 1996). With larger production units come more stressful and shorter weaning periods (Dudink, Simonse, Marks, de Jonge, & Spruijt, 2006).

The swine industry weans piglets at an increasingly earlier age in an effort to increase the efficiency of their breeding stock (Fraser, 1978). According to Fraser, typical weaning age has declined from around 8 weeks to 3-4 weeks, but this can lead to immense problems in health.

For example, piglets are highly receptive to intestinal disorders such as post wean diarrhea (PWD) when weaned early. PWD is a condition mainly related to the moderately immature defense mechanisms of the digestive tract to cope with toxin-producing microorganisms such as *Escherichia coli*. As a result, their growth weight is impaired and mortality may occur from dehydration via diarrhea (Madec, Bridoux, Bounaix, & Jestin, 1998).

Antibiotic growth promoters are used in sub-therapeutic doses in piglet feed to counter act the post weaning diarrhea disease. But, there has been a rise in concerns regarding the role that agriculture has in the transmission of resistant bacteria. A study done by Van den Bogaard, London et al. (2001), focused on determining if it was possible to transmit antibiotic resistant *E.coli* isolates from poultry to humans. The results from the study indicated that transmission of resistant *E.coli* from poultry to humans is very probable. According to Wegener (2003), food animals could serve as a vector for glycopeptide-resistant enterococci infections that was rapidly spreading in the hospitals of America and Europe in 1994. This bacterium was untreatable because it was resistant to all commonly used drugs. Later, it was suggested that this bacteria was associated with avoparein which was used as a growth promoter in food animals (Wegener, 2003). Therefore, there has been a reassessment of the use of antibacterial agents in animal feeds (Casewell et al., 2003). In fact, the microbials that develop resistant genes pose a probable risk to humans if they are transferred. Due to this, the World Health Organization and the European Union determined that the use of antimicrobials in the food animal industry is a public health issue (Castanon, 2007). In 1986, the EU started their plan to wean out antibiotic growth promoters in feed animals and currently it is illegal to use any form of antibiotics as a feed additive when it comes to feed animal (Castanon, 2007). Close (2000) states that this will have an effect on piglet weaning performance and will require the development of new feeding,

management and health strategies. Currently the FDA has unveiled a plan expected to end the use of antibiotics for growth promotion in the U.S. (Charles, 2012).

2.2 Piglet Early Weaning Effect on Growth and Development of Intestinal Disorders

The ever increasing demand from the consumers of pork has forced the weaning age of piglets to decrease to 3-4 weeks of age (Piva et al., 2009). Despite increasing progress to the nutritional formula for nursery pig ration, the time frame of the first week after weaning continues to be a critical production stage in most pork industry shelters characterized by nutritional, environmental and social stresses leading to low weight gain, bad nutritional absorption, diarrhea and gastrointestinal diseases (Biagi, Piva, Moschini, Vezzali, & Roth, 2007). Several studies show that decreasing weaning age increases post weaning stress in piglets. Signs of post weaning stresses include reduce feed intake, high atrophy and diarrhea which results in lower digestive capacity and eventually, reduced weight gain (Zijlstra, Whang, Easter, & Odle, 1996).

Weaning is a stressful event that is takes place a few weeks after being born. Piglets are abruptly separated from their mother, their diet changes from being predominately based on milk to a diet solely based on whole foods. Piglets are usually mixed with unfamiliar piglets and are exposed to a new environment (Dudink et al., 2006). Post weaning nutritional stress may arise from nutritional changes from a lipid to dry diet. The earlier the piglet is weaned the less the offspring is prepared to digest starter diet (Funderburke & Seerley, 1990). As a result of early weaning problems in piglets, economic performance is affected if overall caucus yield decrease or time needed to meet market weight is increased.

The gastrointestinal tract of a pig is an intricate environment. Early weaning periods are met by an immediate but momentary drop in feed intake impairing growth performance. All of

these factors can negatively disturb the immune system and the gut microbial equilibrium, leading piglets to have increase susceptibility to stomach disorders, infections and diarrhea (Gaggìa, Mattarelli, & Biavati, 2010). Moreover, in newborns and weaning pigs their gut experiences changing in size, microbiota, and alteration in digestive and immune function (De Lange, Pluske, Gong, & Nyachoti, 2010). These transformations are influenced by psychological, social, environmental and dietary stresses (Lallès et al., 2004).

One of the leading intestinal disorders that causes mortality and morbidity of piglets in the pork industry is due to post weaning diarrhea (Gaggìa, Mattarelli et al. 2010). Gastrointestinal disturbances in weaned piglets causes large economic loss in the pork industry (Lalles et al., 2007). In the EU, within the pig population total loses of all piglets born amount to 17% and an extensive amount of those can be linked with infection via mucosal surfaces (Lalles et al., 2007).

2.3 Post Weaning Diarrhea- Associated Pathogens

A variety of pathogenic bacteria species colonizes the gastrointestinal tract of piglets. These bacteria can cause clinical diseases and an overwhelming large amount of mortality in weaned piglets in the pork industry (Pluske, Pethick, Hopwood, & Hampson, 2002). Studies show that diarrheal disease caused by entertoxigenic *Escherichia coli* (ETEC) is known to be the most common type of enteric colibacillosis encounter in early weaned piglets (Marquardt et al., 1999).

2.3.1 *Escherichia coli*

Entertoxigenic *Escherichia coli* strains occurs either during the first week of life or between 3–6-weeks of age piglets (Jacek Osek, 2000). The disease is caused after weaning and can be distinguished by dehydration, loss of body weight and sometimes death of contaminated

pigs. Most *E. coli* connected with post-weaning diarrhea belong to a limited number of serogroups, express specific fimbrial adhesins and possess certain toxic properties (J Osek, 2000). *Escherichia coli* serotype O157:H7 is a type of ETEC strain that produces Shiga toxin (Stx), also known as verocytotoxin. It is capable of causing a full spectrum of disease (Wells et al., 1991). Shiga toxin producing *Escherichia coli* are zoonotic pathogens that cause potentially fatal and often epidemic food borne illness. Shiga toxin produces two major serologically distinct types, Stxl and Stx2 (Karmali, 2004). In a study completed by Samadpour, Ongerth et al. (1994), fresh meat pultry and seafood purchased from Seattle grocery stores were examined for the presence of shigatoxin producing *E.coli.* Of the 294 food samples that were tested 17% had colony sequences positive for Stx 1and/or Stx 2 gene.

2.3.2 *Staphylococcus aureus*

Staphylococcus aureus is a gram positive cocci bacterium that is usually found in the nasal area and fecal matter of porcine. In numerous countries *Staphylococcus aureus* is known to be the second most common pathogen causing outbreaks of food poisoning, only outnumbered by Salmonella. Usually the consumption of meat containing staphylococcal enterotoxins is the cause of this illness. Symptoms such as vomiting, abdominal pain and diarrhea usually occur approximately 2–6 h post consumption of food containing enterotoxins (Atanassova, Meindl, & Ring, 2001).

2.3.3 Salmonella

Salmonella infection is a major cause of food-borne illness in humans and is the cause of 1.2 million illnesses, 23,000 hospitalizations and 450 deaths in the United States (Gaggìa, Mattarelli et al. 2010). Meat is an imperative source of food-borne salmonellosis, with poultry and pork implicated more often than any other meat. Strains of salmonella from pork can be

implicated in human food-borne illnesses, and often enter the human food supply chain via pork contamination products (Gaggìa et al., 2010).

2.4 Antibiotic Growth Promoters

In the 1940s the growth-promoting properties of anti-microbial agents for livestock animals were discovered (Close, 2000). During the 1970s close to 50% of all the antibiotics produced in the United States were added directly to the feed of farm animals. The growth promotion of antibiotics is an occurrence of a biological interest in the farm industry in that the feeding of these substances to young farm animals resulted in a greater weight gain per unit of feed and in a more rapid overall growth rate (Novick, 1981).

There are mechanisms that scientist have proposed that explain the growth stimulatory effects of antimicrobial agents. Those mechanisms are that antimicrobial agents increases synthesis of vitamins or other growth factors; they enhance efficiency of absorption and utilization of nutrients because the wall of the intestinal tract is thinner (Visek, 1978). Following these studies feeding farm animals sub-therapeutic doses was successfully adopted. Many positive effects are associated with the inclusion of these sub-therapeutic does such as, reduced nutrient waste, reduction of microbial population in the gastro-intestinal tract, and better nutrient absorption. The most known benefit from these growth promoters are its cost-effectiveness and economic advantages (Close, 2000). On the other hand, following reports about the controversy behind growth promoters, attention grew towards the rise of bacterial resistance to certain antibiotics and the potential harm to both human and animal health (Close, 2000). Alternative strategies for AGP include diet acidification, oligosaccharides, enzymes, herbs, minerals, probiotics, prebiotics and non-starch polysaccharides are being investigated.

2.5 Alternatives to AGP

During the past, the problems of early weaning was neutralized by the use of antibiotic substances that may select antibiotic-resistant genes (Piva et al., 2009). But after the Swann Report in 1968, all attention was drawn to bacteria resistance to different antibiotics and the possible harm to humans and animals (Close, 2000). Due to the widespread concern over this matter, there is an increasing need to find generally recognized safe alternative to antibiotic growth promoters.

Unique diets and management schemes are being investigated and used to overcome problems associated with early weaning (Zijlstra et al., 1996). For example, Piva, Morlacchini et al. (2009) showed that by adding sodium butyrate to weaning feed can significantly increase average daily growth and dry food intake in the first two weeks of weaning; and, Zijlstra, Whang et al. (1996) found that feeding a milk replacer plus a starter diet after weaning increases average daily gain by 30% between 21 and 28 days compared to conventional weaning practices ($P<0.01$). In the Manzanilla et al. (2006) study they used acidifiers, a plant extract antimicrobial to replace antibiotics in piglet diets. The study found that the plant extract increased lymphocyte presence in the colon and had a positive effect on the intestinal composition and microbiota in the piglet. Also in the Katouli, Melin et al. (1999) study, they found that supplementing zinc oxide in weaning piglets diet helped to maintain the permanence of the intestinal microflora and the multiplicity of coliforms during the first two weeks post weaning. A range of feed additives such as organic acids, copper sulphate, zinc oxide, probiotics, prebiotics, and herbs have also been used in newly weaning piglets as an alternative to antibiotics growth promoters (Namkung, Li J. Gong, Yu, Cottrill, & de Lange, 2004).

2.6 Probiotics and Yeast Culture

Probiotic therapy has been examined for its usefulness against a variety of gastrointestinal diseases (Tuohy et al., 2003). A probiotic is considered a live microbial food ingredient that is beneficial to gut health. Most of the probiotic studies dealing with sows belong to the genera Lactobacilli, Bifidobacteria and yeast and helps alleviate diarrhea (Gaggìa et al., 2010). According to a human study by Huang, Bousvaros et al. (2002), bacterial probiotic such as lactobacilli shortens the duration of acute diarrheal illness in children.

Yeast Culture is a fermented product containing *Sacharomyces cerevisiae* yeast. Dietary supplementation of yeast culture has been practiced in grower pigs to improve growth, gut health and used for an alternative supplement to antibiotics (Gosbell, 2004). For example, Shen, Carroll et al. (2011) showed that adding *Saccharomyces cerevisiae* fermentation product to a sow's gestation and lactation diet reduces neutrophil cell count which improves the maternal health status. Studies show that yeast culture supplementation is beneficial to ruminate and pigs by increasing growth performance, milk production, nitrogen balance and nutrient digestion. Also, in a study done by Jurgens, Rikabi et al. (1997), dry yeast supplemented during late gestation increased gamma globulin content of sow's milk and improved post weaning rate and efficiency of weight gain of pigs. Moreover, enzymes, vitamins, and other metabolites produced from yeast fermentation may benefit growth, metabolism, and health of sows and their offspring (Kim et al., 2008).

2.7 Prebiotics and Oats

Prebiotics are non-digestible food ingredients that positively affect the gut by stimulating the growth and activity of beneficial bacteria of the colon. Most identified prebiotics are carbohydrates such as fibers and oligosaccharides (Gaggìa et al., 2010). Evidence shows that

certain prebiotics may mediate important health effects such as improve mineral absorption and reduce the number and size of chemically induced colonic tumors (Costabile et al., 2008). Prior research explains that prebiotics have been used in animal feed industry to enhance the well-being of poultry, swine, horses and dogs (Callaway et al., 2008). In addition, other resent studies show that prebiotics have been used to enhance clearance of zoonotic enteropathogens such as Salmonella in poultry (J. W. Collins, La Ragione, Woodward, & Searle, 2009).

Oats are a type of prebiotic whole grain cereal that is rich in fermentable carbohydrates (Connolly, Lovegrove, & Tuohy, 2010). Fermentable carbohydrates such as dietary fiber have a positive benefit on the intestinal microbiota. Past studies show that feed gain in piglets had an linear increase with amplified population of oats in the diet (Lindemann et al., 1983).

2.8 Synbiotics

Another possibility in gut microflora health is the combination of probiotics and prebiotics in which is called synbiotics (M. D. Collins & Gibson, 1999). It is suggested that a synbiotic diet may give a synergistic result of both prebiotics and probiotics on the growth of piglets (Shim, Verstegen, Kim, Kwon, & Verdonk, 2005). According to Collins and Gibson, the combination of the two could increase the survival of the probiotic organism in which its particular substrate is readily available for its fermentation. This results in the advantage wherein the live microorganism and prebiotic offer. For example, Shim etal. (2005) showed that by feeding suckling piglets a mixture of oligofructose (0.2%) + probiotic (0.3%) as a synbiotic diet can significantly increase the number of bifidobacteria in the colon.

2.9 Impact of Mothers Diet on the Health of Her Offspring

Most of the research done investigates how diets given to piglets at time of weaning promotes health, growth and prevents post weaning diarrhea. Very little investigation has been

done on the effect of dietary supplements given to sows has on her offspring. We know that a mother's diet has the opportunity to positively or negatively affect the health of her offspring (Alexopoulos et al., 2004). The lactation period is the best protection for the offspring after birth and helps to reduce diseases during that period through the consumption of nutrients (Le Huërou-Luron et al., 2010). According to the review colonization is diet dependent, for example breast feeding promotes a less diverse gut flora dominated by bifidobacteria compared to formula feeding (Cilieborg, Boye, & Sangild, 2012). In the Alexopoulos et al. (2004) study, feeding sows/gilts probiotics can improve certain performance pertaining to lactation and gestation period. It can also improve litter health such as, decrease piglet diarrhea score, decrease pre-weaning mortality rate and aid to increase piglet body weight at weaning. Variation in the performance of offspring have also been seen when sows are feed dissimilar diets. In a study conducted by Matysiak et al. (2012), piglets had a significantly higher average daily gain during the suckling period and a higher body weight at weaning when their mother was supplemented with a mixture of plant extract consisting of 5.4% carvacrol (oregano), 3.2% cinnamaldehyde (cinnamon) and 2.2% capsicum oleoresin (Mexican pepper). This study also reveals that the mortality of piglets during the suckling period was significantly lower in the extract group than in the control group.

The goal of this study was to evaluate the effect that inclusion of oat (a prebiotic source), or yeast culture (a probiotic source), or combination of oat+ yeast (a symbiotic source) into gestation and lactation diets had on gut bacteria colonization, weight gain and incidence of PWD in piglets.

CHAPTER 3

Materials and Methods

3.1 Animals and Housing

Two experimental trials were conducted during the summer months of 2013. In each trial 16 sows (Duroc, Landrace, Yorkshire, and Berkshire) of first – third parity and 80 piglets were used. All animals were housed at the Swine Research Unit of the North Carolina A&T State University Farm. Sows and nursing piglets were housed together in gestation pens and weaned piglets were housed in the nursery. The Institutional Animal Care and Use Committee (IACUC) of North Carolina A&T State University approved all activities.

3.2 Feed

Four feed formulations, for both the gestation and lactation periods, were used in the two trials. They were (1) control diet, (2) (prebiotic) control + Diamond V. Mills yeast culture (YC) (5g/kg), (3) (probiotic) control + ground whole oat (15%), and (4) (symbiotic) control + Diamond V. Mills YC + ground whole oat. All of the feed was formulated to meet the nutritional demands of the sows and was milled by NC State Feed Mill. A detailed list of gestational and lactation diet feed formulation can be found in Table 1. Gestation feed was administered beginning on the last 30 days of gestation and ending on day of farrowing and the lactation feed was administered beginning on day of farrowing and ending on the day piglets were weaned. Sows received the same feed supplementation during gestation and lactation. Upon weaning, all piglets were fed the same nursery ration formulated to meet all nutritional requirements. All animals had free access to feed and water at all times.

Table 1

Gestational and Lactation Diet Feed Breakdown

	Gestation (% of diet)			
Feed Ingredients	**Control**	**YC**	**Oat**	**YC + Oat**
11102 NCDA Corn (Rolled)	80.3	79.9	65.3	65
Soybean Meal	13.8	13.8	13.8	13.8
Diamond V Mills YC	n/a	0.5	n/a	0.5
Ground Whole Oat 15%	n/a	n/a	15	15
Corn NCDA	n/a	n/a	n/a	n/a
Corn (1/8) Micro-Flush	1	1	1	1
Limstone Fine	1.11	1.1	1.1	1.1
MON-CAL 21% P	2.05	2.04	2.05	2.03
Salt	0.5	0.5	0.5	0.5
Swine TM PX (KSU)	0.15	0.15	0.15	0.15
Swine Sow-Pig VIT	0.04	0.4	0.037	0.04
Threonine	n/a	n/a	n/a	n/a
BIOLYS 50%	n/a	n/a	n/a	n/a
Swine TM Prmx (KSU)	n/a	n/a	n/a	n/a
Swine VTM Prmx	n/a	n/a	n/a	n/a
Poultry Fat	1	1	1	1
	Lactation (% of diet)			
Feed Ingredients	**Control**	**YC**	**Oat**	**YC + Oat**
11102 NCDA Corn (Rolled)	n/a	n/a	n/a	n/a
Diamond V Mills YC	n/a	0.5	n/a	0.5
Ground Whole Oat 15%	n/a	n/a	15	15
Soybean Meal	17.6	17.5	10.85	10.8
Corn NCDA	73	72.6	64.8	64.5
Corn (1/8) Micro-Flush	1	1	1	1
Limstone Fine	1.1	1.07	1.07	1.1
MON-CAL 21% P	2.4	2.37	2.38	2.37
Salt	0.5	0.5	0.5	0.5
Swine TM PX (KSU)	n/a	n/a	n/a	n/a
Swine Sow-Pig VIT	n/a	n/a	n/a	n/a
Threonine	0.01	0.009	0.009	0.009
BIOLYS 50%	0.25	0.25	0.25	0.25
Swine TM Prmx (KSU)	0.15	0.15	0.15	0.15
Swine VTM Prmx	0.04	9.7	0.04	0.4
Poultry Fat	4	4	4	4

3.3 Weaning

At time of weaning, 80 piglets (18-21 days on average) were selected by determining their litter group average weight and choosing the 20 piglets closest to that median number. The chosen piglets were moved into nursery pens (5 piglets per pen) based on the feed that their mother received. For both trials there were a total of 16 pens (one pen for each of the four diet groups) (Figure 1). Pens were divided between two nursery rooms with eight pens per room. Piglet placement into feed-specific pens was random.

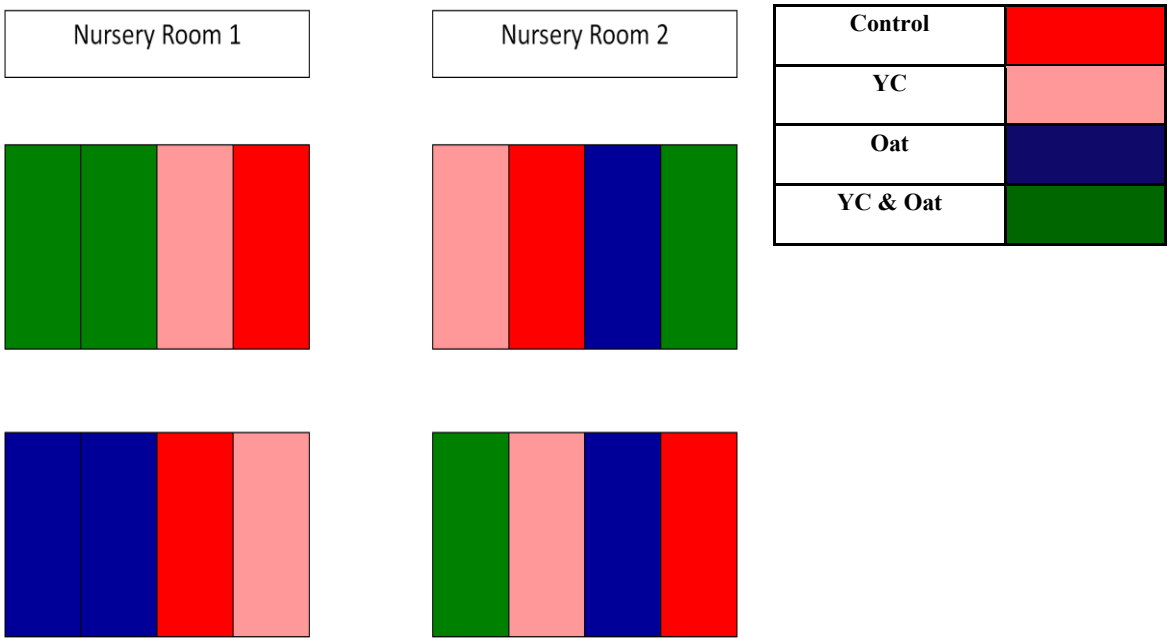


Figure 1. Diagram of Nursery Pen Layout. There were four pens per diet groups (16 pens total). Pens were divided between two nursery rooms with 8 pens per room. The diagram displays the location of each pen.

3.4 Feed Intake

Every day for the first week after weaning, half a pound of feed/ per piglet was added to feeders in each of the 16 pens. During the second week, daily feed amounts per pen were increased to one pound per piglet. To calculate the average feed intake for the week, the amount

of feed left (orts) in the feeders in each pen was vacuumed out and weighed. The ort weights were then subtracted from the total amount of feed offered during the week.

3.5 Weight Measurements

Weights of each individual piglets were taken at birth, day of weaning, and days 7 and 14 post-weaning by securely tying the rear hocks of the piglets with a rope and suspending the piglet in the air with a hand scale (Figure 2). From birth through weaning and post weaning 20 piglets per diet were monitored for weight gain.

Figure 2. Picture of Assistants Weighing the Piglets.

3.6 Fecal Scoring

Fecal samples from a total of 160 weaned piglets in the two experimental trials (80 piglets per trial) (averaging 21 days old) were scored for diarrhea. Five participants, not associated with the study were recruited to visually score the feces using pictures and scoring criteria. 1) firm fecal pellets 2) normal pellets 3) soft pellets 4) soft no pellets but not runny 5) soft and runny fecal. For pictures and scoring criterion see Figure 3. The observations were recorded on day 0, 1, 7 and 14 post-weaning, and were made in a blinded fashion whereby the

observers did not know the diet group associated with the pen or the piglets. The average scores were calculated and graphed.

Table 2

Fecal Scoring Rubric

Fecal	Score	Description	Abnormal/ Normal/ Diarrhea
	1	Firm fecal pellets	Abnormal
	2	Normal pellets	Normal
	3	Soft pellets	Normal
	4	soft no pellets but not runny	Diarrhea
	5	Soft and runny fecal	Diarrhea

3.7 Fecal Collection, Plating and Colony Counting Determination

Fecal samples were collected from 96 of the total 160 piglets directly from the large intestine (rectum) using cotton swabs on day 0, 4, 7 and 14 post-weaning. Fecal samples were also collected from all 16 of the sows in trial 2, (rectally) during day 84 of gestation (before giving sows gestation diet), on farrowing day, and on weaning day. For bacterial plating, fecal

samples were diluted 1 gram/10 microliters of peptone water into a stomacher filter bag. Next the samples were homogenized by placing the bag into a Stomacher 3500 machine (150 RPM) from Seward Limited (Long Island, NY, USA) for one minute. One milliliter of the homogenate was pipeted into eppendorf tubes and plated on five separate enrichment agars; (Sorbitol MacConkey agar for *Escherichia coli*, Baird–Parker agar for *Staphylococcus aureus*, M.R.S agar for Lactobacillus, XLD agar for Salmonella sp. and Reinforced Clostridial agar for bifidobacterium sp. Bacteria were plated using an auto spiral plate machine (Auto plate 4000) from Spiral Biotech (Norwood, MA, USA) and direct plating was also used for confirmation. Plates were left right side up until dried. For colony counting, plates were placed upside-down into the Isotemp Standard Lab Incubator from Fisher Scientific (Pittsburg, PA, USA) for 16 hours in 37 degrees Celsius. Bacteria colonies were counted using the Color Colony Counter then formulated into colony formulating unit per gram (CFU/g).

3.8 DNA Extraction

Deoxyribonucleic acid extraction was performed using the boil DNA extraction method. Presumptive positive bacterial colonies were transferred with an inoculation loop to test tubes with Tryptic Soy Broth and grown overnight in an incubator shaker at 35 degrees Celsius. One milliliter of bacteria culture was then dispensed into 1.5 milliter eppendorf tube and centrifuged for 5 minutes. The supernatant was then removed and the pellet was reconstituted with 200 microliters of sterile double filtered deionized water. The pellet was broken up by pipeting it up and down. The supernatant was boiled for 10 minutes before placing it into -80 degrees freezer for 30 minutes. Once the supernatant thawed the tubes were centrifuged for 5 minutes. Lastly the supernatant was stored in -20 degrees Celsius until ready to use in PCR analysis.

3.9 PCR

In the laboratory, polymerase chain reaction was conducted using DNA extracted from each presumptive positive sample. PCR was performed using the BioRad cycler from BioRad (Hercules, CA, USA) according to the Promega usage information form for a 50 microliters reaction volume, with a master mix consisting of 25 microliters of GoTaq Green, one microliter each of Stx-1F (forward), Stx-1R (reverse), Stx-2F (forward Stx-2R (reverse) from Integrated DNA Technologies (Coralville, Iowa) Table 3, and 20 microliters of nuclease-free water. The master mix was added with one microliter of the sample DNA and then briefly mixed by vortexing.

Table 3

PCR Primer Sequences

Primer	Sequence	Amplification Size(bp)
Shiga Toxin 1 Primer Forward	5'AA TCG CCA TTC GTT GAC TAC TTCT 3'	370bp
Shiga Toxin 1 Primer Reverse	3'GCC ATT CTG GCA ACT CGC GAT GCA 5'	
Shiga Toxin 2 Primer Forward	5'CAG TCG TCA CTC AACT GGT TTC ATCA3'	283bp
Shiga Toxin 2 Primer Reverse	3'GGA TAT TCT CCC CAC TCT GAC ACC5'	

3.10 Gel Electrophoresis

Gel electrophoresis was completed on the porcine fecal bacteria samples to detect shiga toxin 1 and 2. The BioRad sub-cell model 96 was used to conduct this procedure. A 2% gel was formulated from two grams of molecular grade certified agar and 200 milliliters of

Tris/Borate/EDTA. The liquid was then microwave for 90 seconds until the substance was clear and boiling. Next the liquid was poured into the electrophoresis apparatus and gel comb was placed inside until the box was half way full. After the liquid solidified into a gel, 7.5 microliters of ladder/dye mix, 7.5 microliters of positive *E.coli* O157:H7 control, 7.5 microliters of DNA samples, and 7.5 microliters of the negative control (without *E.coli* DNA) were pipetted into the wells. The gels ran at 60 volts for 40 minutes. The gel was then extracted and placed into ethidium bromide for 20 minutes. Lastly the gel was imaged and read by the Bio Rad ChemiDoc MP imaging system from Bio Rad (Hercules CA).

3.11 Statistical Analysis

Statistical analysis was completed on the fecal score population and bacteria counts using GraphPad Prism. In GraphPad Prism, a one-way ANOVA with a Bonferroni post-test was completed on the samples.

CHAPTER 4

Results

4.1 Weight Gain of Piglets

Piglet weight gains were taken on day of farrowing, day of weaning, day 1, 4, 7, and 14 of post weaning. Throughout this time 20 piglets per diet were monitored for weight gain. Piglets whose dams were feed a CON+O diet were heaviest on day of birth (Figure 3). There was no significant difference between the piglets average weight gain during suckling period (Figure 4). Piglets weaned from sows that were fed CON+O lost significantly less weight then piglets weaned from sows that were fed CON+YC and CON+YC+O throughout the weaning process (Figure 5). Piglets weaned from sows that were fed CON+YC lost the most weight throughout the weaning process (Figure 6). Together our data show that the piglets whose mothers consumed oats in their diet weighed the most at birth and lost the least amount of weight during the post-weaning period, suggesting that feeding sows a diet containing oat may be better for sustaining growth of her piglets.

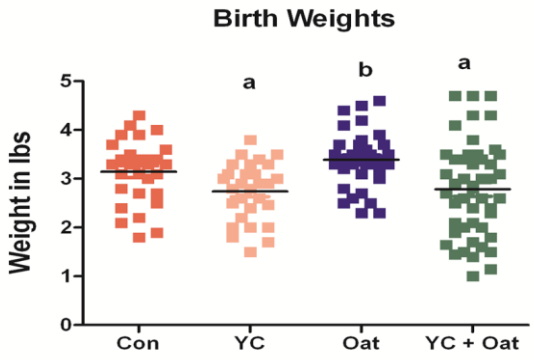


Figure 3. Birth Weights of Piglets. Average birth weights of piglets were each square represents an animal. The bars indicate the mean weight (n=29 for CON and YC, n= 35 for Oat and n = 47 for YC + Oat) Significant differences ($p<0.05$) using 1 way ANOVA were found between *a* and *b*.

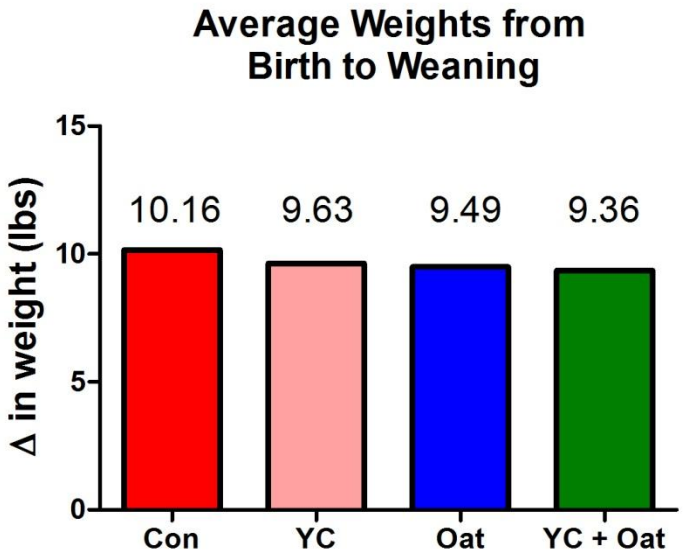


Figure 4. Average Weights from Birth to Weaning. The change in weights from birth to weaning (avg. weight at weaning – avg. weight at birth).

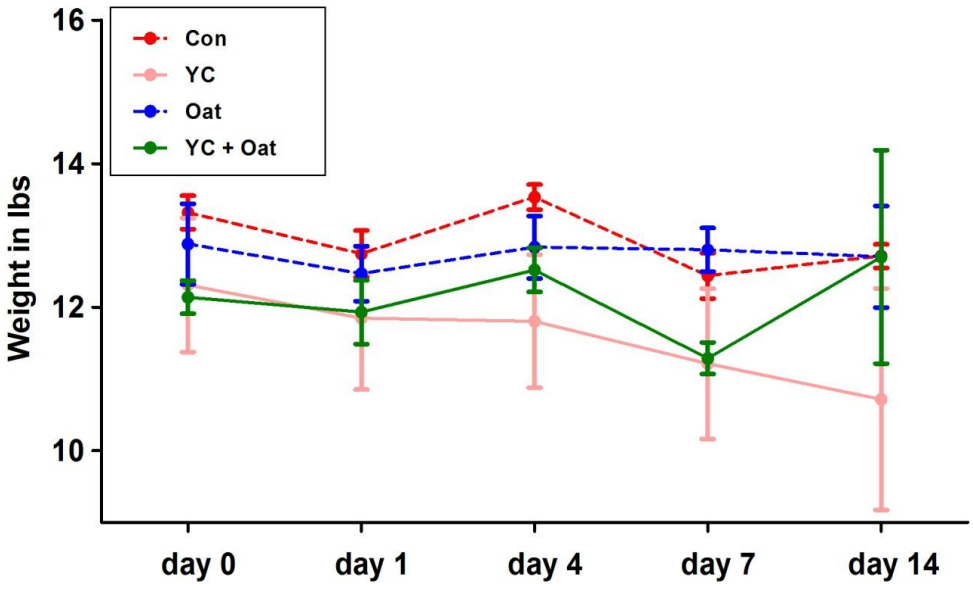


Figure 5. Average Weights Post Weaning. Lines with different patters (dashed vs. solid) indicate significant differences except in the case of CON+O vs YC+O, which was not significantly different as determined by repeated measures ANOVA with Bonferroni post-test.

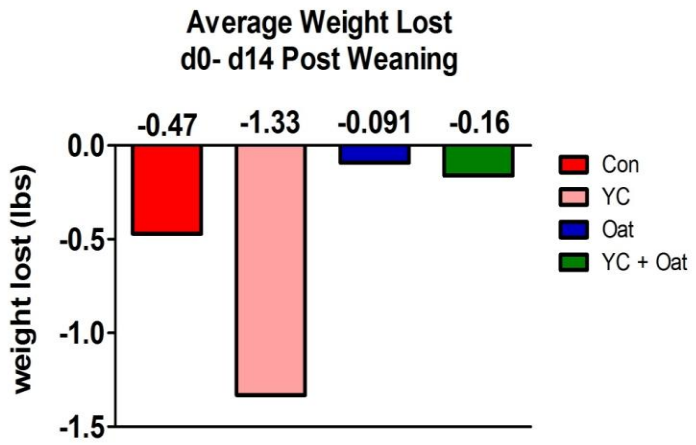
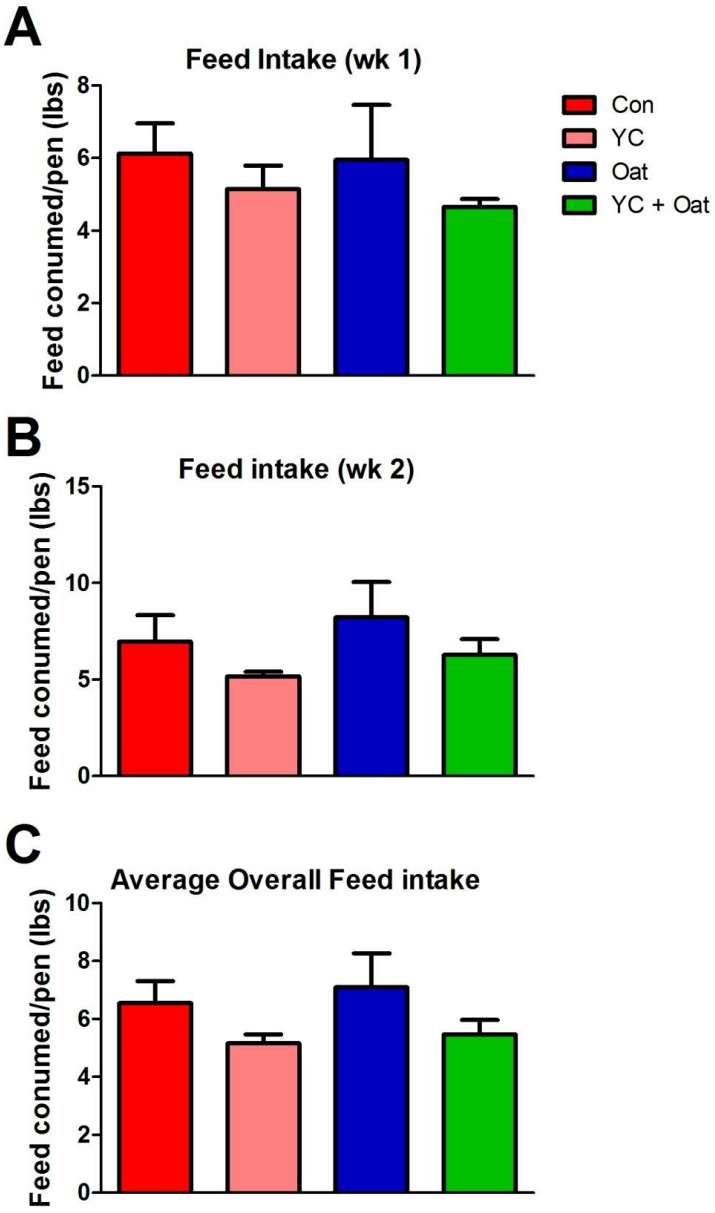


Figure 6. Average Weight Lost Post Weaning. Average weight lost from day 0 post weaning through day 14 post weaning.

4.2 Feed Intake of Piglets

Piglet feed intake was monitored from birth until 14 days post weaning (Figure 7). Piglets were fed a nursery ration formulated to meet all nutritional requirements. All animals had free access to feed and water at all times. Every day for the first week post weaning, half a pound of feed/ per piglet was added to feeders in each of the 16 pens. During the second week, daily feed amounts per pen were increased to one pound per piglet. To calculate the average feed intake for the week, the amount of feed left (orts) in the feeders in each pen was vacuumed out and weighed. The ort weights were then subtracted from the total amount of feed offered during the week. The total feed consumed in week 1 and 2 post weaning showed no significant difference between all the piglets. This data suggest that feeding a CON+YC, CON+O or CON+YC+O diet to gestational sows 30 days prior to farrowing may not affect feed intake.



Figures 7. Average Feed Intake. The measurement of total feed consumed by animal was recorded on day 7 and 14 post weaning. The data are on an average of four pens per diet group (**A**) and (**B**) and eight pens per diet group (**C**). Statistical analysis was completed using ANOVA with Bonferroni post-test multiple comparisons.

4.3 Fecal Scoring

Throughout the fourteen day trials, the fecal scores of piglets born of sows on oat supplemented diet did not suffer from severe diarrhea, averaging a score of three (Figure 8). On day seven fecal scores of piglets born of sows on oat supplemented diet were significantly less than those born of sows that consumed control and yeast + oat diets. This observation suggests that feeding sows a diet consisting of 15% oat may prevent post wean diarrhea in their offspring during weaning.

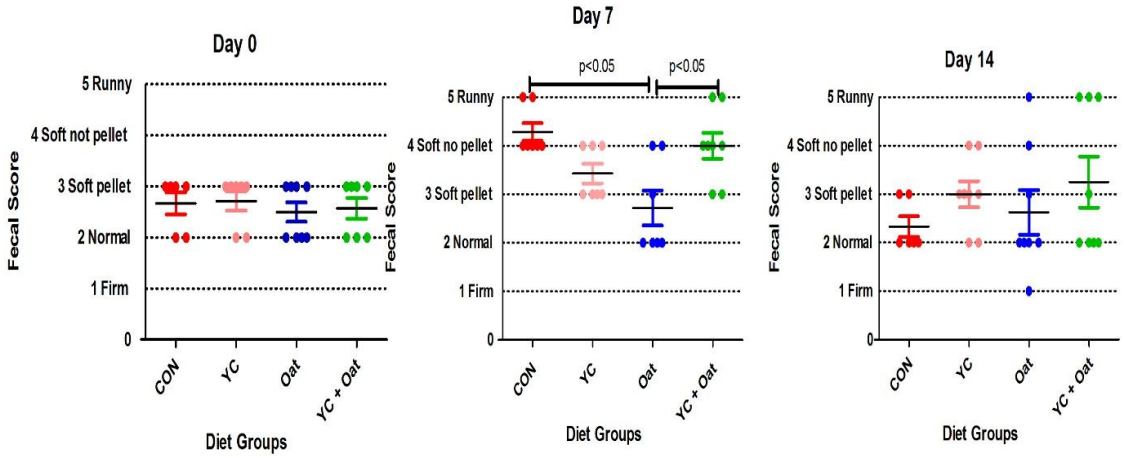


Figure 8. Fecal Score. The fecal scores were observed by 5 recruited participants who were given a fecal scoring rubric on day 0, 7 and 14. Scoring was done in a blind fashion. The participants did not know the origin of the piglets or the diets associated with the pens. Data is on an average of n=7. A one way ANOVA was used to determine the significance. The bar line represents the means of significant differences.

4.4 Bacteria Population of Offspring

Fecal samples were plated to evaluate the population of *Staphylococcus arueus*, Salmonella, *Escherichia coli*, Lactobacillus, and Bifidobacterium in the small intestines of the piglets. Three piglets from each diet group was sacrificed and fecal swabs were collected on

weaning day 0, day 4, day 7, and day 14. There was no noticeable or significant difference between the bacteria population of the piglets. For graphs see appendix B. This suggest that feeding sows a supplemental diet consisting of CON+O, CON+YC or CON+YC+O diet has no effect on bacteria population of their piglets.

4.5 *Escherichia coli* Population of Sows

Fecal samples were also collected from all 16 of the sows on day 84 of gestation, farrowing day, and weaning day. Samples were collected from colon of the sows. There was no significant difference ($p < 0.05$) in *E.coli* population between any of the sow diet groups during gestation day 84 (Figure 9A). Sows that were fed a synbiotic diet, had significantly less ($p<0.01$)($p<0.05$) *E.coli* than all other diet groups during farrowing (Figure 9B) and had significantly less ($p < 0.05$) *E.coli* than sows that were fed CON+YC and CON diet during weaning day (Figure 9C). There was no significant difference in *E.coli* population between gestation day 84, farrowing day and weaning day of sows that were fed the synbiotic diet (Figure 9D) but there was a trend towards a decrease after gestation day 84 up until farrowing day. This suggest that feeding sows a diet consisting of 5g/kg Diamond V Mills Yeast Culture and 15% oat as a synbiotic 30 days before farrowing may affect *E.coli* colonization in the gut.

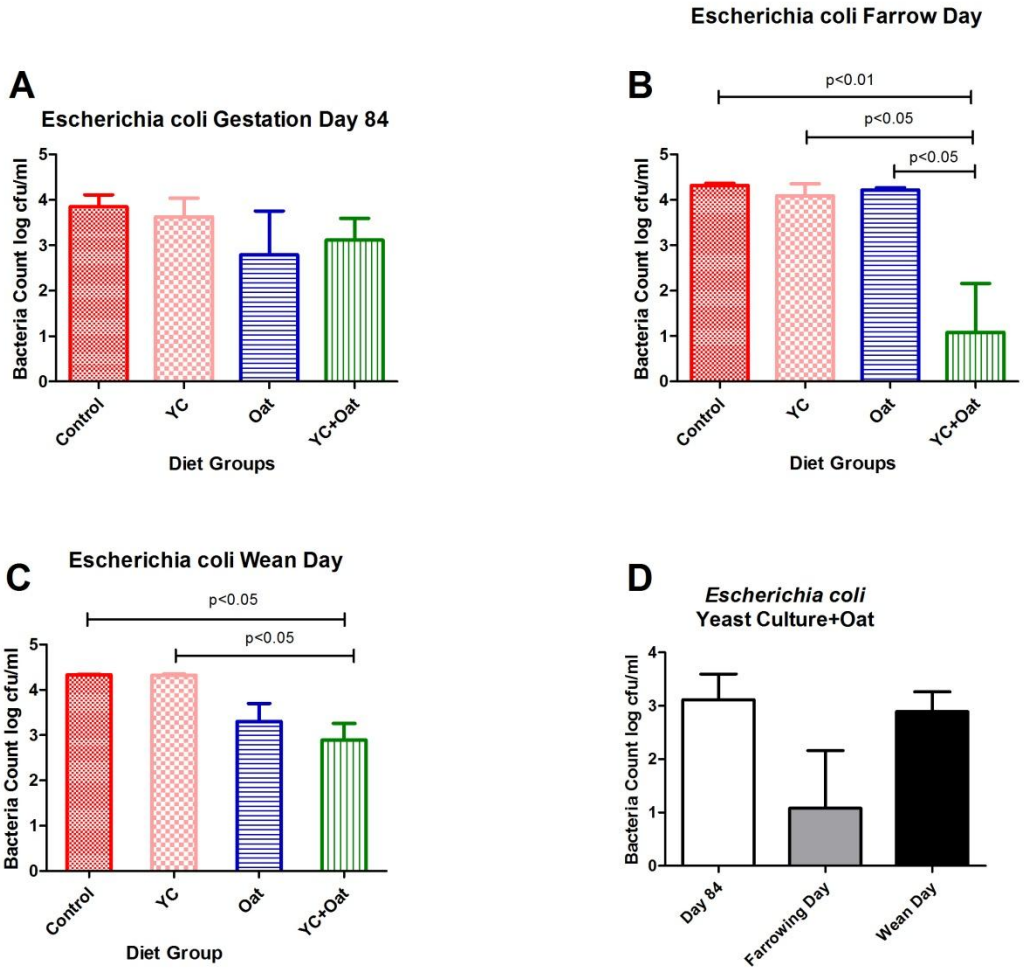


Figure 9. E.coli Population in Sows During Gestation to Weaning Day.

4.6 *Staphylococcus aureus* Population of Sows

There was no significant difference ($p < 0.05$) in *Staphylococcus aureus* population from gestation day 84 through weaning day of sows that were fed the different diets (Figure 10A-C). There was a trend towards a decrease throughout the period. On weaning day there was no *Staphylococcus aureus* colonies detected in sows whom consumed the CON+O diet (Figure C). This data proposes that feeding sows a diet consisting of CON+O while gestating may potentially eliminate *Staphylococcus aureus* in the digestive track of sows.

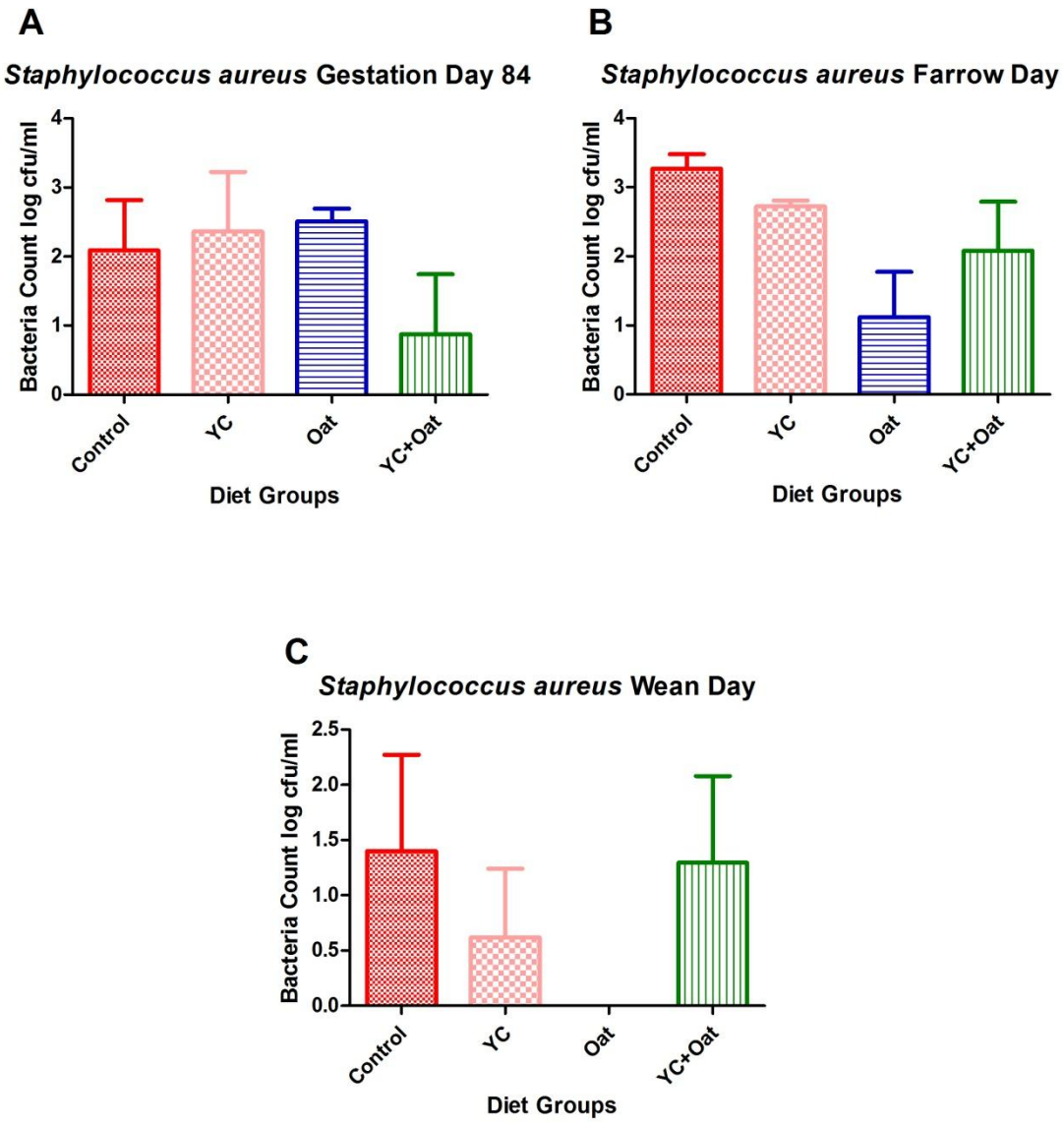


Figure 10.Staphylococcus aureus Population in Sows During Gestation to Weaning Day.

4.7 *Escherichia coli* Shiga Toxin Genotyping

Ninety six piglets and 48 sows *Escherichia coli* bacteria samples were isolated and cultured for detection of shiga toxin genes. Polymerase chain reaction and gel electrophoresis technique was used to identify *Escherichia coli* shiga toxin. Qualitative analysis was used to detect gene specific *E.coli.* Table 4 shows that in the case of sows, all of the diet groups except control had a fewer colonies of toxigenic *E.coli present.* This suggests that feeding sows a diet

consisting of either 15% oat, 0.5% Diamond V. Mills Yeast Culture alone or in combination may prevent high population of shiga toxin producing *Escherichia coli* in the gut (Table 4).

Table 4

E.coli Detection in Sows

	Detection of *E.coli* O157:H7 (Sows)							
Diet Group	Day	Sows fecal was swabbed	Successfully Isolated *E.coli* Sample	(+) Shiga Toxin 1	(+) Shiga Toxin 2	(+) Both	Total (+)	% Shiga Toxin Isolated
Control	Gest 84	4	4	0	1	3	4	100
	Farrow	4	4	0	0	2	2	50
	Weaning	4	4	3	0	0	3	75
YC	Gest 84	4	4	0	1	3	4	100
	Farrow	4	4	0	2	1	3	75
	Weaning	4	4	0	0	1	1	25
Oat	Gest 84	4	4	0	2	1	3	75
	Farrow	4	4	0	1	3	4	100
	Weaning	4	4	1	0	0	1	25
YO	Gest 84	4	**4**	1	1	1	3	75
	Farrow	4	1	0	1	0	1	100
	Weaning	4	2	0	0	0	0	0
Total		48	43	29				67.4

In the case of piglets although we did not detect shiga toxin positive *E.coli* in the feces of piglets early after weaning (day 0), as time progressed positive toxigenic *E.coli* isolates were detected throughout all of the diet groups. As table 5 shows nearly all of the isolates of shiga toxin positive *E.coli* were detected at day 14 post weaning (Table 5). This suggests that the piglets acquired the *E.coli* from the environment. (For gels images see appendix C.)

Table 5

E.coli Detection in Piglets

	Detection of *E.Coli* 0157:H7 (Piglets)							
Diet Group	Day	Piglets fecal was swabbed	Successfully Isolated *E.coli* Sample	(+) Shiga Toxin 1	(+) Shiga Toxin 2	(+) Both	Total (+) Shiga Toxin	% Shiga Toxin Isolated
Control	Day 0	6	6	0	0	0	0	0
	Day 4	6	5	0	0	0	0	0
	Day 7	6	5	0	0	0	0	0
	Day 14	6	6	0	2	0	2	33.3
YC	Day 0	6	6	0	0	1	1	16.7
	Day 4	6	5	0	0	0	0	0
	Day 7	6	6	0	0	0	0	0
	Day 14	6	6	0	0	2	2	33.3
Oat	Day 0	6	5	0	0	0	0	0
	Day 4	6	4	0	0	0	0	0
	Day 7	6	4	0	0	0	0	0
	Day 14	6	6	0	2	2	4	66.7
YC+Oat	Day 0	6	5	0	0	0	0	0
	Day 4	6	5	0	0	0	0	0
	Day 7	6	6	0	0	1	1	16.7
	Day 14	6	5	0	2	0	2	40
Total	Total	96	85	12				14.1

CHAPTER 5

Discussion and Future Directions

Our study demonstrated that, feeding sows feed supplemented with either 15% oat, or 5g/kg Diamond V. Mills Yeast Culture or together as a synbiotic diet from gestation until farrowing day may lower the *Escherichia coli* and *Staphylococcus aureus* population overall and prevent high population of shiga toxin producing *Escherichia coli* in the gut. It also finds that offspring of the mothers that consume 15% oat supplemented feed do not suffer from PWD and PWD associated weight loss to the same degree as those born of sows that consumed non-supplemented feed or feed supplemented with YC.

5.1 Impact on Mother's Diet on Offspring Health

There are several studies that focus on the impact of a mother's diet on the health of her offspring but there are few studies that emphasize feed additives (probiotic or a prebiotic) as a substitute to using antibiotic growth promoters in those diets. More specifically, the few studies that focuses on the impact of a mother's diet on the health of her offspring by way of probiotic and/or prebiotic such as Alexopoulos et al. (2004) feed the sow and the offspring the probiotic diet. The study that was conducted feeds only the sow the feed additive diet. It was hypothesized that feeding oat (prebiotic), yeast culture (probiotic) or oat+ yeast (synbiotic) to gestational and lactation sows would improve their offspring gut health and potentially overall post weaning performance.

5.2 Feed Intake/Weight Gain

In a study done by Shen, Piao et al. (2009), piglets that were feed Diamon V Mills YC (5g/kg) had higher average daily weight gain then piglets that were feed 2.5 g/kg, 10 g/kg and 20g/kg ($P<0.05$). The 5g/kg YC ration also proved to increase jejunal villus height and crypt

depth ratio. This was the reason our study used a 5g/kg YC ratio. Even though, in the study piglets weaned from sows that were fed CON+YC (5g/kg) lost the most weight throughout the weaning process on a feed consumption that showed no significant difference throughout the four diets; our study was consistent with others (Kornegay, Rhein-Welker, Lindemann, & Wood, 1995) stating that piglets weaned from sows that were fed CON+YC (5g/kg) would have lower overall average daily gain then other diets.

5.3 Whole Grain Oat (15%)

Throughout the study, we examined that feeding sows a diet consist of 15% whole grain oat had a positive influence on the sows health and the overall health of the offspring. The piglets whose mothers consumed oats in their diet weighed the most at birth, lost the least amount of weight and had less incident of diarrhea throughout the post-weaning period. In this study we also observed that feeding sows a diet consisting of 15% whole grain oat after 84 days of gestation may potentially eliminate *staphylococcus aureus* in the digestive track of sows.

5.4 Synbiotic

To our knowledge there have not been any studies that focus on the combination of Oat and Yeast Culture as a synbiotic additive in a sow's diet in hopes to positively affect their offspring gut health. Therefore, this section of the research was not able to compare to other studies. As a result, findings on the positive impact of a synbiotic diet (15% oat and 5g/kg Yeast Culture) on sows and their offspring health will need to be further researched to assure reliable results. Even though our study didn't show significant difference in feeding a synbiotic diet to sows in hopes to affect the offspring health in comparison to any other diet in the experiment; the diet did positively affect the sows gut health by dramatically decreasing *E.coli* bacteria population from gestation to farrowing day.

5.5 Future Directions

In this study we collected weight data at time of birth. Our data showed variance in piglet weights between individual piglets within the same diet groups as well as variance between piglets in different diet groups. Although our data suggest that diet supplementation with either oat or YC contributed to the increase in birth weight other factors could be at play. For example, in our study the sows had different parities, and genetic breed backgrounds. In addition, we did not account for litter sizes nor did we measure feed intake or weight gain of the sows. Therefore, future studies should monitor for sows weight gain and feed intake, keeping sow breed and parity consistent as well as take in account the size of their litters.

In addition our fecal scoring data showed some variance in scores of piglets within the same diet groups. One variable that may have contributed to this could have been that we conducted these trials during the summer months of June and July when there were high temperatures in the unit. In instances of high tempters pigs will consume less feed and more water. The large consumption of water could have played a part in the fluctuation in fecal score variance. It would be interesting to repeat these studies in different seasons of the year where temperatures are not as hot.

Throughout the project pathogenic and beneficial bacteria were tested. Even though *Staphylococcus aureus* was plated and quantitatively tested, it was not tested for the presence of MRSA. Methicillin-resistant *Staphylococcus aureus* is a large spread issue in human medicine and is among the most significant infections in hospitalized individuals (Weese & van Duijkeren, 2010). In further studies it would be interesting to also test for the presence of MRSA.

While plating for *Escherichia coli*, we only tested for one specific shiga-like toxin-producing *Escherichia coli* which was O157: H7, but there have been other reports of isolated

serotypes that produce severe diarrhea in piglets as well as several foodborne outbreaks of hemorrhagic colitis in humans. These isolated serogroups include O1, O2, O4, O5, O6, O22, O23, O26, O38, O45, O48, O50, O55, O73, O75, O91, O100, O103, O104, O105, O111, O113, O114, O115, O117, O118, O119, O121, O125, O126, O128ab, O132, O145, O153, O163, O165, and O166 (Samadpour et al., 1994). In this case it would be necessary to use the denaturing gradient gel electrophoresis (DGGE) method to identify and screen for a population of pathogenic *Escherichia coli.*

For shiga toxin population detection polymerase chain reaction was conducted to replicate the DNA *E.coli* bacteria samples and gel electrophoresis was conducted to determine the presence of enterotoxigenic *Escherichia coli*. The bands of some samples showed a more prominent presence of the *E. coli* strain while other bands were not as prominent making the detection of some of the specific DNA reading inadequate. This may have been caused by the variation of DNA castration in the samples. In further studies, it will be helpful to use a NanoDrop and a DNA concentration formula to conduct a more accurate band detection reading.

CHAPTER 6

Conclusion

Post-weaning diarrhea caused by *Escherichia coli* is a widespread problem in the pork industry. This diarrhea is accountable for economic losses due to mortality, morbidity, decreased growth rate, and cost of medication (Fairbrother, Nadeau et al. 2005). The disease occurs shortly after weaning and is distinguished by watery diarrhea, dehydration, loss of body weight and sometimes death of infected pigs (Pluske, Hampson, & Williams, 1997). Shiga toxin producing *Escherichia coli*, such as O157 H7, are zoonotic pathogens that cause fatal and frequently epidemic food or waterborne illness (Karmali, 2004). The current practice of industries in America is to use antibiotic growth promoters in sub-therapeutic doses to feed piglets to counter act the post wean diarrhea disease, although there has been increasing concern regarding the transmission of resistant bacteria which has led to a reassessment of the use of antibacterial agents in animal feeds (Casewell et al., 2003).

The ultimate goal of this project was to investigate whether the addition of alternative dietary supplements into the gestational and lactation diets will positively affect the health of weaned pigs by promoting growth and gut health. Although this study suggests that including oats into the gestational and lactation diets of sows may positively affect the health of weaned pigs by promoting growth and decreasing post weaning diarrhea, it is recommended that additional work is completed before concluding that the diet does affect the immunity of the piglets and is a good substitute to using antibiotics.

References

Alexopoulos, C., Georgoulakis, I., Tzivara, A., Kritas, S., Siochu, A., & Kyriakis, S. (2004). Field evaluation of the efficacy of a probiotic containing Bacillus licheniformis and Bacillus subtilis spores, on the health status and performance of sows and their litters. *Journal of animal physiology and animal nutrition, 88*(11-12), 381-392.

Atanassova, V., Meindl, A., & Ring, C. (2001). Prevalence of *Staphylococcus aureus* and staphylococcal enterotoxins in raw pork and uncooked smoked ham—a comparison of classical culturing detection and RFLP-PCR. *International Journal of Food Microbiology, 68*(1), 105-113.

Biagi, G., Piva, A., Moschini, M., Vezzali, E., & Roth, F. (2007). Performance, intestinal microflora, and wall morphology of weanling pigs fed sodium butyrate. *Journal of animal science, 85*(5), 1184-1191.

Callaway, T., Edrington, T., Anderson, R., Harvey, R., Genovese, K., Kennedy, C., . . . Nisbet, D. (2008). Probiotics, prebiotics and competitive exclusion for prophylaxis against bacterial disease. *Animal Health Research Reviews, 9*(02), 217-225.

Casewell, M., Friis, C., Marco, E., McMullin, P., & Phillips, I. (2003). The European ban on growth-promoting antibiotics and emerging consequences for human and animal health. *Journal of Antimicrobial Chemotherapy, 52*(2), 159-161.

Castanon, J. (2007). History of the use of antibiotic as growth promoters in European poultry feeds. *Poultry science, 86*(11), 2466-2471.

Charles, D. (2012). FDA Launches voluntary plan to reduce use of antibiotics in animals, from http://www.npr.org/blogs/thesalt/2012/04/11/150432234/fda-launches-voluntary-plan-to-reduce-use-of-antibiotics-in-animals

Cilieborg, M. S., Boye, M., & Sangild, P. T. (2012). Bacterial colonization and gut development in preterm neonates. *Early human development, 88*, S41-S49.

Close, W. H. (2000). Producing pigs without antibiotic growth promoters. *Advances in pork production, 11*, 47-56.

Collins, J. W., La Ragione, R. M., Woodward, M. J., & Searle, L. E. (2009). Application of prebiotics and probiotics in livestock *Prebiotics and probiotics science and technology* (pp. 1123-1192): Springer.

Collins, M. D., & Gibson, G. R. (1999). Probiotics, prebiotics, and synbiotics: approaches for modulating the microbial ecology of the gut. *The American Journal of Clinical Nutrition, 69*(5), 1052s-1057s.

Connolly, M. L., Lovegrove, J. A., & Tuohy, K. M. (2010). *In vitro* evaluation of the microbiota modulation abilities of different sized whole oat grain flakes. *Anaerobe, 16*(5), 483-488.

Costabile, A., Klinder, A., Fava, F., Napolitano, A., Fogliano, V., Leonard, C., . . . Tuohy, K. M. (2008). Whole-grain wheat breakfast cereal has a prebiotic effect on the human gut microbiota: a double-blind, placebo-controlled, crossover study. *British Journal of Nutrition, 99*(01), 110-120.

De Lange, C., Pluske, J., Gong, J., & Nyachoti, C. (2010). Strategic use of feed ingredients and feed additives to stimulate gut health and development in young pigs. *Livestock Science, 134*(1), 124-134.

Dudink, S., Simonse, H., Marks, I., de Jonge, F. H., & Spruijt, B. M. (2006). Announcing the arrival of enrichment increases play behaviour and reduces weaning-stress-induced behaviours of piglets directly after weaning. *Applied Animal Behaviour Science, 101*(1), 86-101.

Fairbrother, J. M., Nadeau, É., & Gyles, C. L. (2005). Escherichia coli in postweaning diarrhea in pigs: an update on bacterial types, pathogenesis, and prevention strategies. *Animal Health Research Reviews, 6*(01), 17-39. doi: doi:10.1079/AHR2005105

Fraser, D. (1978). Observations on the behavioural development of suckling and early-weaned piglets during the first six weeks after birth. *Animal Behaviour, 26*, 22-30.

Funderburke, D., & Seerley, R. (1990). The effects of postweaning stressors on pig weight change, blood, liver and digestive tract characteristics. *Journal of animal science, 68*(1), 155-162.

Gaggìa, F., Mattarelli, P., & Biavati, B. (2010). Probiotics and prebiotics in animal feeding for safe food production. *International Journal of Food Microbiology, 141*, S15-S28.

Gosbell, I. B. (2004). Methicillin-Resistant Staphylococcus Aureus: Impact on Dermatology Practice. [Article]. *American Journal of Clinical Dermatology, 5*(4), 239-259.

Honeyman, M. (1996). Sustainability issues of US swine production. *Journal of animal science, 74*(6), 1410-1417.

Huang, J. S., Bousvaros, A., Lee, J. W., Diaz, A., & Davidson, E. J. (2002). Efficacy of probiotic use in acute diarrhea in children: a meta-analysis. *Digestive Diseases and Sciences, 47*(11), 2625-2634.

Hurt, C. (1994). Industrialization in the pork industry. *Choices, 9*(4), 9-13.

Jurgens, M., Rikabi, R., & Zimmerman, D. (1997). The effect of dietary active dry yeast supplement on performance of sows during gestation-lactation and their pigs. *Journal of animal science, 75*(3), 593-597.

Karmali, M. A. (2004). Prospects for Preventing Serious Systemic Toxemic Complications of Shiga Toxin–Producing Escherichia coli Infections Using Shiga Toxin Receptor Analogues. *Journal of Infectious Diseases, 189*(3), 355-359.

Katouli, M., Melin, L., Jensen-Waern, M., Wallgren, P., & Möllby, R. (1999). The effect of zinc oxide supplementation on the stability of the intestinal flora with special reference to composition of coliforms in weaned pigs. *Journal of applied microbiology, 87*(4), 564-573.

Kim, S. W., Brandherm, M., Freeland, M., Newton, B., Cook, D., & Yoon, I. (2008). Effects of yeast culture supplementation to gestation and lactation diets on growth of nursing piglets. *Asian-Australasian J Anim Sci, 21*, 1011-1014.

Kornegay, E., Rhein-Welker, D., Lindemann, M., & Wood, C. (1995). Performance and nutrient digestibility in weanling pigs as influenced by yeast culture additions to starter diets containing dried whey or one of two fiber sources. *Journal of animal science, 73*(5), 1381-1389.

Lalles, J.-P., Bosi, P., Smidt, H., & Stokes, C. R. (2007). Nutritional management of gut health in pigs around weaning. *Proceedings of the Nutrition Society, 66*(02), 260-268.

Lallès, J.-P., Boudry, G., Favier, C., Le Floc'h, N., Luron, I., Montagne, L., . . . Sève, B. (2004). Gut function and dysfunction in young pigs: physiology. *Animal Research, 53*(4), 301-316.

Le Huërou-Luron, I., Blat, S., & Boudry, G. (2010). Breast-v. formula-feeding: impacts on the digestive tract and immediate and long-term health effects. *Nutrition research reviews, 23*(01), 23-36.

Lindemann, M., Meade, R., Cornelius, S., Hanke, H., Miller, K., & Rust, J. (1983). Evaluation of high protein oats as a replacement for corn in diets fed to growing-finishing swine. *Journal of animal science, 56*(4), 853-857.

Madec, F., Bridoux, N., Bounaix, S., & Jestin, A. (1998). Measurement of digestive disorders in the piglet at weaning and related risk factors. *Preventive veterinary medicine, 35*(1), 53-72.

Manzanilla, E., Nofrarias, M., Anguita, M., Castillo, M., Perez, J., Martin-Orue, S., . . . Gasa, J. (2006). Effects of butyrate, avilamycin, and a plant extract combination on the intestinal equilibrium of early-weaned pigs. *Journal of animal science, 84*(10), 2743-2751.

Marquardt, R. R., Jin, L., Kim, J. W., Fang, L., Frohlich, A. A., & Baidoo, S. K. (1999). Passive protective effect of egg-yolk antibodies against enterotoxigenic Escherichia coli K88+ infection in neonatal and early-weaned piglets. *FEMS Immunology & Medical Microbiology, 23*(4), 283-288.

Matysiak, B., Jacyno, E., Kawęcka, M., Kotodziej-Skalska, A., & Pietruszka, A. (2012). The effect of plant extracts fed before farrowing and during lactation on sow and piglet performance. *South African Journal of Animal Science, 42*(1), 15-21.

McBride, W. D. (1997). *Change in US Livestock Production, 1969-92*: US Department of Agriculture, ERS.

Namkung, H., Li J. Gong, M., Yu, H., Cottrill, M., & de Lange, C. (2004). Impact of feeding blends of organic acids and herbal extracts on growth performance, gut microbiota and digestive function in newly weaned pigs. *Canadian journal of animal science, 84*(4), 697-704.

National Pork Producers Council. (2012). Pork Facts, from http://www.nppc.org/pork-facts/

NC Department of Agriculture and Consumer Services. (2013). Agricultural Statistics.

Novick, R. P. (1981). The development and spread of antibiotic-resistant bacteria as a consequence of feeding antibiotics to livestock. *Annals of the New York Academy of Sciences, 368*(1), 23-60.

Osek, J. (2000). Clonal analysis of Escherichia coli strains isolated from pigs with post-weaning diarrhea by pulsed-field gel electrophoresis. *FEMS microbiology letters, 186*(2), 327-331.

Osek, J. (2000). Virulence factors and genetic relatedness of *Escherichia coli* strains isolated from pigs with post-weaning diarrhea. *Veterinary microbiology, 71*(3), 211-222.

Pan, C., & Kinsey, J. (2002). *The supply chain of pork: US and China*: Food Industry Center, University of Minnesota.

Piva, A., Morlacchini, M., Casadei, G., Gatta, P. P., Biagi, G., & Prandini, A. (2009). Sodium butyrate improves growth performance of weaned piglets during the first period after weaning. *Italian Journal of Animal Science, 1*(1), 35-42.

Pluske, J. R., Hampson, D. J., & Williams, I. H. (1997). Factors influencing the structure and function of the small intestine in the weaned pig: a review. *Livestock production science, 51*(1), 215-236.

Pluske, J. R., Pethick, D. W., Hopwood, D. E., & Hampson, D. J. (2002). Nutritional influences on some major enteric bacterial diseases of pig. *Nutrition research reviews, 15*(02), 333-371.

Samadpour, M., Ongerth, J. E., Liston, J., Tran, N., Nguyen, D., Whittam, T. S., . . . Tarr, P. (1994). Occurrence of Shiga-like toxin-producing Escherichia coli in retail fresh seafood, beef, lamb, pork, and poultry from grocery stores in Seattle, Washington. *Applied and environmental microbiology, 60*(3), 1038-1040.

Shen, Y., Carroll, J., Yoon, I., Mateo, R., & Kim, S. (2011). Effects of supplementing Saccharomyces cerevisiae fermentation product in sow diets on performance of sows and nursing piglets. *Journal of animal science, 89*(8), 2462-2471.

Shim, S., Verstegen, M., Kim, I., Kwon, O., & Verdonk, J. (2005). Effects of feeding antibiotic-free creep feed supplemented with oligofructose, probiotics or synbiotics to suckling piglets increases the preweaning weight gain and composition of intestinal microbiota. *Archives of animal nutrition, 59*(6), 419-427.

Stein, H. H., & Kil, D. Y. (2006). Reduced use of antibiotic growth promoters in diets fed to weanling pigs: dietary tools, part 2. *Animal biotechnology, 17*(2), 217-231.

Tuohy, K. M., Probert, H. M., Smejkal, C. W., & Gibson, G. R. (2003). Using probiotics and prebiotics to improve gut health. *Drug Discovery Today, 8*(15), 692-700. doi: 10.1016/s1359-6446(03)02746-6

Van den Bogaard, A., London, N., Driessen, C., & Stobberingh, E. (2001). Antibiotic resistance of faecal Escherichia coli in poultry, poultry farmers and poultry slaughterers. *Journal of Antimicrobial Chemotherapy, 47*(6), 763-771.

Visek, W. (1978). The mode of growth promotion by antibiotics. *Journal of Animal Science, 46*(5), 1447-1469.

Weese, J. S., & van Duijkeren, E. (2010). Methicillin-resistant *Staphylococcus aureus* and *Staphylococcus pseudintermedius* in veterinary medicine. *Veterinary Microbiology, 140*(3), 418-429.

Wegener, H. C. (2003). Antibiotics in animal feed and their role in resistance development. *Current opinion in microbiology, 6*(5), 439-445.

Wells, J., Shipman, L., Greene, K., Sowers, E., Green, J., Cameron, D., . . . Ostroff, S. (1991). Isolation of Escherichia coli serotype O157: H7 and other Shiga-like-toxin-producing E. coli from dairy cattle. *Journal of Clinical Microbiology, 29*(5), 985-989.

Zijlstra, R. T., Whang, K.-Y., Easter, R. A., & Odle, J. (1996). Effect of feeding a milk replacer to early-weaned pigs on growth, body composition, and small intestinal morphology, compared with suckled littermates. *Journal of animal science, 74*(12), 2948-2959.

Appendix A

Protocols

Directions for making Peptone water

1. Dissolve 15 g of the medium in one liter of distilled water.

2. Mix thoroughly.

3. Autoclave at 121°C for 15 minutes.

Directions for making Sorbitol MacConkey agar for detecting E.Coli

1. Dissolve 51.5 g of the medium in one liter of distilled water
2. Mix thoroughly in a 1500ml flask
3. Use a hot plate to bring to a boil
4. put flask in hot bath for 5-10min
5. Add 4mL of Cerfixime Tellurite/1L of agar
6. Autoclave for 121°C for15min
7. Pure onto agar plate

Directions for making Difco Baird-Parker for detecting S.aureus

1. Dissolve 63 g of the medium in 950mL of distilled water
2. Mix thoroughly in a 1500ml flask
3. Use a hot plate to bring to a boil
4. put flask in hot bath for 5-10min
5. Autoclave for 121°C for15min
6. Pure onto agar plate

Directions for making M.R.S agar for detecting Lactobacillus

1. Dissolve 62 g of the medium in one liter of distilled water
2. Mix thoroughly in a 1500ml flask
3. Use a hot plate to bring to a boil
4. put flask in hot bath for 5-10min
5. Autoclave for 121°C for15min
6. Pure onto agar plate

Directions for making XLD agar for detecting Salmonella

1. Dissolve 55 g of the medium in one liter of distilled water
2. Mix thoroughly in a 1500ml flask
3. Use a hot plate to bring to a boil
4. put flask in hot bath for 5-10min
5. Autoclave for 121°C for15min
6. Pure onto agar plate

Directions for making Reinforced Clostridial agar for detecting Bifidobacterium

1. Dissolve 52.5 g of the medium in one liter of distilled water
2. Mix thoroughly in a 1500ml flask
3. Use a hot plate to bring to a boil
4. put flask in hot bath for 5-10min
5. Autoclave for 121°C for15min
6. Pure onto agar plate

Appendix B

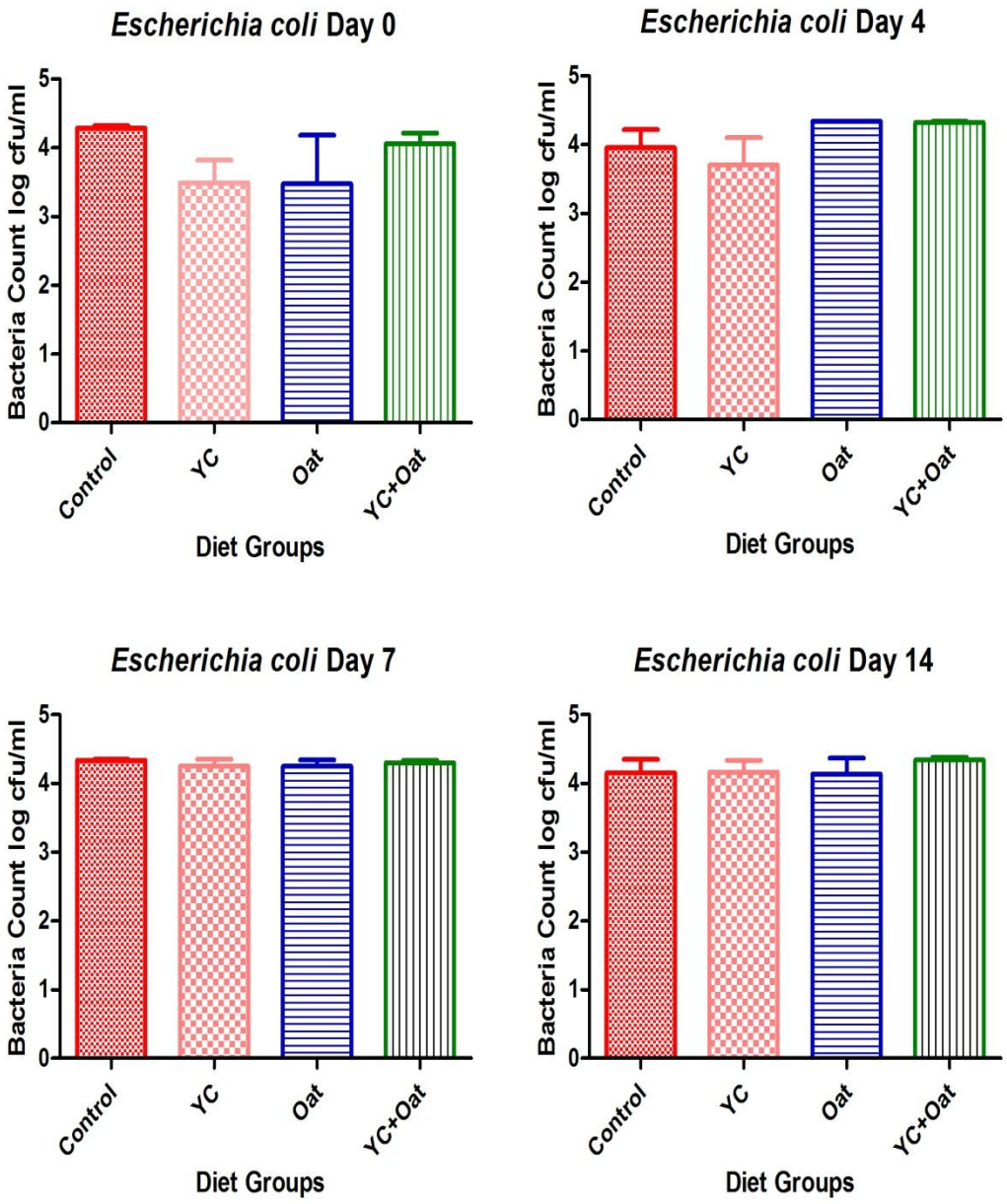


Figure 11. Piglet Escherichia coli Bacteria Population.

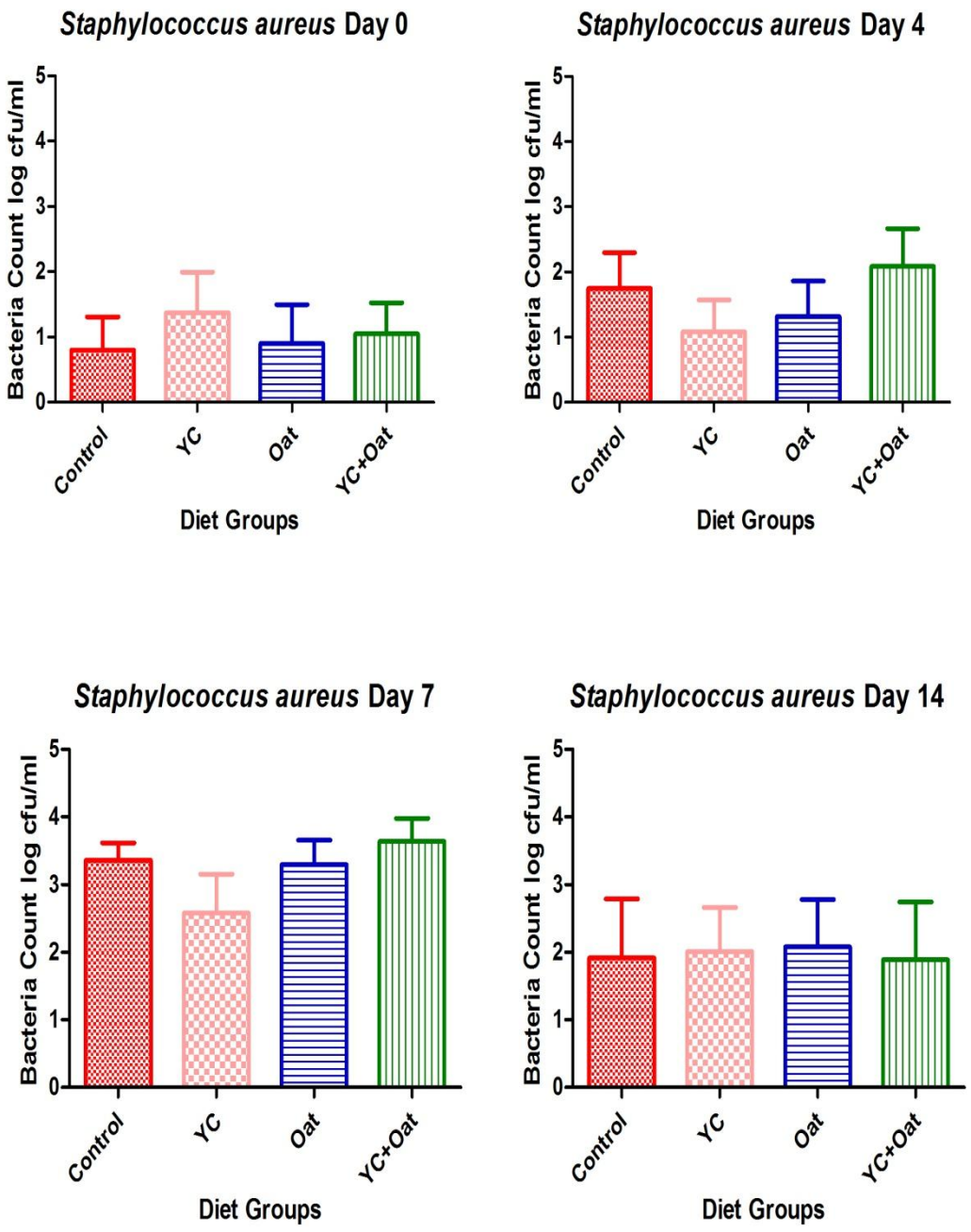


Figure 12. Piglet Staphylococcus aureus Bacteria Population.

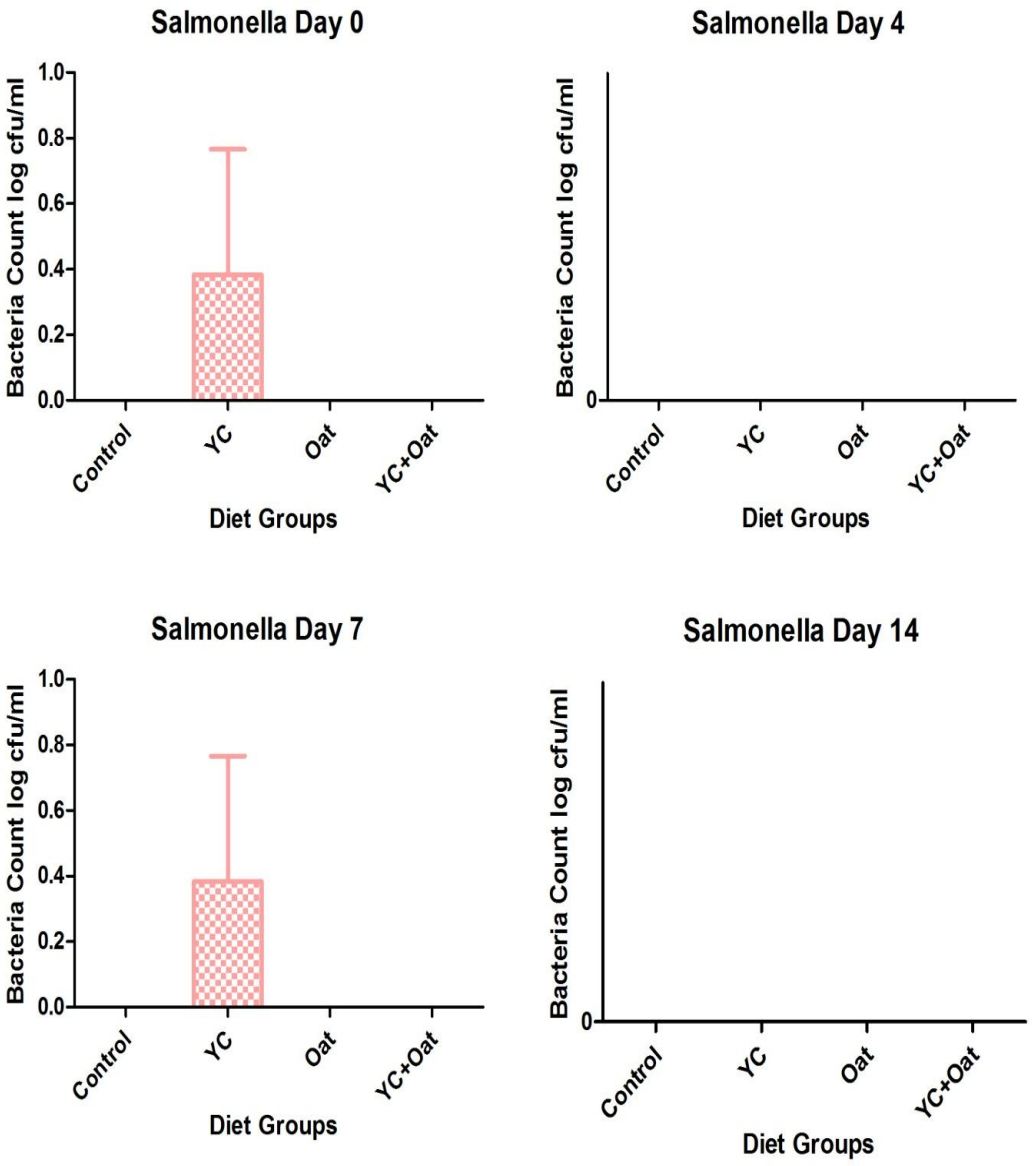


Figure 13. Piglet Salmonella Bacteria Population.

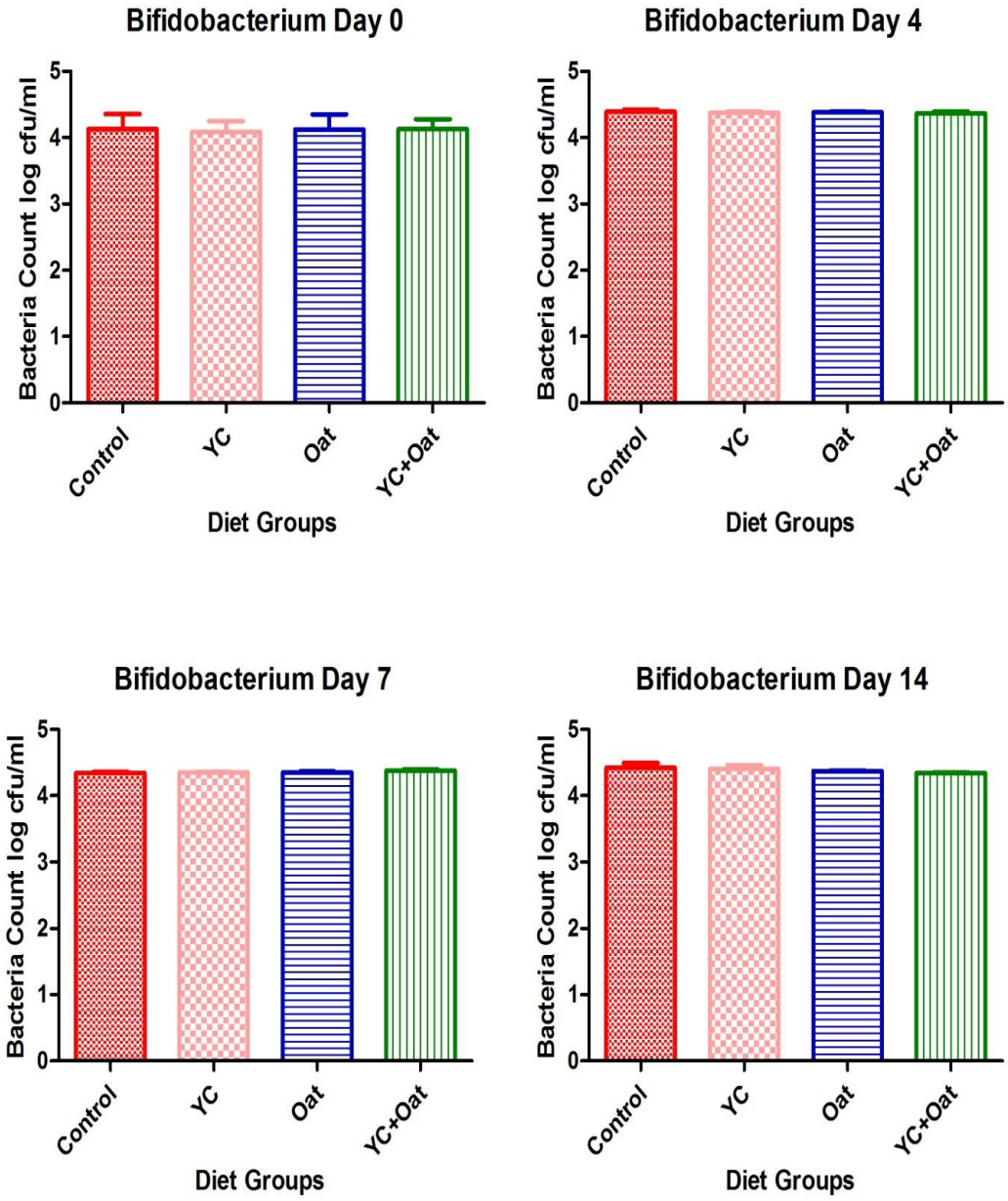


Figure 14. Piglet Bifidobacterium Bacteria Population.

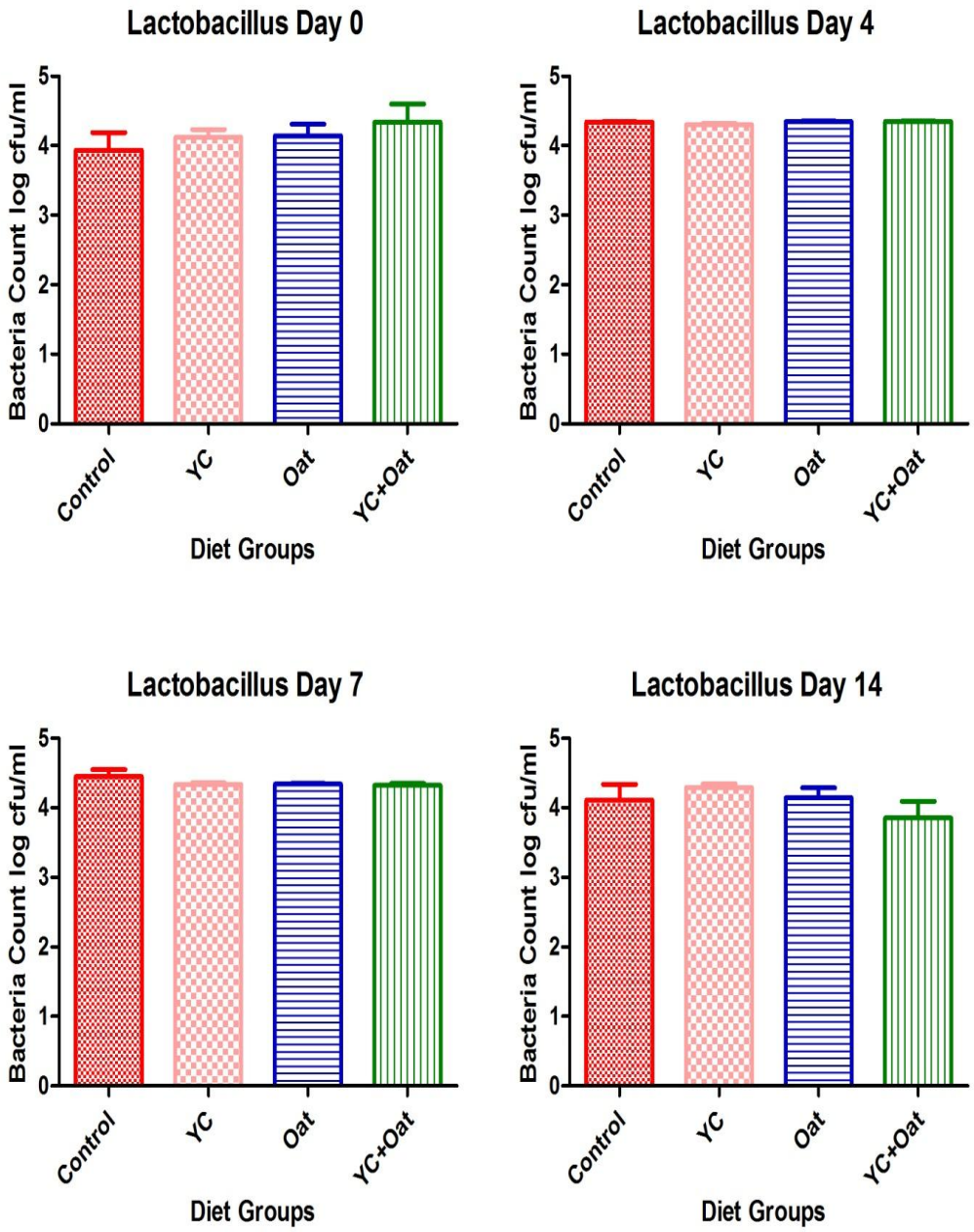


Figure 15. Piglet Lactobacillus Bacteria Population.

Appendix C

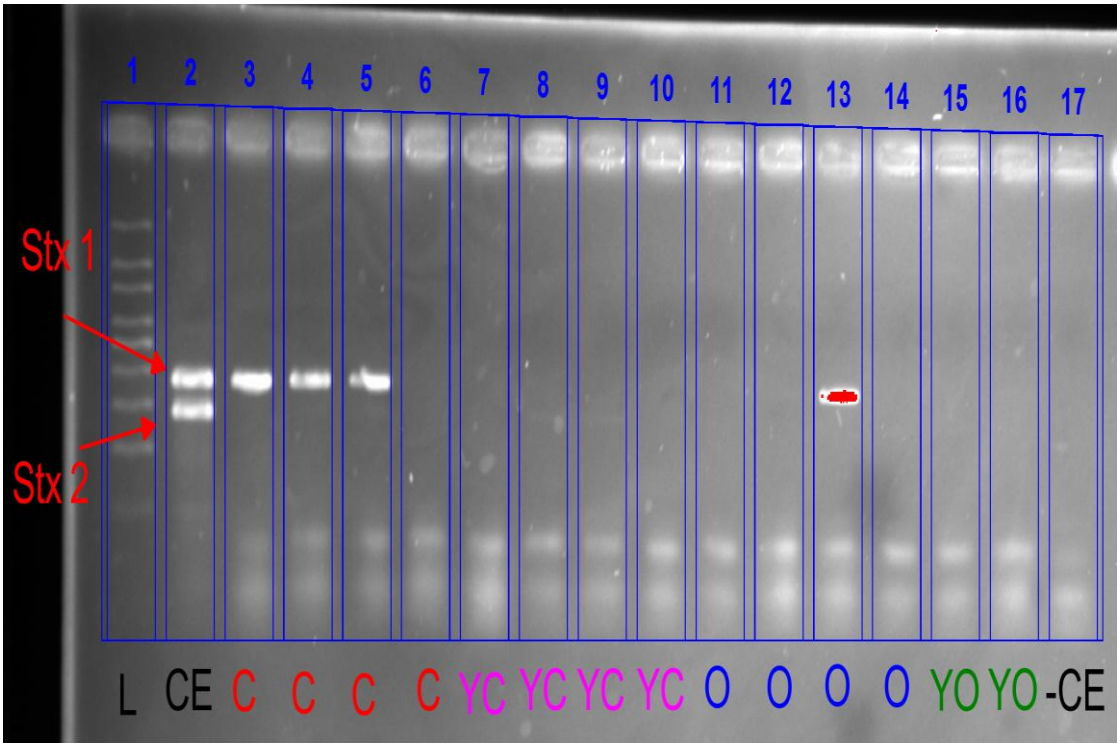


Figure 16. Representative Image of Sows Gel Electrophoresis. Image shows that lane 3-5 and 13 is positive for Primer 1 (Shiga Toxin 1). Lane 1: Marker Lane 2: Positive control for Shiga Toxin 1and 2, Lane 3-6: Piglet whose mother was fed Control diet, Lane 7-10: Piglet whose mother was fed yeast culture diet, Lane 11-14: Piglet whose mother was fed oat diet, Lane 15 and 16: Piglet whose mother was fed yeast culture + oat diet, Lane 17: Negative control for Shiga Toxin 1 and 2. Positive samples matched first (Stx 1) (370 bp) or second (Stx 2) (283bp) band of lane 2 or both.

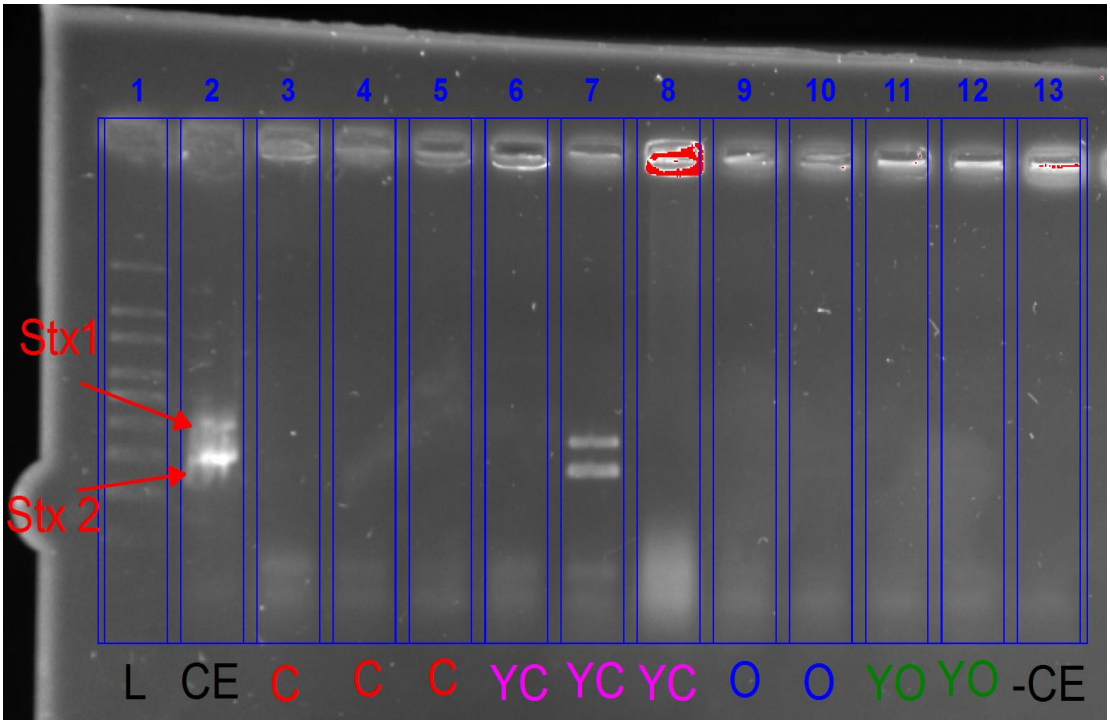


Figure 17. Gel Electrophoresis Trial 1 Day 0 Piglets.

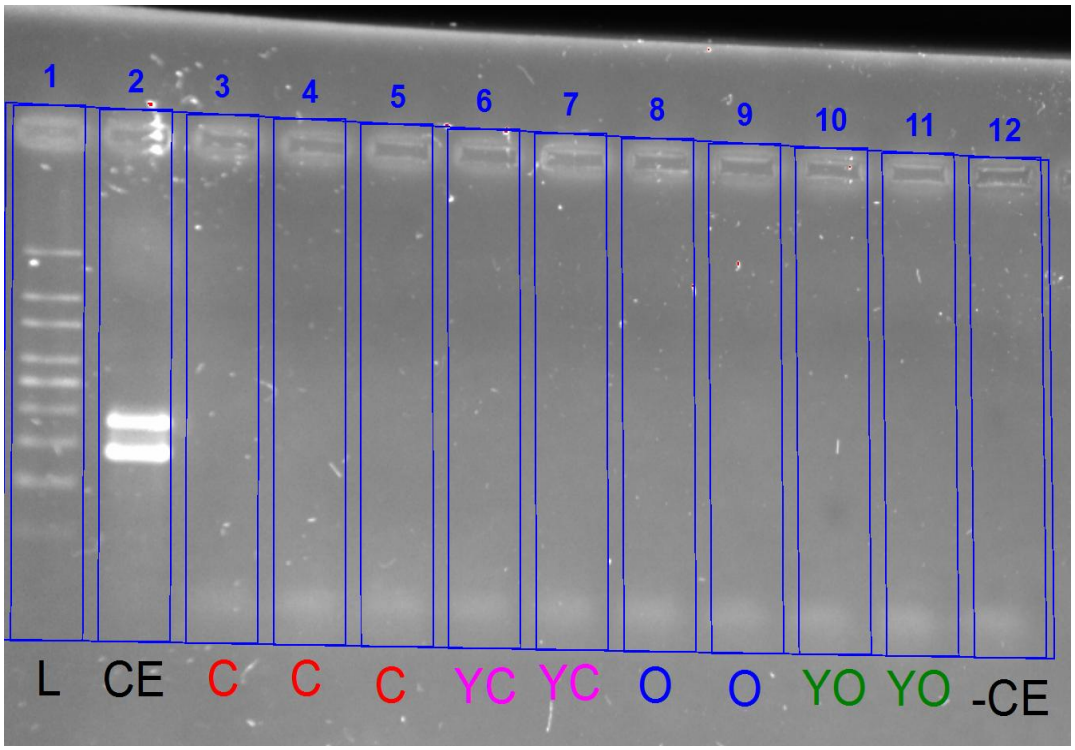


Figure 18. Gel Electrophoresis Trial 1 Day 4 Piglets.

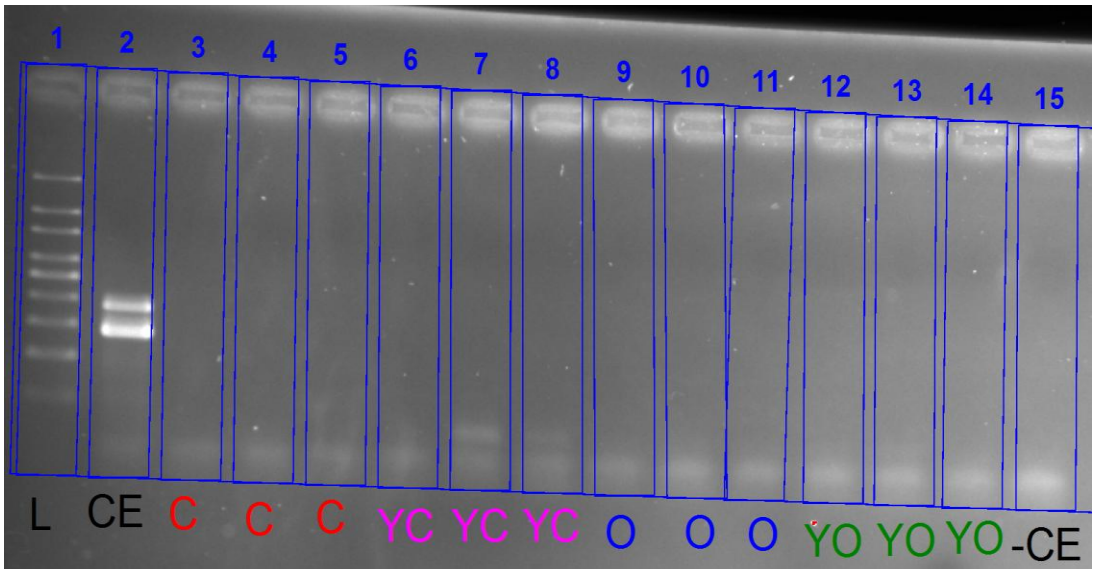


Figure 19. Gel Electrophoresis Trial 1 Day 7 Piglets.

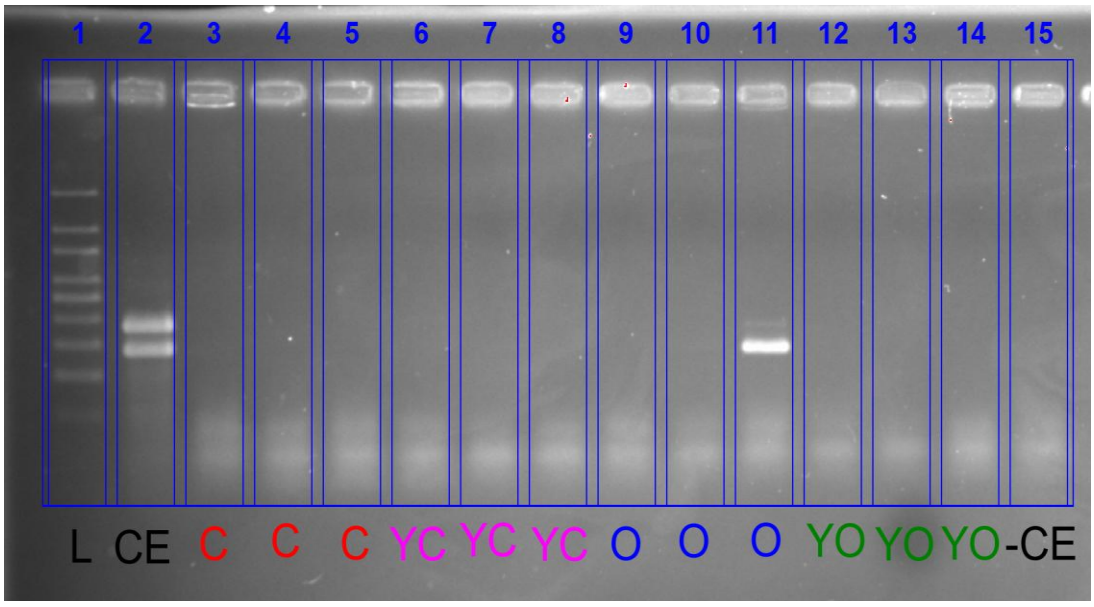


Figure 20. Gel Electrophoresis Trial 1 Day 14 Piglets.

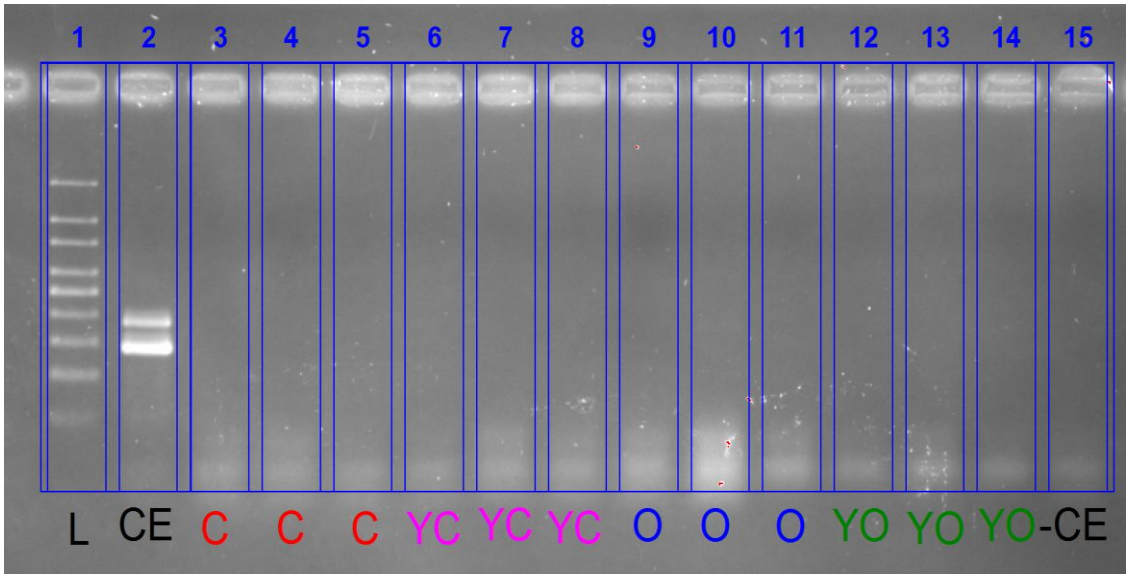


Figure 21. Gel Electrophoresis Trial 2 Day 0 Piglets.

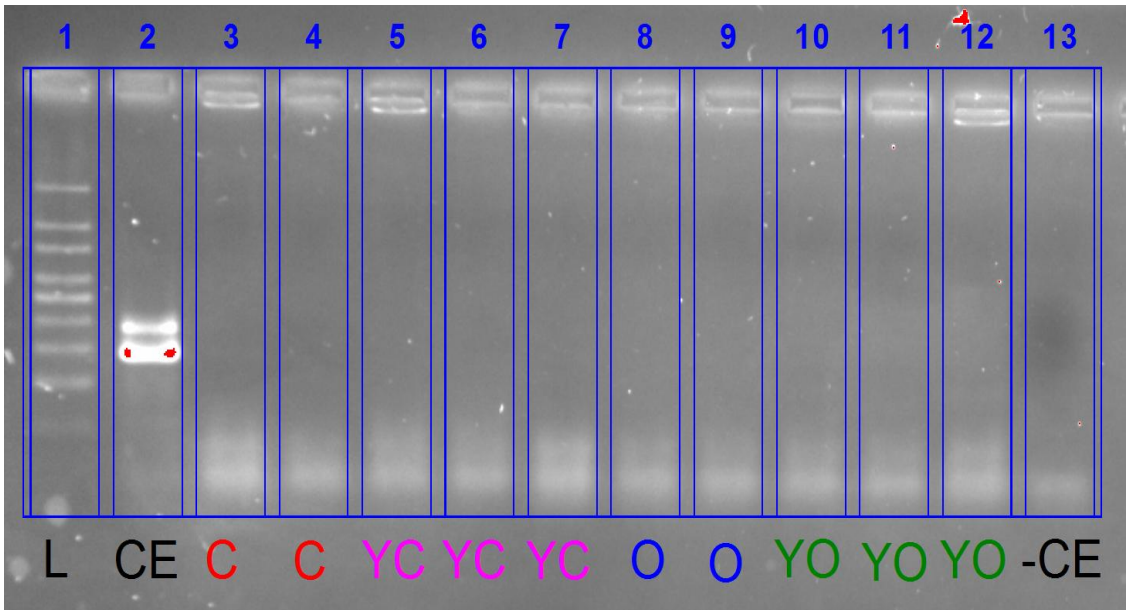


Figure 22. Gel Electrophoresis Trial 2 Day 4 Piglets.

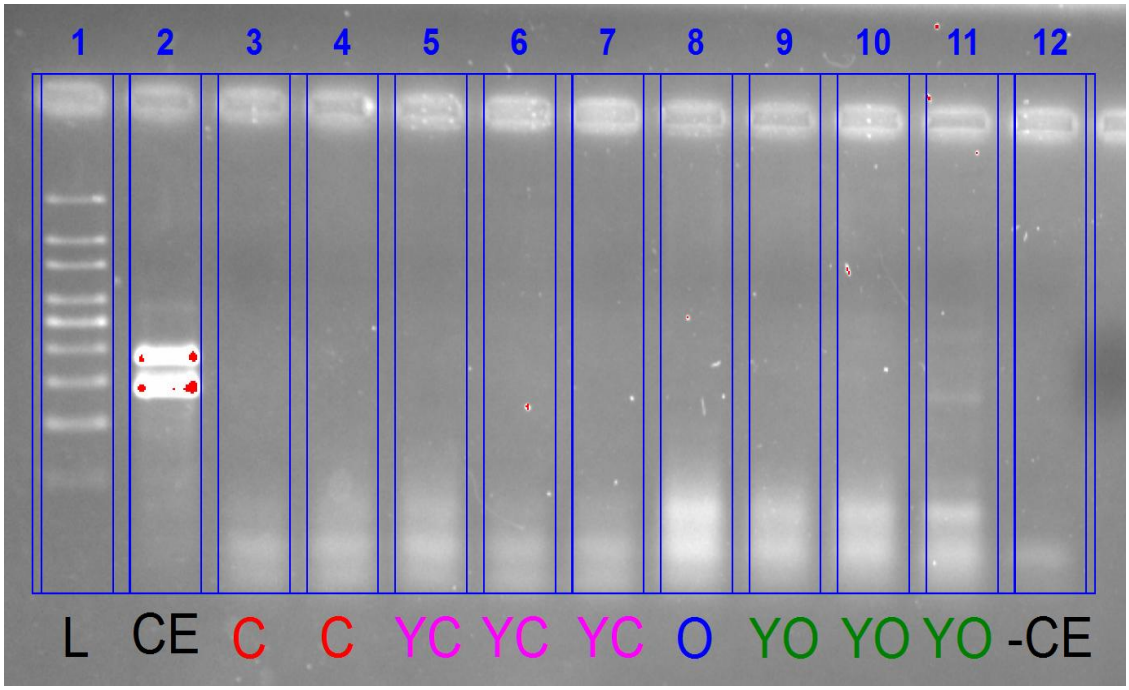


Figure 23. Gel Electrophoresis Trial 2 Day 7 Piglets.

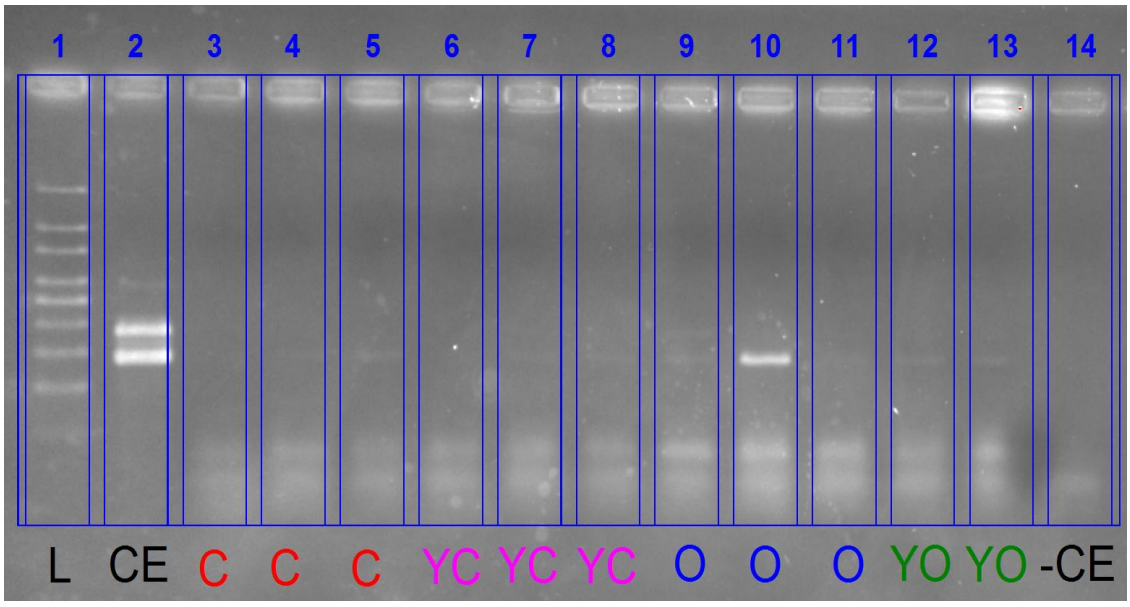


Figure 24. Gel Electrophoresis Trial 2 Day 14 Piglets.

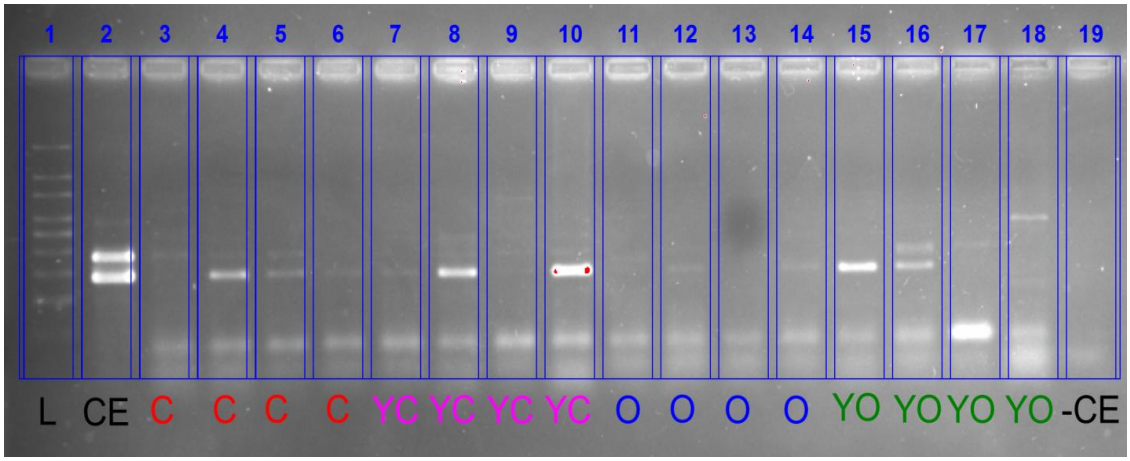


Figure 25. Gel Electrophoresis Trial 2 Gestation Day 84 Sows.

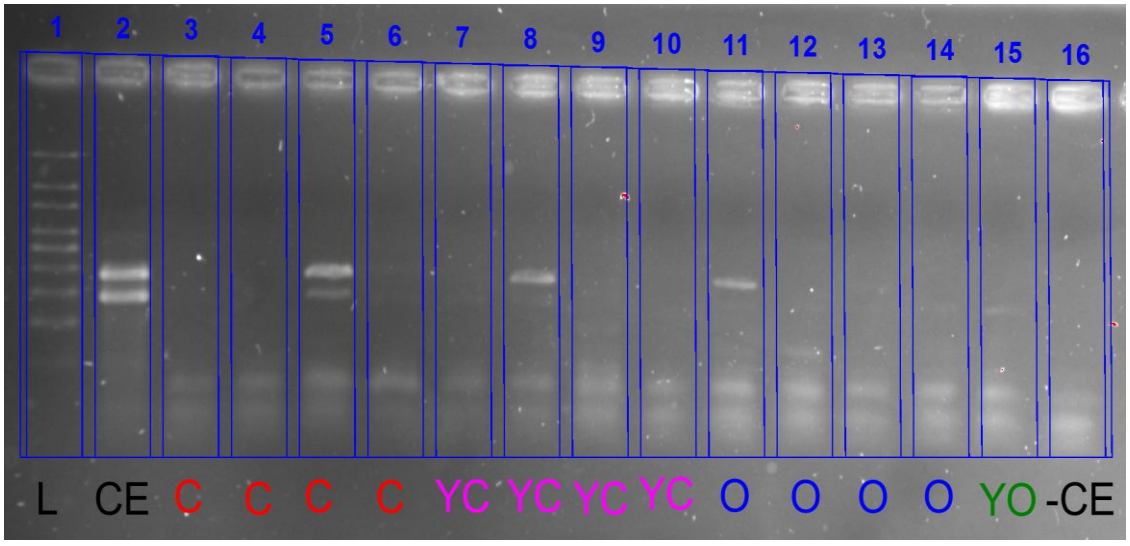


Figure 26. Gel Electrophoresis Trial 2 Farrowing Day Sows.

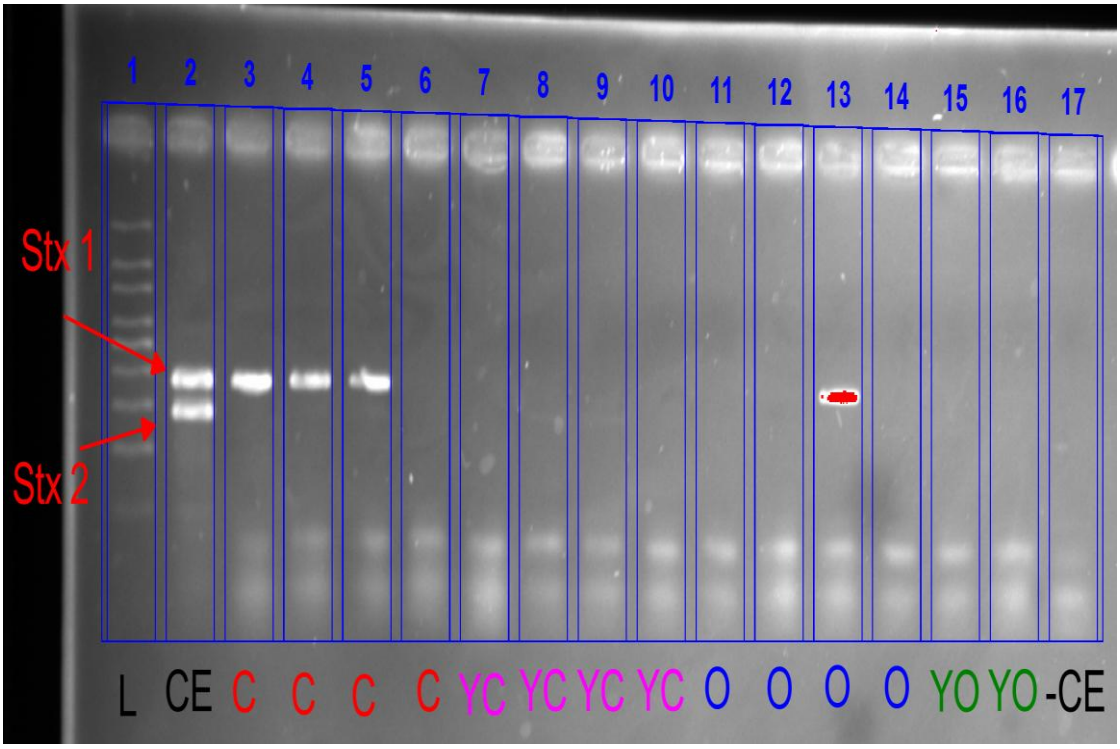


Figure 27. Gel Electrophoresis Trial 2 Weaning Day Sows.