5-2017

EFFECTS OF EGG YOLK ON PIGLET GROWTH PERFORMANCE, CIRCULATING IMMUNOGLOBULINS, BIOMARKERS OF INTESTINAL INTEGRITY, AND MICROBIAL COMMUNITY

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By

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A THESIS

Presented to the Faculty of

The Graduate College at the University of Nebraska

In Partial Fulfillment of Requirements

For the Degree of Master of Science

Major: Animal Science

Under the Supervision of Professor Phillip S. Miller

Lincoln, NE

May, 2017

EFFECTS OF EGG YOLK ON PIGLET GROWTH PERFORMANCE, CIRCULATING IMMUNOGLOBULINS, BIOMARKERS OF INTESTINAL INTEGRITY, AND MICROBIAL COMMUNITY

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University of Nebraska, 2017

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A total of 72 crossbred pigs weaned at 24 days were assigned to a completely randomized design (CRD) arrangement of treatments to determine the effects of egg yolk on a nursery piglet's growth performance, circulating immunoglobulins, biomarkers of intestinal integrity, and microbial community. Pigs were provided with spray-dried plasma (SDP), spray-dried egg yolk (SDEY), or a control diet. During Phase III, ADG was affected by treatment ($P < 0.10$). Pigs consuming the egg yolk diet had greater ($P < 0.05$) ADG vs. the plasma group for Phase III. Circulating immunoglobulins (IgA and IgG) were not affected by dietary treatment. C-reactive protein (CRP) and haptoglobin (Hp) concentrations remained constant during the experiment. Treatment did effect Glugacon like peptide-2 (GLP-2) concentration ($P < 0.01$) where pigs fed egg yolk had the greatest concentration. Microbial OTUs were similar among treatments ($P > 0.8$), but there were changes over time; from d 0 to Phase I and II there was an increase in diversity ($P < 0.01$) followed by a decrease in diversity ($P < 0.01$) in Phase III. Dominance was higher ($P < 0.01$) in Phase III when compared to the other phases. Treatments had no effect ($P = 0.33$) on bacterial community composition, but phases had a significant effect ($P < 0.001$) on bacterial community composition. Cost analysis of the diets showed that egg yolk was $2.30/lb and plasma was $2.16/lb. Egg yolk was the least

expensive diet during Phase II, and the most expensive during Phase I. These results indicate that performance, circulating immunoglobulins, biomarkers of intestinal integrity, and microbial communities are similar in pigs receiving nursery diets containing either egg yolk or spray-dried plasma. Therefore, based upon these results the type of protein utilized in a nursery pig diet may be determined by cost.

ACKNOWLEDGEMENTS

I would first like to express my sincere appreciation and gratitude to Dr. Phillip S. Miller, my advisor, for his constant encouragement and support during my time and for giving me this rarified opportunity to complete my master program. I would also like to thank my other thesis committee members, Dr. Thomas E. Burkey and Dr. Samodha Fernando for their guidance and advice, both of which greatly added to the value of this work.

I would also like to thank my fellow non-ruminant animal science graduate students as well as those in the Fernando lab, without your willingness to help and your considerable knowledge this project would not have been possible.

Finally, I would like to thank my family and friends, especially my husband Caleb A. Cunningham for supporting me during my pursuit of this degree. Thank you for always being there for me.

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LITERATURE REVIEW

INTRODUCTION

During weaning, piglets go though a variety of life-altering changes. They experience stress, completely change diets, develop their own immune system, gut microbiota, and all the while are expected to grow as efficiently as possible.

Weaning stressors include psychological, environmental, nutritional and transportation (Funderburke et al., 1989; Garcia et al., 2016). These stressors cause villous atrophy and crypt hyperplasia (Horn et al. 2014), which leads to malabsorption of nutrients, dehydration, diarrhea and enteric infections (Cera et al., 1988). Therefore, a high-quality protein is essential for piglets during weaning. Both spray-dried egg (SDEY) and spray-dried plasma

(SDP) products can increase growth performance (Coffey et al., 1995; Song et al., 2012), improve health, and inhibit the onset of certain diseases (Jin et al., 1998; Niewold et al., 2007; Mathew et al., 2009) which is excellent because pigs are not born with antibodies leaving them vulnerable to disease.

Biomarkers of intestinal integrity also maintain pig health. C-reactive protein (CRP) is responsible for removing damaged cells and infectious agents (Saco et al., 2016). Haptoglobin (Hp) acts as an antioxidant by removing free radicals (Andersen et al., 2012). Glucagon-like peptide-2 (GLP-2) inhibits intestinal cell apoptosis, increases intestinal transport and absorption of nutrients, and increases intestinal growth and development (Chai et al., 2015).

Gut microbiota aid the pig in creating both intestinal and immune systems. However, they are continually evolving over the course of the pig's lifetime in response to diet changes and infectious agents (Zhao et al., 2015).

Overall, diets or more specifically the protein source, have a significant effect on a nursery pig's growth, development, and health. This can be observed through changes in performance, microbial diversity, antibody production, and biomarkers of intestinal integrity.

STRESSORS IN WEANING PIGS

Weaning is one of the most stressful events a pig will endure in its life, and is comprised of several different sources of stress: environmental, nutritional, and psychological. These stressors result in a transient decrease in feed intake, which is evident by villous atrophy and crypt hyperplasia which lead to both short- and long-term challenges for the pig, impacting growth performance, nutrient absorption, gut secretions, scours, and disease susceptibility (Funderburke et al., 1990; David et al., 2002; Horn et al., 2014).

The environmental stressors (high temperature, regrouping with new pigs, and restricted space allowance) directly negatively impact feed intake and gain. However, it was shown that when combined, the impact of the stressors is additive; so if it is possible to control for even one of these stressors, growth performance would improve (Hyun et al., 1998). Housing change is another significant environmental stressor that occurs during weaning. This change is made because removing piglets from the dam will significantly decrease the chances of disease transfer from the dam to the piglets (Davis et al., 2006; Sutherland et al., 2014).

Transport also causes the pig a significant amount of stress. According to Hicks et al. (1998) shipping results in a 5% decrease in BW. Some of the stress that occurs during transport is inevitable, although making certain the pigs have access to feed and

especially water, if the trip is a duration of 24 h or more, can make a difference (Garcia et al., 2015; Garcia et al., 2016). When piglets arrive at the new facility, they must establish a new dominance order, which in the beginning will involve a significant amount of fighting, but will decrease within 24 h. It is also important to note that in this study, the regrouping process did not result in any long-term performance setbacks (McGlone et al., 1987).

In contrast, nutritional stressors do have a negative impact on growth performance. It is quite common for a piglet to consume very little feed the first few d after weaning (Dybkjaer et al., 2006). In fact, Horn et al. (2014) reported that during the first 48 h nearly 10% of pigs consume no feed. Based on numerous data sets, Edge et al. (2005) concluded that pigs do not meet their maintenance requirements for metabolizable energy until approximately five d after weaning. Bruininx et al. (2002) stated that during the first wk post weaning, the energy requirements for maintenance increase. These observations are due to sudden diet changes; the pig goes from being solely dependent on a liquid diet from the dam to an entirely solid diet in less than 24 h (Hötzel et al., 2011).

It is natural that it will take the pig time to mentally adjust to getting its nutrients from 2 separate locations instead of relying solely on its mother and to physically adjust to its major energy source transitioning from fat and lactose in milk to carbohydrates in grain (Dybkjaer et al., 2006; Bomba et al., 2014). The biggest impact this diet adjustment has on the pig is on the pig's gastrointestinal health; more specifically, villous atrophy of the small intestine (Cera et al., 1988; Bruininx et al., 2002; Bomba et al., 2014), which leads to a malabsorption syndrome resulting in gut disorders (i.e., diarrhea; Bomba et al., 2014).

Psychological stress also stems from being separated from the dam. It is a result of relocation to a new environment and the introduction of new penmates (Funderburke et al., 1990; Davis et al., 2006; Pastorelli et al., 2012). As previously mentioned, establishing dominance causes environmental stress, but it also causes psychological stress. While there is some initial stress caused to all the pigs during the establishment of dominance, it is the subordinate pigs that may be chronically stressed as a result of their newly established dominant penmates (McGlone et al., 1987).

SPRAY-DRIED PLASMA

Inclusion of Spray-dried Plasma in Nursery Diets

Spray drying animal plasma (SDAP) is a multi-step process. First, blood from slaughter facilities is collected and pooled. Animals are determined to be healthy and fit for human consumption by government officials. Next, a centrifuge separates the blood so that the plasma may be separated from the cellular portion of the blood. Finally, the plasma fraction is spray-dried resulting in a powdery substance that may easily be added to feed (Pujols et al., 2008; Gerber et al., 2014).

SDAP whether it comes from bovine, ovine, or avian species is a commonly used protein source that is included in many nursery diets, especially during Phase I (Torrallardona et al., 2003; Pierce et al., 2005; Zhang et al., 2015a). Because in addition to supplying the pig with a source of protein, SDAP can help improve growth performance, gut integrity, and immunity (Torrallardona et al., 2003; Tran et al., 2014).

The impact of SDAP on growth performance is mixed. In some studies, SDAP greatly improves pig growth performance while in others it has little to no effect. According to a study by Grinstead et al. (2000), the effects of diets with increasing

amounts of whey protein product (WPP) and SDAP (2.5 to 5%) were compared. It was discovered, that during the first wk (d 0 to 7) pigs fed SDAP had greater average daily gain (ADG) and average daily feed intake (ADFI), which increased with increasing SDAP. However, during wk 2 (d 7 to 14), there were no significant differences in performance among treatments. Tran et al. (2014) conducted an experiment in which SDPP had a positive effect on performance. The pigs were fed either a SDPP diet containing 5 and 2.5 % (Phase I and II, respectively) or a control diet. At the end of the experiment, it was determined that the pigs fed the SDPP diet had greater BW (18.15 vs. 17.19 kg), ADG (424.1 vs. 397.6), and ADFI (601.1 vs. 564.4 g) than the control pigs.

Two different studies by Van Dijk et al. (2001; 2002) report that SDPP had no significant effect on ADG or ADFI. The study by Van Dijk et al. (2002) compared SDPP and casein diets fed to unweaned pigs and reported that a potential reason for the lack of a SDPP effect could be due to the casein diet producing a sufficient ADG and ADFI. The study by Van Dijk et al. (2001) also compared a casein diet to a SDPP which resulted once again in there being no significant effects from SDPP on ADG or ADFI. However, in this case, the explanation was different than for the previous study. In this case, Van Dijk et al. (2001) reported that the lack of a SDPP effect on performance was most likely due to the exceedingly hygienic conditions during the experiment performed. According to Coffey et al. (1995), pig performance is affected more in commercial-type nursery conditions vs. a controlled off-site experimental environment.

Effects of Spray-dried Plasma on Health of Pigs

It has been well documented that spray-dried plasma has multiple positive effects on the overall gut health of pigs. Spray-dried porcine plasma has been recognized as an effective way to improve intestinal barrier function, reduce inflammation, and eliminate diarrhea (Peace et al., 2011).

In an experiment by Zhang et al. (2015b) it was hypothesized that spray-dried chicken plasma (SDCP) would also have beneficial effects on weanling pigs because it was similar in composition to SDBP and SDPP. Four dietary treatments were created; a control, 5% SDPP, 2.5% SDPP + 2.5% SDCP, and 5% SDCP. The results of this study indicated that the pigs in the SDCP, SDCP + SDPP, and SDPP had greater villus height and a villus height to crypt ratio in the duodenum and ileum compared to the control pigs. It was also shown that the SDCP and SDPP pigs had enhanced apparent total tract digestibility (ATTD) of crude protein (CP), ether extract (EE), calcium, and ash: these pigs also had a lower incidence of diarrhea. These results indicate that SDPP and SDCP inclusion in a nursery diet resulted in an improvement in nutrient digestibility and enhanced digestive, intestinal, and absorptive function.

A study conducted by Niewold et al. (2007) was completed with the intent of discovering the effects SDPP and spray-dried immune plasma powder (SDIPP) have on piglets infected with enterotoxigenic *E. coli*. Three diets were created, a control (soybean and whey), 8% SDPP, and 8% SDIPP, which came from pigs that were immunized with 2 mL of *Porcilis coli*. It was shown that the pigs fed either the SDIPP or the SDPP diets had a decreased incidence of enterotoxigenic *E. coli* (ETEC) F4 post-weaning diarrhea (PWD), which was assessed by looking at their fecal percent dry matter

(DM) values indicating that SDIPP produced feces with the greatest DM closely followed by SDPP and the control which had the lowest % DM. Results also showed that after inoculation with ETEC, the control pigs shed the greatest amount of ETEC, followed by SDPP pigs with the SDIPP pigs shedding very little ETEC. These results support SDPP as an excellent ingredient to include in nursery diets to prevent PWD.

Potential Infectious Agents in Plasma Products

There are numerous illnesses that threaten the health and lives of pigs, especially during weaning when their immune systems are immature. These include: porcine epidemic diarrhea virus (PEDV), enterotoxigenic *Escherichia coli* (ETEC), Porcine circovirus type 2 (PCV2), and mycotoxins (Owusu-Asiedu et al., 2002; Pujols et al., 2008; Pasick et al., 2014; Weaver et al., 2014).

PEDV is a single stranded, positive-sense, enveloped RNA virus, belonging to the *Coronaviridae* family. It was first discovered in the United Kingdom in 1971, where it was infecting pigs with devastating results; however, the disease was not named until 1978 in Belgium. PEDV did not spread to North America until April of 2013, where it spread through Mexico, Canada, and the United States with great speed and had a mortality rate of 100% in suckling pigs (Gerber et al., 2014; Trujillo-Ortega et al., 2016).

Many possibilities as to how this deadly disease was spreading have been investigated; and one such investigation conducted in Ontario, Canada by Pasma et al. (2016) did so by reviewing surveillance of transportation, processing plants, and assembly yards in addition to testing the feed from the first 6 farms or animals infected by PEDV. After extensive testing, it was discovered that none of the infected farms had any risk factors such as biosecurity, transportation, rendering companies, or service

providers in common. So, further investigating was undertaken and it was determined that 21 of the 25 outbreaks of PEDV used a single feed company and all contained nursery feed containing SDPP in common. Thus, samples from the feed company and from the company that made the SDPP to create the feed were evaluated. The feed and the SDPP were tested by RT-PCR for presence of PEDV genetic material and tested positive. However, upon more rigorous evaluation it was determined through bioassay that although PEDV was infectious through SDPP, it was not transmissible through the complete feed coated in the SDPP.

A study conducted by Opriessnig et al. (2014) further investigated the potential of SDPP to infect pigs with PEDV. This study involved feeding pigs 5 different diets; 1) no SDPP in the feed and pigs not inoculated with PEDV; 2) no SDPP in the feed and pigs inoculated with PEDV; 3) 5% commercial SDPP in the feed from d post inoculation (dpi) 0 to 28 pigs not inoculated with PEDV; 4) 5% commercial SDPP in the feed from dpi -4 to 28 and pigs inoculated with PEDV; and 5) no SDPP in the feed and 5 mL/d orally from dpi -4 to 6 of EPFX/EGCO; and inoculating the pigs with PEDV. The results of this study showed that the pigs consuming the diet containing the 5% SDPP that were not inoculated with PEDV, did not develop any symptoms consistent with PEDV and did not shed the virus in their feces or seroconvert. However, the pigs inoculated with the PEDV virus did develop symptoms, shed the virus in their feces, and seroconverted. These results are consistent with Opriessnig et al. (2014) that showed a commercially created PEDV positive SDPP feed would not transmit the PEDV virus to pigs.

Porcine circovirus (PCV-2) is a common disease occurring in nursery pigs. It is a single-stranded DNA virus, which belongs to the Circoviridae family and is considered to

be the critical infectious agent in postweaning multisystemic wasting syndrome (PMWS) and is ubiquitous amongst swine and extremely lethal (Pujols et al., 2008). PCV-2 is very resistant to inactivation by various methods such as chemical, physical, and dry-heat treatments; as a result, concerns have been raised that PCV-2 may be transmissible through commercially created SDP (Polo et al., 2013).

In a study conducted by Patterson et al. (2010), 12 pigs were separated into 3 groups; a negative control group that was not inoculated, a group inoculated intraperitoneally or by oral gavage with SDP infected with reconstituted PCV-2, and a group inoculated intraperitoneally with plasma from a pig infected with PCV-2. The results of this study were such that by 35 dpi all the inoculated pigs had significantly more PCV-2 DNA than the negative control pigs. These results demonstrated that through intraperitoneal and oral gavage, it is possible to infect naïve pigs with experimentally created PCV-2 infected SDP. However, Patterson et al. (2010) stated that it is inappropriate to extrapolate these results and apply them to commercially produced SDPP.

An experiment by Polo et al. (2013) can be applied to commercial SDPP product and processes. The objective of the study was to evaluate the potential of neutralizing antibodies (NA), which are present in pooled liquid plasma, to act against PCV-2. The results confirmed that NA are critical for inactivating the PCV-2 virus and it was shown that the pooled plasma samples diluted 1:256 were able to inactivate 100 to 200 tissue culture infectious dose (TCID) of the PCV-2 virus particles. Thus, according to this study, it is safe to conclude that commercially-created SDP will not transmit PCV-2 to naïve pigs and can help fight off the disease.

EGG YOLK

Composition of Egg Yolk

Because nursery pigs have an immature digestive system at weaning, they require a complex and highly digestible diet as they make the transition from a milk diet to a solid diet (Bras et al., 2003). Eggs are an excellent source of high-quality protein, fat, and both essential and non-essential vitamins and minerals (Tang et al, 2015). While all 7 classes of nutrients are essential to include in a pigs diet, the most important feed ingredient in a nursery diet is the protein source. The protein source has the greatest effect on the pig's growth performance and health as both are strongly influenced by it (Bras et al., 2003). It has also been discovered that eggs possess other beneficial components, such as IgY, which is an IgG like antibody that can be found in egg yolk (Rossi et al., 2013).

Usage of Egg Yolk in Nursery Pig Diets

Post weaning nursery pigs are very susceptible to a growth performance lag and while SDP has proven to be an effective protein choice to alleviate this lag, it is quite costly and an alternative less costly option would benefit the swine industry. Spray-dried egg is an excellent protein source and is readily available. The process of spray-drying does not alter the composition of egg yolk. It consists of the liquid yolk droplets coming into contact with extremely hot air (167 to 173°C), thus instantly drying the droplets out. The droplets are separated from air in a cyclone separator, where the time required depends on how large or small the droplet is and how hot or cool the temperature of the droplet is initially (Froning et al., 1998; Ignário et al., 2007).

The effects of including SDE in nursery pig diets on growth performance are mixed. In a series of experiments conducted by Song et al. (2012), the value of including

SDE as a protein source in nursery pig diets was evaluated. The first 2 experiments involved a 10-d study and 2 diets, one control and one including 5% SDE. In both experiments the SDE increased ADG and ADFI when compared with the control diets. However, there was no effect of SDE on G:F. In the 4th experiment SDE and SDP were compared to see if the former could replace the latter. The study lasted 6 wk and consisted of 4 phases and the concentration of SDE or SDP in each phase were as follows: 6%, 4%, 2%, 0%, respectively. The inclusion of SDE during Phase I (6%) increased ADFI compared to the other diets. These experiments show that inclusion of SDE in the diets of nursery pigs has positive effects on performance and may be an effective ingredient.

Another experiment was conducted to assess whether SDE would be an appropriate replacement for SDP and soybean meal (SBM) in a nursery pig diets. The dietary treatments were formulated based on the level of egg protein (3 or 6%) added to Phase I (d 0 to 14) in place of either SBM or SDPP. The treatments were as follows: 1) control 2) 3% SDE in place of SBM. 3) 6% SDE in place of SBM 4) 3% SDE in place of SDPP 5) 6%SDE in place of SDPP. A 6th treatment was designated to replace SDPP and consisted of 4% egg protein blend (EPB). In the 2nd Phase (d 14 to 28) a common diet was fed to all the pigs to see if there were any carryover effects.

During Phase I, pigs fed either the 3% SDE for SDPP or 3% or 6% SDE for SBM had similar ADG. However, pigs fed the 6% SDE for SDPP or 4% EPB for SDPP had decreased ADG. Feed efficiency was decreased when SDE replaced SBM at 6% and 3%. The 6% SDE replacing SDPP had decreased ADG ($P<0.01$) and ADFI ($P<0.01$) compared to the other diets. These results indicate that while replacing SDPP with SDE

at 6% will have negative ramifications, it would be acceptable to replace SDPP with SDE at 3% without a significant effect on growth performance (Owen et al., 1993).

While the results as to whether or not SDE products are a viable replacement for SDP products in a nursery diet have been variable, it has been discovered that SDE products are as affordable or slightly more than SDP products. A study done by Norin et al. (1998) was completed in order to determine if there were cost effective alternatives to SDP for segregated early-weaned (SEW) pigs. The experiment conducted focused on replacing SDP with a SDE product. The first phase was from d 14 to 28 and consisted of a complex diet, a simple diet + 6% SDPP, a simple diet + 3% SDPP + 6% SDE and a simple diet + 12% SDE. During the first wk of the experiment, pigs fed the 12% SDE diet gained at a slower (P <0.05) rate than pigs fed the other diets. There were also no price differences among treatments on a feed cost/pound gain basis during the first wk. During wk 2 there were no performance differences among treatments; however, there were cost differences. The SDE diet cost less (P<0.05) per pound of gain vs. the complex and the simple + 6% SDPP diets. It was also shown that at the end of the experiment there were no performance differences among the 4 treatments. This indicates that SEW pigs are capable of using SDE as a protein source to achieve adequate growth performance.

An experiment performed by James et al. (2000) investigated the effects of SDP, an inedible egg product, and a combination of the 2 would have upon a nursery pig's growth performance, and whether or not the egg product could supplement or even replace SDP in order to decrease diet cost. There were 5 treatments: 1) negative control; 2) 4% SDP; 3) 6.4% inedible egg product; 4) 2% SDP + 3.15% inedible egg product; and

5) 2% SDP. The study lasted for 4 wk and during the first 2 wk the pigs were fed one of the treatments and for the second 2 wk, the pigs were fed a common diet to establish any carry over effects. The results indicate that while the 6.3% inedible egg product failed to significantly improve ADG, ADFI, or G:F when compared to the other diets during the first 2 wk, the combination of inedible egg product and SDP did numerically improve the ADG and G:F of the pigs. Pigs consuming inedible egg product did not perform as well as pigs consuming the SDP. However, the inedible egg product was an adequate protein source, and was more affordable than the SDP, which cost $2.00/kg while the egg cost $0.40/lb.

Interaction of Gut Health and Egg Yolk

When piglets are weaned they are extremely vulnerable to illnesses because they lose their immune protection from sow's milk. This is why it is critical that piglets receive a diet rich in immune globulins to help provide immune protection. Egg yolk contains immune globulins called IgY, at a concentration of approximately 12,000 ppm (Harmon et al., 2002). These IgY antibodies can be created to aid the weanling pigs in fighting off certain diseases. Some examples of diseases that IgY has been tested against with differing results are *E. coli*, *Salmonella,* and *Rotavirus*. Specific IgY antibodies can be created by injecting chickens with organisms that cause a specific disease, which results in the chicken creating IgY antibodies against that specific pathogen (Thacker, 2013).

Economic losses due to intestinal disease are significant to the swine industry; in fact, enterotoxigenic *E. coli* (ETEC) is not only the most common diarrheal disease in nursery pigs it also accounts for 50% of the 10 million pigs that die each year (Owusu-

Asiedu et al., 2003). The most prevalent type of ETEC is the type that expresses the K88+ fimbrial antigen, which occurs most often in pigs housed in large numbers (Marquardt et al., 1999). An in vitro experiment to determine whether or not spray-dried egg yolk (SDEY) containing K88+ antibodies could prevent adhesion of *E. coli* K88+ was conducted by Jin et al. (1998). A competitive adhesion test was performed in vitro to simulate in vivo conditions and to discover whether the egg yolk control (non-immunized chickens) or egg yolk antibodies (EYA;immunized chickens) could prevent *E. coli* K88+ from adhering to the intestinal mucus and at which dilution (10, 20, 40, 100, 250, 500, 1,000 cfu mL^{-1}). The results demonstrated that EYA at 10- to 100- dilution were effective at preventing the adhesion of *E. coli* K88+ to the intestinal mucosa with a prevention of (84.6 to 97.0%). Unfortunately, the control antibodies were not nearly as effective and became even less so as their dilution rates increased (Jin et al., 1998).

Specific EYA (SEYA) is more effective at protecting pigs from ETEC-K88 than untreated EYA. SEYA are also more effective than SDPP and could even replace antibiotics. According to Owusu-Asiedu et al. (2003b), not only do SEYA have greater anti-K88 antibody titers (600,000) vs. SDPP (18,000 anti-K88 antibody titers). These results indicate that to achieve the same safety for the piglets from ETEC-K88, pig diets would require 10% SDPP and only 0.33% treated egg yolk in their diet. Antibiotic resistance and the possibility that antibiotics in feed will be reduced or even eliminated all together is a strong motivator for swine producers to find antibiotic alternatives. In a study by Li et al. (2008) an experiment was performed on pigs to compare non-encapsulated IgY with a microencapsulated IgY and an antibiotic commonly used to treat pigs for ETEC (aureomycin). All of the pigs were challenged with 5 mL of K88+ *E. coli.*

and were treated 3 times a d the first d and twice a d the second and third d, respectively. The results showed that non-encapsulated IgY and the antibiotic treated pigs developed ETEC symptoms to the same degree after the challenge. However, the pigs given the non-encapsulated IgY recovered 72 h post challenge while only 50% of the antibiotic pigs were recovered at this point. Microencapsulated IgY pigs had the best recovery time though; it took only 24 h before all the pigs in this group ceased to show clinical symptoms. Thus, IgY is an effective treatment for ETEC-K88+ and it is even more effective when it is encapsulated.

Salmonella is a much more invasive pathogen than *E. coli* because it is capable of invading both the vascular system and lymph nodes in addition to the intestines. Therefore, it is not as susceptible to dietary intervention because it is able to avoid the gut. Thus, egg yolk treatments (Anti-*Salmonella* egg yolk powder, ASEY and Egg yolk control, EY) are far less effective than the antibiotic treatments (Apramycin and carbadox and Oxytetracycline) at preventing the shedding of *Salmonella* (Mathew et al., 2008).

Rotavirus is a virus that has been found in both humans and animal species and is similar to *E. coli* because like *E. coli* it is found most often in newborn animals (Kaminjolo et al., 1994). In fact, of the diagnosed rotavirus infections, 80% are found in unweaned pigs. Unfortunately, the morbidity rate is between 50 and 80% and the mortality is 0 to 15% (Schwartz et al., 1978). It was shown in a study by Vega et al. (2012) that IgY antibodies were an effective and affordable treatment for rotavirus. The experiment used neonatal gnotobiotic (Gn) pigs infected with Rotavirus and given milk supplemented with varying concentrations of IgY Rotavirus specific antibodies. The results indicated that the IgY Rotavirus specific antibodies were able to successfully

protect all of the animals in that treatment against developing rotavirus diarrhea and from shedding the virus.

INDICATORS OF GASTROINTESTINAL HEALTH

IMMUNOGLOBULINS

IgA and IgG

Immunoglobulins (Ig) or antibodies are a type of glycoprotein molecule generated by B-lymphocytes, and their purpose is bind to antigens usually with high affinity and specificity (Abbas et al., 2015). There are several different classes of immunoglobulins, each with its own unique structure and purpose in the body. IgA is made up of α heavy chains and is found in both monomeric and dimeric forms, while IgG is comprised of γ heavy chains and is only found in a monomeric form (Murphy et al., 2008).

The pig does not receive any maternal antibodies in utero because the placenta is not permeable to maternal immunoglobulins. Therefore, immediately after the pig is born it is critical to consume as much colostrum as possible in order to obtain the necessary immunoglobulins. The pig's gut during the first 2 d after birth is capable of absorbing macromolecules in their entirety with no alterations (Werhahn et al., 1981). Thus, the pig can absorb critical antibodies such as IgG, which is the most essential globulin during the first few wk of the piglet's life (Kielland et al., 2015) and secretory IgA (sIgA) which provides the pig with a first line of defense against bacterial pathogens in the gut (Butler et al., 2016).

The sow transfers the immunoglobulins to her offspring through the use of Ig receptors; polymeric Ig receptor (pIgR), and the neonatal fixed chain receptor (FcRn), which during late gestation and immediately after birth bind to IgA and IgG respectively

and transport the Ig molecules into the colostrum, which the piglets then consume (Danielsen et al., 2006). Unfortunately, this transfer will only last the piglets until "gut closure" occurs. Gut closure may occur any time from 12 to 36 h after birth resulting in the piglet no longer being capable of indiscriminately absorbing intact proteins through their columnar enterocytes. Gut closure is timed with the moment when the sow is no longer able to transfer IgG from their blood to their colostrum or milk (Butler et al., 2006).

Colostrum provides piglets with passive immunity immediately following birth, and colostrum consumed within the first 24 h plays a significant role in pigs developing their own antibodies. However, both IgA and IgG have very short half-lives; blood IgG has a half-life of approximately 12 to 14 d and IgA from colostrum in the intestines disappears after 7 d. Therefore, by the time pigs reach weaning age they must be synthesizing their own IgG and IgA (Ogawa et al., 2016).

IgG occurs in 2 different isotypes (IgG_1 and IgG_2), which allows for diverse isotype-related functions such as binding to various Fc receptors and activation of complement. The expression of IgG_1 and IgG_2 is controlled by Type 1 (IFN-γ, IL-12) and Type 2 (IL-4, IL-12) cytokines (Crawley et al., 2003). The major source for production of IgA and secretory IgA (sIgA) in the gastrointestinal tract and the gut lumen as well as IgA in the blood is from the upper ileal (IPP) and jejunal peyers patches (JPP). The IPP are present in fetal pigs; however, the JPP are not present because they require antigen exposure in order to develop (Butler et al., 2016).

IgA and IgG are essential antibodies for the pig to develop because of their ability to eliminate pathogens. IgG is known for its ability to eliminate parvovirus in crypt cells

and may play a role in eliminating PEDV by either neutralizing it in the lumen of the intestine or helping transfer the antigen through receptors on apical surfaces of microfold cells. Immunoglobulin A also plays a role in eliminating PEDV infection in neonatal pigs because of its presence in maternal IgA milk, which is transferred to the neonate (Poonsuk et al., 2016b).

BIOMARKERS OF INTESTINAL INTEGRITY

C-Reactive Protein

C-reactive protein (CRP) is an acute-phase protein (APP) created by the liver in response to activity of cytokines (Barbé et al. 2011). The cytokines that regulate CRP transcription are IL-1β, IL-6, and TNF-α (Saco et al., 2015). Structurally, CRP is made up of 5 identical subunits arranged as a cyclic pentamer. CRP has multiple different binding sites; the first is located on the binding face and can bind to 2 calcium atoms, the second also located on the binding face can bind to phosphocholine, which is commonly found on pathogenic bacteria. The third binding sites are located on the A face and are able to bind Clq, which then activates the C pathway, which then interact with FcγRs on phagocytic cells, thus mediating phagocytosis (Mold et al., 2002).

C-reactive protein is classified as a major positive APP because the concentration of it dramatically increases in the blood in response to a stimulus (Christoffersen et al., 2015). In fact, CRP levels in human blood can increase from one tenth to several hundred μg/mL in a 24 h period in response to stimuli (Mortensen et al., 2000). Some of the various stimuli CRP will respond to are infections, tissue damage, neoplastic growth, surgical trauma, inflammation, immunological disorders, and stress (Murata et al., 2004; Salamano et al., 2008). An average concentration of CRP in a pig's serum is below 22

μg/mL (Pomorska-Mól et al., 2013). This concentration will increase dramatically after one of the aforementioned stimuli is applied, thus making CRP a very good indicator of swine health status.

Serum Haptoglobin

Haptoglobin (Hp) plays an important role in the innate immune system. Haptoglobin like CRP is a major positive acute-phase protein and is synthesized by hepatocytes in the liver (Wyns et al., 2015). Plasma Hp acts as an antioxidant by binding to hemoglobin so an accumulation of free radicals do not build up in the body (Saco et al., 2016). Although hemoglobin is a critical molecule for transporting oxygen, the heme portion can be toxic. Therefore, hemoglobin is attached to Hp through a non-covalent protein-protein bond, which forms the haptoglobin-hemoglobin complex (Andersen et al., 2012). This complex is recognized by the specific surface receptor on macrophages, CD163, which is then disposed of by phagocytes (Murata et al., 2004).

Haptoglobin is an excellent indicator of disease because the reaction time after the onset of any illness compromising tissue homeostasis or injury is only 6 to 12 h, and can be measured easily in a blood sample (Heegaard et al., 2011). In fact, serum Hp concentrations of pigs infected with PRRS virus were elevated at 7 to 14 d post-inoculation (Gómez-Laguna et al., 2010). In a study by Pomorska-Mól et al. (2013) pigs were infected with *Pasteurella multocida* (Pm) and/or swine influenza virus (SIV) where Hp was one of the APP's used to determine the severity of infection. The Hp concentration in serum prior to inoculation was (0.83 mg/mL) and was the greatest at 5 d post inoculation (dpi) with both PM and SIV at (4.97 mg/mL). Serum Hp concentration began to increase at 2 d post inoculation and remained elevated until the completion of

the study, which makes Hp serum concentration an appropriate marker to monitor ongoing infection in pigs.

Glucagon-like peptide 2

Glucagon-like peptide 2 (GLP-2) is produced by the proglucagon gene and is made up of 35 AA, which are different from other mammals such as humans, rats, and cows, which only have 33 AA. Pigs possess an additional 2 neutral AA, Serine and Leucine, which were discovered through MALDI-TOF and TOF-TOF analyses (Pedersen et al., 2008).

Glucagon-like peptide 2 functions as a peptide hormone and is created through posttranslational processing of proglucagon in enteroendocrine L cells (Burrin et al., 2006; Vegge et al., 2013). There are glucagon-like peptide receptors (GLP-2R), which are part of the G-protein-coupled receptor family and are found in the hypothalamus and the gastrointestinal tract with the majority present in the jejunum (Paris et al., 2004; Chai et al., 2015).

There are numerous functions that GLP-2 serves in the body, amongst them are to enhance nutrient absorption, increase energy balance and gut barrier function, and to reduce inflammation in the intestine. All of which, can greatly improve the overall health of the animal. The release of GLP-2 into circulation is stimulated through the consumption of nutrients and when an intestinal injury occurs (Connor et al., 2016). The half-life of GLP-2 in circulation is extremely short lasting only 7 to 8 min in a pig.

Several studies have been done evaluating the effects various concentrations and forms of GLP-2 have on the gastrointestinal tract. In a study completed by Sigalet et al. (2014) the effects of GLP-2 on intestinal development were investigated in pigs from

suckling to weaning. It was shown that while a greater dose of natural GLP-2 did result in increased villus height and crypt depth in the jejunum and ileum, the control pigs did produce enough GLP-2 naturally to have adequate growth and intestine development.

Because GLP-2 has a short half-life there have been studies to evaluate the effect acylated GLP-2 peptide has on pig gastrointestinal tracts, since it has a longer half-life than GLP-2. Thymann et al. (2014) conducted a study to determine the effects acylated GLP-2 in low-sanitary conditions could have on weanling pigs. Results showed that because acylated GLP-2 had a half-life of approximately 19 h, gut adaptation during weaning was improved. However, in another experiment conducted by Thymann et al. (2014), which used native GLP-2 in weanling pigs, it was shown that native GLP-2 had no effect on weanling pigs ability to absorb nutrients. Although the reason may not be entirely to do with the short half-life, but because after weaning the number of GLP-2R are greatly reduced.

MICROBIOME

Function of Microbiome in Pigs

The gastrointestinal system of the pig is populated by a diverse and highly compact group of microorganisms whose numbers may exceed 10^{14} and many are bacteria (Dai et al., 2011). These microorganisms make up a complex community called the microbiome, which is made up of more organisms than our somatic and germ cells combined. The vast majority of culturable bacteria in the swine intestine are obligate anaerobes and gram-positive bacteria such as: *streptococci*, *lactobacilli*, *eubacteria*, *clostridia* and *peptostreptococci*. Only approximately 10% of the culturable microbes are gram-negative (Leser et al., 2000). Although there are numerous species that can be investigated through cell culture, there are a great deal of microbes that are incapable of growing in culture. However, they can be evaluated though a molecular approach called broad range sequencing of 16S ribosomal RNA (rRNA) genes (Hooper et al., 2001).

This advanced technology has exposed the diversity of the gastrointestinal microbial community in swine species (Lamendella et al., 2011). In fact, a study by Leser et al. (2002) was conducted to demonstrate just how unique the microbiota of pigs gastrointestinal tract are utilizing 16s rDNA sequence technology. Samples were obtained from 24 pigs each representing various ages, health status, and diets in order to encompass all the different microbes that populate the pig gut microbiome. This study discovered that based upon a 97% similarity criterion, there are 375 different phylotypes populating the pig GI tract.

These bacteria that populate the gastrointestinal tract of swine have several critical roles in the pig; the microbes help fight pathogens, extract nutrients, and assist

with immune and epithelium development (Looft et al., 2012). Microbes are able to help swine develop an immune system and eliminate pathogens because the microbes in a pigs' small intestine are essential for development of gut associated immune tissue (GALT), and have the ability to elicit adaptive antibody responses (Potockova et al., 2015). For example, according to a study by Mach et al. (2015) piglets expressing a higher concentration of *Prevotella* species at 70 d, had a higher concentration of serum IgA which could decrease their energy expenditure on fighting off pathogens thus, increasing performance.

Another demonstration of microbial impact on the gut is performed on germ-free mice using DNA microarray analysis to reveal that after colonization with just one microbe, *B. thetaiotaomicron,* the host's genes will be altered to regulate nutrient absorption and metabolism (Hooper et al., 2001). Yan et al. (2013) investigated the impact gut microbes could have upon metabolism and fat storage and concurred that gut microbes do in fact play a critical role. While gut microbes can greatly alter the way the digestive system functions, the microbes do play a significant role on their own in the hindgut of the pig. They are capable of breaking down the indigestible products in the swine diet that could not be hydrolyzed in the small intestine, such as the large quantities of cellulose they consume after they have reached maturity (Bayley, 1978). The microbes are also capable of recycling bile salts and providing their host with vitamin K and exogenous alkaline phosphatases (Kim et al., 2015).

Microbes are also able to provide both essential and non-essential AA to the pig, through the recycling of nitrogen back into the gut in order for the microbes to create AA. However, these AA may not be significant source for the host, unless the host is not

consuming enough AA (Bergen, 2015). Although, a study by Yang et al. (2014) demonstrated that the AA contributed by tightly attached bacteria in the gut may in fact compensate for the utilization of AA by the luminal bacteria, thus providing the pig with a source of AA.

Factors Affecting Gut Microbiota

The establishment of different microbes begins immediately after birth. A great deal of the neonate's microbiota comes from the sow: skin, feces, milk, and from the birth canal. Basically, the piglet is dependent upon the sow to obtain microbes (Mach et al., 2015). However, as the pig matures it obtains microbes on its own from various sources such as the environment and solid feed, which help to create the pig's more stable community of microbes. Microbes fluctuate constantly in the gut and the amount and type of microbe are dependent upon several factors: life stage, diet, health, and location in the gastrointestinal tract (Kim et al., 2015; Zhao et al., 2015).

As microbes populate the pig, many factors impact the success or failure in colonizing the gastrointestinal tract with healthy microbiota. One of these factors is stress, which coincides with weaning, which is the time pigs are starting to develop their microbiota making them vulnerable to its negative ramifications. According to Benis et al. (2015) exposure to stress early in a pig's development will greatly impact the host's microbiota development and have long-lasting effects on a pig's ability to develop a functional mucosal and systemic immune system. A very comprehensive review by Pajarillo et al. (2014) supports this conclusion that stress, in particular during the weaning process, results in poor microbial development.

This review also discusses another important factor that impacts the microbiome (i.e., diet). A dramatic change in diet (milk to solid) can have a significant impact on the microbiota. The most significant change is a shift from mostly *Bacteroides* species pre-weaning to mainly *Prevotella* species post-weaning. This may be due to the fact that *Bacteroides* are capable of utilizing mono and oligosaccharides found in milk, while *Prevotella* can utilize hemicelluloses and xylans present in plants. A study by Frese et al. (2015) also investigated the impact diet has upon swine microbiota. The microbiota of pigs were closely monitored using 16s rRNA technology for 7 wk (from birth until weaning) in order to see how much the microbial population changed as their diet was altered. Feed changes were in fact the major contributor to species variation as can be seen in an alpha diversity analysis, which clearly showed a difference in number of species as the pigs were changed from a milk to a solid diet. This study by Frese et al. (2015) also evaluated time as a factor that could potentially impact microbial populations. It was observed through an alpha diversity study that the diversity of the pig's microbiota slowly increased with time as major factor.

Disease is another factor that can drastically alter the pig microbiome. In a study by Koh et al. (2015), the microbiota from the large intestine of 14 pigs were evaluated, half of which were healthy and the other half which were infected with PEDV. A disparity in how microbes populated the guts of the 2 different groups of pigs was discovered. The normal group was mostly made up of commensal bacteria such as *Psychrobacter, Prevotella, and Faecalibacterium.* While the PEDV infected group were populated with a considerable amount of pathogenic bacteria such as *Fusobacterium and*

Escherichia. This study successfully demonstrated that when pigs are infected with a virus such as PEDV there is a marked microbial imbalance in the gastrointestinal tract.

CONCLUSION

Of all the events in a pig's life weaning is one of the most influential. The pig endures numerous stressors such as being transported to a completely new environment, mixing with new piglets, losing maternal immunity, and adapting to a solid diet. In spite of all these stressors, and with considerable assistance from producers who provide a high-quality diet and try to minimize the number of stressors the piglets are exposed too, piglets are still able to thrive. The pigs develop their own immune system to fight pathogens, alter their gut microbiota to prevent villous atrophy and decrease crypt depth, and consume enough feed to grow.

LITERATURE CITED

Andersen, C. B. et al. 2012. Structure of the haptoglobin-haemoglobin complex. Nature 489: 456-459.

Barbé, F., K. Atanasova, and K. Van Reeth. 2011. Cytokines and acute Phase proteins associated with acute swine influenza infection in pigs. Vet. J. 187: 48-53.

Bayley, H. S. 1978. Comparative physiology of the hindgut and its nutritional significance. J. Anim. Sci. 46: 1800-1802.

Benis, N. et al. 2015. Network analysis of temporal functionalities of the gut induced by perturbations in new-born piglets. BMC Genomics 16: 556.

Bergen, W. G. 2015. Small-intestinal or colonic microbiota as a potential amino acid source in animals. Amino Acids 47: 251-258 DOI: 10.1007/s00726-014-1875-z.
.

Bomba, L. et al. 2014. Gut response induced by weaning in piglet features marked changes in immune and inflammatory response. Funct. Integr. Genomics. 14: 657-671.

Bras, R. 2003. Spray-dried egg for weanling pigs. Zootec. 32:1901-1911.

Bruininx, E. M. et al. 2002. A prolonged photoperiod improves feed intake and energy metabolism of weanling pigs. J. Anim. Sci. 80: 1736-1745.

Burrin, D. G. et al. 2007. GLP-2 rapidly activates divergent intracellular signaling pathways involved in intestinal cell survival and proliferation in neonatal piglets. Am J Physiol Endocrinol Metab 292: E281-291.

Butler, J. E. et al. 2016. Antibody repertoire development in fetal and neonatal piglets. XXIV. Hypothesis: The ileal Peyer patches (IPP) are the major source of primary, undiversified IgA antibodies in newborn piglets. Dev. Comp. Immunol. 65: 340-351.

Butler, J. E., J. Sun, N. Wertz, and M. Sinkora. 2006. Antibody repertoire development in swine. Dev. Comp. Immunol. 30: 199-221.

Cera, K. R., D. C. Mahan, R. F. Cross, G. A. Reinhart, and R. E. Whitmoyer. 1988. Effect of age, weaning and postweaning diet on small intestinal growth and jejunal morphology in young swine. J. Anim. Sci. 66: 574-584.

Chai, Y. L., H. M. Ma, and J. Jiang. 2015. Molecular characterization, tissue expression profile and SNP analysis of porcine GLP2R. Genet. Mol. Res. 14: 12931-12941.

Christoffersen, B. et al. 2015. Age- and Sex-Associated Effects on Acute-Phase Proteins in Göttingen Minipigs. Comp. Med. 65: 333-341.

Coffey, R. D., and G. L. Cromwell. 1995. The impact of environment and antimicrobial agents on the growth response of early-weaned pigs to spray-dried porcine plasma. J. Anim. Sci. 73: 2532-2539.

Connor, E. E. et al. 2016. Glucagon-like peptide 2 and its beneficial effects on gut function and health in production animals. Domest. Anim. Endocrinol. 56 Suppl: S56-65.

Crawley, A., and B. N. Wilkie. 2003. Porcine Ig isotypes: function and molecular characteristics. Vaccine 21: 2911-2922.

Dai, Z. L., G. Wu, and W. Y. Zhu. 2011. Amino acid metabolism in intestinal bacteria: links between gut ecology and host health. Front. Biosci. (Landmark Ed) 16: 1768-1786.

Danielsen, M. et al. 2006. Proteome profiles of mucosal immunoglobulin uptake in inflamed porcine gut. Proteomics 6: 6588-6596.

David, J. C., J. F. Grongnet, and J. P. Lalles. 2002. Weaning affects the expression of heat shock proteins in different regions of the gastrointestinal tract of piglets. J. Nutr. 132: 2551-2561.

Davis, M. E., S. C. Sears, J. K. Apple, C. V. Maxwell, and Z. B. Johnson. 2006. Effect of weaning age and commingling after the nursery Phase of pigs in a wean-to-finish facility on growth, and humoral and behavioral indicators of well-being. J. Anim. Sci. 84: 743-756.

Dybkjaer, L., A. Jacobsen, F. Togersen, and H. Poulsen. 2006. Eating and drinking activity of newly weaned piglets: Effects of individual characteristics, social mixing, and addition of extra zinc to the feed. J. Anim. Sci. 84: 702-711.

Edge, H., J. Dalby, P. Rowlinson, and M. Varley. 2005. The effect of pellet diameter on the performance of young pigs. Livestock Production Science 97: 203-209.

Frese, S. A., K. Parker, C. C. Calvert, and D. A. Mills. 2015. Diet shapes the gut microbiome of pigs during nursing and weaning. Microbiome 3: 28.

Froning, G. W., R. L. Wehling, S. Cuppett, and L. Niemann. 1998. Moisture content and particle size of dehydrated egg yolk affect lipid and cholesterol extraction using supercritical carbon dioxide. Poult. Sci. 77: 1718-1722.

Funderburke, D. W., and R. W. Seerley. 1990. The effects of postweaning stressors on

pig weight change, blood, liver and digestive tract characteristics. J. Anim. Sci. 68: 155-162.

Garcia, A. et al. 2015. Effect of Provision of Feed and Water during Transport on the Welfare of Weaned Pigs. Animals (Basel) 5: 407-425.

Garcia, A. et al. 2016. Impact of Providing Feed and/or Water on Performance, Physiology, and Behavior of Weaned Pigs during a 32-h Transport. Animals (Basel) 6.

Gerber, P. F. et al. 2014. The spray-drying process is sufficient to inactivate infectious porcine epidemic diarrhea virus in plasma. Vet. Microbiol. 174: 86-92.

Grinstead, G. S. et al. 2000. Effects of a whey protein product and spray-dried animal plasma on growth performance of weanling pigs. J. Anim. Sci. 78: 647-657.

Gómez-Laguna, J. et al. 2010. Haptoglobin and C-reactive protein as biomarkers in the serum, saliva and meat juice of pigs experimentally infected with porcine reproductive and respiratory syndrome virus. Vet. J. 185: 83-87.

Harmon, B.G., M. Latour, and S. Norberg. Spray-dried eggs as a source of immune globulins for SEW pigs. 2002. Purdue University Swine Research Report. pp. 31-35.

Heegaard, P. M. et al. 2011. Optimal combinations of acute Phase proteins for detecting infectious disease in pigs. Vet. Res. 42: 50.

Hicks, T. A., J. J. McGlone, C. S. Whisnant, H. G. Kattesh, and R. L. Norman. 1998. Behavioral, endocrine, immune, and performance measures for pigs exposed to acute stress. J. Anim. Sci. 76: 474-483.

Hooper, L. V., and J. I. Gordon. 2001. Commensal host-bacterial relationships in the gut. Science 292: 1115-1118.

Horn, N., F. Ruch, G. Miller, K. M. Ajuwon, and O. Adeola. 2014. Impact of acute water and feed deprivation events on growth performance, intestinal characteristics, and serum stress markers in weaned pigs. J. Anim. Sci. 92: 4407-4416.

Hötzel, M. J., G.P.P. de Souza, O. A. Dalla Costa, L.C.P. Machado Filho. 2011. Disentangling the effects of weaning stressors on piglets' behavior and feed intake: Changing the housing and social environment. Applied Animal Behaviour Science. 135:44-50.

Hyun, Y., M. Ellis, G. Riskowski, and R.W. Johnson. 1998. Growth performance of pigs subjected to multiple concurrent environmental stressors. J. Anim. Sci. 76: 721-727.

Ignário, R. M., S. C. da Silva Lannes. 2007. Preparation of powdered egg yolk using a mini spray dryer. Cienc. Tecnol. Aliment. 27:729-732.

James, B.W., J.C. Sparks, M.H. Jurgens, and D.R. Zimmerman. 2000. Comparison of inedible egg product and spray-dried plasma as sources of protein in weanling pigs. Iowa State University: Nutrition Research Report ASL-R1658. Online. Available: http://www.ipic.iastate.edu/reports/ 99swinereports/asl-1658.pdf.

Jin, L. Z., S. K. Baidoo, R. R. Marquardt, and A. A. Frohlich. 1998. In vitro inhibition of adhesion of enterotoxigenic Escherichia coli K88 to piglet intestinal mucus by egg-yolk antibodies. FEMS Immunol. Med. Microbiol. 21: 313-321.

Kaminjolo, J. S. and A. A. Adesiyun. 1994. Rotavirus infection in calves, piglets, lambs and goat kids in Trinidad. Br. Vet. J. 150:293-299.

Kielland, C., V. Rootwelt, O. Reksen, and T. Framstad. 2015. The association between immunoglobulin G in sow colostrum and piglet plasma. J. Anim. Sci. 93: 4453-4462.

Kim, H. B., and R. E. Isaacson. 2015. The pig gut microbial diversity: Understanding the pig gut microbial ecology through the next generation high throughput sequencing. Vet. Microbiol. 177: 242-251.

Koh, H. W., M. S. Kim, J. S. Lee, H. Kim, and S. J. Park. 2015. Changes in the Swine Gut Microbiota in Response to Porcine Epidemic Diarrhea Infection. Microbes. Environ. 30: 284-287.

Lamendella, R., J. W. Domingo, S. Ghosh, J. Martinson, and D. B. Oerther. 2011. Comparative fecal metagenomics unveils unique functional capacity of the swine gut. BMC Microbiol. 11: 103.

Leser T. D. et. al. 2000. Changes in bacterial community structure in the colon of pigs fed different experimental diets and after infection with *Brachyspira hyodsenteriae.* Appl. Environ. Microbiol. 66: 3290-3296.

Leser, T. D. et al. 2002. Culture-independent analysis of gut bacteria: the pig gastrointestinal tract microbiota revisited. Appl. Environ. Microbiol. 68: 673-690.

Li, X. et. al. 2008. Chitosan-alginate microcapsules for oral delivery of egg yolk immunoglobulin (IgY): In vivo evaluation in a pig model of enteric colibacillosis. J. Vet. Immunol. and Immunopath. 129:132-136.

Looft, T. et al. 2012. In-feed antibiotic effects on the swine intestinal microbiome. Proc.

Natl. Acad. Sci. U S A 109: 1691-1696.

Mach, N. et al. 2015. Early-life establishment of the swine gut microbiome and impact on host phenotypes. Environ. Microbiol. Rep 7: 554-569.

Marquardt, R. R. et. al. 1999. Passive protective effect of egg-yolk antibodies against enterotoxigenic *Escherichia coli* K88+ infection in neonatal and early-weaned piglets. FEMS. Immunol. And Med. Microbiol. 23:283-288.

Mathew, A. G., S. Rattanatabtimtong, C. M. Nyachoti, and L. Fang. 2009. Effects of in-feed egg yolk antibodies on Salmonella shedding, bacterial antibiotic resistance, and health of pigs. J. Food. Prot. 72: 267-273.

McGlone, J. J., W.F. Stansbury, and L.F. Tribble. 1987. Effects of heat and social stressors and within pen weight variation on young pig performance and agonistic behavior. J. Anim. Sci. 65: 456-462.

Mold, C., W. Rodriguez, B. Rodic-Polic, and T. W. Du Clos. 2002. C-reactive protein mediates protection from lipopolysaccharide through interactions with Fc gamma R. J. Immunol. 169: 7019-7025.

Mortensen, R. F., and W. Zhong. 2000. Regulation of phagocytic leukocyte activities by C-reactive protein. J. Leukoc. Biol. 67: 495-500.

Murata, H., N. Shimada, and M. Yoshioka. 2004. Current research on acute Phase proteins in veterinary diagnosis: an overview. Vet. J. 168: 28-40.

Niewold, T. et al. 2007. Dietary specific antibodies in spray-dried immune plasma prevent enterotoxigenic Escherichia coli F4 (ETEC) post weaning diarrhoea in piglets. Veterinary Microbiology 124: 362-369.

Norin, S. L., P.S. Miller, A. Lewis, and D. Reese. 1998. Protein sources of segregated early weaned (SEW) pigs. Nebraska Swine Reports. Paper 1555.

Ogawa, S. et al. 2016. The effect of colostrum ingestion during the first 24 hours of life on early postnatal development of piglet immune systems. J. Anim. Sci.

Opriessnig, T., C. T. Xiao, P. F. Gerber, J. Zhang, and P. G. Halbur. 2014. Porcine epidemic diarrhea virus RNA present in commercial spray-dried porcine plasma is not infectious to naïve pigs. PLoS One 9: e104766.

Owen, K. Q., J. L. Nelssen, M. D. Tokah, R. D., Goodband, S. S. Dritz, and L. J. Kats. 1993. Spray-dried egg protein diets for early-weaned starter pigs. Swine Day. SRP695. pp. 50-53.

Owusu-Asiedu, A., S. K. Baidoot, C. M. Nyachoti, and R. R. Marquardt. 2002b. Response of early-weaned pigs to spray-dried porcine or animal plasma-based

diets supplemented with egg-yolk antibodies against enterotoxigenic Escherichia colil. J. Anim. Sci. 80: 2895-2903.

Owusu-Asiedu, A., C. M. Nyachoti, S. K. Baidoo, R. R. Marquardt, and X. Yang. 2003a. Response of early-weaned pigs to an enterotoxigenic Escherichia coli (K88) challenge when fed diets containing spray-dried porcine plasma or pea protein isolate plus egg yolk antibody. J. Anim. Sci. 81: 1781-1789.

Owusu-Asiedu, A., C. M. Nyachoti, and R. R. Marquardt. 2003b. Response of early-weaned pigs to an enterotoxigenic Escherichia coli (K88) challenge whenfed diets containing spray-dried porcine plasma or pea protein isolate plus egg yolk antibody, zinc oxide, fumaric acid, or antibiotic. J. Anim. Sci. 81: 1790-1798.

Pajarillo, A.B., E., J. P. Chae, M. P. Balolong, H. Bum Kim, and D. K. Kang. 2014. Assessment of fecal bacterial diversity among healthy piglets during the weaning transition. J. Gen. Appl. Microbiol. 60: 140-146.

Paris, M. C. et al. 2004. Plasma GLP-2 levels and intestinal markers in the juvenile pig during intestinal adaptation: effects of different diet regimens. Dig. Dis. Sci. 49: 1688-1695.

Pasick, J. et. al. 2014. Investigation into the role of potentially contaminated feed as a source of first-detected outbreaks of porcine epidemic diarrhea in Canada. Transboundary and Emerging Diseases. 61:397-410.

Pasma, T., M. C. Furness, D. Alves, and P. Aubry. 2016. Outbreak investigation of porcine epidemic diarrhea in swine in Ontario. Can. Vet. J. 57: 84-89.

Pastorelli, H. et al. 2012. Sanitary housing conditions modify the performance and behavioural response of weaned pigs to feed- and housing-related stressors. Animal 6: 1811-1820.

Patterson, A. R. 2010. Efficacy of experimentally produced spray-dried plasma on infectivity of porcine circovirus type 2^{1} No. 88. p 4078–4085 J. Anim. Sci.

Peace, R. M. et al. 2011. Spray-dried porcine plasma influences intestinal barrier function, inflammation, and diarrhea in weaned pigs. J. Nutr. 141: 1312-1317.

Pedersen, N. B. et al. 2008. Porcine glucagon-like peptide-2: structure, signaling, metabolism and effects. Regul. Pept. 146: 310-320.

Pierce, J. L., G. L. Cromwell, M. D. Lindemann, L. E. Russell, and E. M. Weaver. 2005. Effects of spray-dried animal plasma and immunoglobulins on performance of early weaned pigs. J. Anim. Sci. 83: 2876-2885.

Polo, J. et al. 2013. Neutralizing antibodies against porcine circovirus type 2 in liquid pooled plasma contribute to the biosafety of commercially manufactured spray-dried porcine plasma. J. Anim. Sci. 91: 2192-2198.

Pomorska-Mól, M., I. Markowska-Daniel, K. Kwit, K. Stępniewska, and Z. Pejsak. 2013. C-reactive protein, haptoglobin, serum amyloid A and pig major acute Phase protein response in pigs simultaneously infected with H1N1 swine influenza virus and Pasteurella multocida. BMC Vet. Res. 9: 14.

Poonsuk, K. et al. 2016. Does Circulating Antibody Play a Role in the Protection of Piglets against Porcine Epidemic Diarrhea Virus? PLoS One 11: e0153041.

Potockova, H., J. Sinkorova, K. Karova, and M. Sinkora. 2015. The distribution of lymphoid cells in the small intestine of germ-free and conventional piglets. Dev. Comp. Immunol. 51: 99-107.

Pujols, J. et al. 2008. Lack of transmission of porcine circovirus type 2 to weanling pigs by feeding them spray-dried porcine plasma. Vet. Rec. 163: 536-538.

Rossi, M. et al. 2013. Developments in understanding and assessment of egg and egg product quality over the last century. Worlds Poultry Science Journal 69: 414-429.

Saco, Y. et al. 2016. C-reactive protein, haptoglobin and Pig-Major acute Phase protein profiles of pigs infected experimentally by different isolates of porcine reproductive and respiratory syndrome virus. Vet. Microbiol. 183: 9-15.

Salamano, G. et al. 2008. Changes in haptoglobin, C-reactive protein and pig-MAP during a housing period following long distance transport in swine. Vet. J. 177: 110-115.

Schwartz, K. J., and R. D. Glock. 1978. Rotavirus infections in swine. Iowa State University Veterinarian. Vol. 40 Iss. 3, Article 7.

Sigalet, D. L. et al. 2014. Effects of chronic glucagon-like peptide-2 therapy during weaning in neonatal pigs. Regul. Pept. 188: 70-80.

Song, M. et al. 2012. Effects of dietary spray-dried egg on growth performance and health of weaned pigs. J. Anim. Sci. 90: 3080-3087.

Sutherland, M. A., B. L. Backus, and J. J. McGlone. 2014. Effects of Transport at Weaning on the Behavior, Physiology and Performance of Pigs. Animals (Basel) 4: 657-669.

Tang, S. G. et al. 2015. Chemical Compositions of Egg Yolks and Egg Quality of Laying Hens Fed Prebiotic, Probiotic, and Synbiotic Diets. J. Food. Sci. 80: C1686-

1695.

Thacker. P. A. 2013. Alternatives to antibiotics as growth promoters for use in swine production: a review. J. Anim. Sci. and Biotech. 4(1):35.

Thymann, T. et al. 2014. Glucagon-like peptide 2 treatment may improve intestinal adaptation during weaning. J. Anim. Sci. 92: 2070-2079.

Torrallardona, D., M. R. Conde, I. Badiola, J. Polo, and J. Brufau. 2003. Effect of fishmeal replacement with spray-dried animal plasma and colistin on intestinal structure, intestinal microbiology, and performance of weanling pigs challenged with Escherichia coli K99. J. Anim. Sci. 81: 1220-1226.

Tran, H. et al. 2014. Effects of spray-dried porcine plasma on growth performance, immune response, total antioxidant capacity, and gut morphology of nursery pigs. J. Anim. Sci. 92: 4494-4504.

Trujillo-Ortega, M. E. et al. 2016. Isolation and characterization of porcine epidemic diarrhea virus associated with the 2014 disease outbreak in Mexico: case report. BMC Vet. Res. 12: 132.

van Dijk, A. J. et al. 2002. Small intestinal morphology and disaccharidase activities in early-weaned piglets fed a diet containing spray-dried porcine plasma. J. Vet. Med. A. Physiol. Pathol. Clin. Med. 2:81-86.

van Dijk, A. J. et al. 2001. Small intestinal morphology in weaned piglets fed a diet containing spray-dried porcine plasma. Res. Vet. Sci. 71: 17-22.

Vega, C. G. et al. 2012. IgY antibodies protect against human Rotavirus induced diarrhea in the neonatal gnotobiotic piglet disease model. PLoS One 7: e42788.

Vegge, A. et al. 2013. Glucagon-like peptide-2 induces rapid digestive adaptation following intestinal resection in preterm neonates. Am. J. Physiol. Gastrointest. Liver. Physiol. 305: G277-285.

Weaver, A. C., J. M. Campbell, J. D. Crenshaw, J. Polo, and S. W. Kim. 2014. Efficacy of dietary spray-dried plasma protein to mitigate the negative effects on performance of pigs fed diets with corn naturally contaminated with multiple mycotoxins. J. Anim. Sci. 92: 3878-3886.

Werhahn, E., F. Klobasa, and J. E. Butler. 1981. Investigation of some factors which influence the absorption of IgG by the neonatal piglet. Vet. Immunol. Immunopathol. 2: 35-51.

Wyns, H., E. Plessers, P. De Backer, E. Meyer, and S. Croubels. 2015. In vivo porcine lipopolysaccharide inflammation models to study immunomodulation of drugs.

Vet. Immunol. Immunopathol. 166: 58-69.

Yan, H. et al. 2013. Dietary fat content and fiber type modulate hind gut microbial community and metabolic markers in the pig. PLoS One 8: e59581.

Yang, Y. X., Z. L. Dai, and W. Y. Zhu. 2014. Important impacts of intestinal bacteria on utilization of dietary amino acids in pigs. Amino Acids 46: 2489-2501.

Zhang, S. et al. 2015a. Comparison of spray-dried egg and albumen powder with conventional animal protein sources as feed ingredients in diets fed to weaned pigs. J. Anim. Sci. 86: 772-781.

Zhang, Y. et al. 2015b. Spray-dried chicken plasma improves intestinal digestive function and regulates intestinal selected microflora in weaning piglets. J. Anim. Sci. 93: 2967-2976.

Zhao, W. et al. 2015. The dynamic distribution of porcine microbiota across different ages and gastrointestinal tract segments. PLoS One 10: e0117441.

Table 1.1 Feed Ingredient Composition (adapted from American Egg Board 1999)

Composition, %	Whole Egg (dried)	Egg Yolk (dried)	Egg White (dried)
Crude protein	47.35	34.25	81.1
Dry Matter	96.3	97.3	93.5
Total lipid	39.2	52.9	0.4
Ash	3.4	3.3	3.6
Calcium	0.236	0.307	0.104
Phosphorus	0.629	1.04	0.104
Lysine	3.36	2.477	5.94
Methionine	1.503	0.86	3.013
Tryptophan	0.78	0.393	1.437
Threonine	2.143	1.55	3.653

CHAPTER 2: EFFECTS OF EGG YOLK ON GROWTH PERFORMANCE, CIRCULATING IMMUNOGLOBULINS, AND BIOMARKERS OF INTESTINAL INTEGRITY OF NURSERY PIGS

ABSTRACT

An experiment was conducted to determine the effects of egg yolk compared to spray-dried plasma on growth performance, circulating immunoglobulins, and biomarkers of intestinal integrity in nursery pigs. Crossbred pigs (n = 72) were weaned at 24 d (7 ± 0.54 kg) and divided into 3 dietary treatments in a completely randomized design (CRD), resulting in 6 pens per treatment with 4 pigs per pen (2 barrows, 2 gilts). The 3 dietary treatments were standard nursery diets supplemented with spray-dried plasma (2.36%), egg yolk (6%), and a control, which were fed in 2 phases (Phase I: d 0 to 7; Phase II: d 7 to 21). In the third Phase (Phase III: d 21 to 28), all pigs were fed the same diet. Pigs and feeders were weighed and blood samples were collected on d 0, 7, 14, 21, and 28. Growth performance (ADG, ADFI, G:F) was analyzed using PROC GLIMMIX of SAS. Circulating immunoglobulins (IgA and IgG) and biomarkers of intestinal integrity (CRP, GLP-2, Hp) were analyzed using repeated measures by time. For Phase I and II, growth performance was not affected by dietary treatment. Pigs consuming the egg yolk diet had greater ($P < 0.05$) ADG vs. the plasma group for Phase III. Circulating immunoglobulins were not affected by dietary treatment. The concentration of IgA increased from d 0 (0.17 ng/mL) to 28 (0.3 ng/mL; time; $P < 0.01$). The concentration of IgG did not respond ($P > 0.10$) over the course of the 28-d trial. CRP and Hp concentrations remained constant during the experiment. Treatment did effect GLP-2 concentration ($P < 0.01$) where egg yolk fed pigs had the greatest concentration (1.19 ng/mL) followed by plasma and control (0.72 and 0.51 ng/mL; respectively). Cost analysis of the diets showed that egg yolk ($2.30/lb) was the least costly diet during Phase II and the most costly during Phase I, while plasma ($2.16/lb)

was the reverse. Based upon these results it is evident that growth performance, circulating immunoglobulins, and biomarkers of intestinal integrity are similar in pigs receiving diets containing egg yolk or spray-dried plasma.

Key words: cost analysis, egg yolk, growth performance, immunological response, nursery pigs

INTRODUCTION

Weaning is an extremely stressful time in a pig's life (Lallès et al., 2004), and most commonly results in poor growth performance, malabsorption of nutrients, and increased susceptibility to disease (Hicks et al., 1998; Horn et al., 2014).

A high-quality protein is a crucial component of a weaning pig's diet. A common source of protein in a nursery diet is SDAP, which according to numerous studies can help weaning pigs to overcome the stressors they may experience during weaning (Coffey et al., 1995; Patterson et al., 2010; Polo et al., 2013). However, due to the spread of PEDV and the fear that SDAP may have been a factor in transmission, combined with its relatively high price, a need for alternative proteins, such as SDEY was created.

Egg has an excellent AA profile (Owen et al., 1993) and is high in nutrients (Tang et al., 2015). Nursery diet studies have shown that egg has improved growth performance, overall health status of pigs, and may be a suitable replacement for antibiotics (Jin et al., 1998; Hong et al., 2004; Song et al., 2012).

Pigs are capable of absorbing immunoglobulins such as IgA and IgG immediately after birth, however these antibodies have very short half-lives, thus the pig must begin creating its own post weaning in order to survive (Werhahn et al., 1981; Ogawa et al., 2016). Another way to measure pig health are biomarkers of intestinal integrity. CRP removes damaged cells and toxins and Hp acts as an antioxidant removing free radicals (Andersen et al., 2012; Saco et al., 2016). GLP-2 acts specifically on the gastrointestinal tract to improve absorption of nutrients, and reduce inflammation (Connor et al., 2016).

This study investigates whether or not spray-dried egg yolk can be used as a replacement protein for spray-dried plasma in nursery pig diets. Growth performance (ADG, ADFI, and G:F) and health (biomarkers of intestinal integrity and circulating immunoglobulins) were investigated throughout the course of this experiment in order to see if this replacement was a possibility.

MATERIALS AND METHODS

Animals and Experimental Design

The experimental protocol was reviewed and approved by the Institutional Animal Care and Use Committee of the University of Nebraska, Lincoln. Seventy-two, 21 to 24 d-of-age weaned pigs were sorted by sex and initial BW and randomly allocated to 1 of 3 dietary treatments. The average initial BW was 7.0 ± 0.54 kg and there were 6 replicates per treatment with 2 gilts and 2 barrows per pen. The pigs had ad libitum access to feed and water. Heat lamps were placed in each pen to maintain a room temperature of 27° C for the first 2 wk and subsequently the temperature was gradually decreased by 2 degrees each wk until the study was completed. The study consisted of a 4-wk feeding experiment divided into 3 Phases: Phase I (d 0 to 7), Phase II (d 7 to 21), and Phase III (d 21 to 28) which consisted of only the control (CTR) diet to investigate any carry over effects.

Dietary Treatments

Treatments included diets containing the following: 1) Plasma (2.36%); 2) Egg yolk (6%); and 3) Control (CTL; no egg yolk or spray-dried plasma). The ingredient composition and calculated analysis of experimental diets are presented in Tables 2.1 and 2.2. Phase I, II, and III dietary treatments were formulated to contain 1.50%, 1.35% and

1.23% standard ileal digestible Lys, respectively. Total Lys was 1.66% in Phase I, 1.52% in Phase II and 1.39% in Phase III diets. Diets were formulated to meet or exceed the NRC (2012) requirements. The spray-dried egg yolk was provided by 2 different companies, Sparboe (Litchfield, MI) and Nepco (Social Circle, GA), and was provided in equal amounts in the egg yolk treatment for both Phase I and II diets. Sparboe egg yolk contained 34.65% CP and 3.05% Lys while Nepco egg yolk contained 37.20% CP and 3.14% Lys, and the 50/50 mixture contained 35.86% CP and 3.08% Lys. Rose Acre Farms (Seymour, IN) provided the spray-dried plasma for both Phase I and II. The third Phase was a common diet provided among treatment groups to investigate any carry over affects.

Data and Sample Collection

Individual pig and feeder weights were recorded over the course of the experiment on d 0, 7, 14, 21, and 28, in order to calculate average daily gain (ADG), average daily feed intake (ADFI), and feed efficiency (gain:feed; G:F). Blood samples were obtained from every pig via jugular venipuncture, and serum was collected after centrifugation (20 min at 1,500×*g*). Blood samples were obtained from every pig at the same time points that pig BW measurements were taken. The serum samples were stored at -20 °C for subsequent analysis.

Serum Immune Measurements

In order to analyze the concentrations of circulating immunoglobulins IgA and IgG, a porcine specific enzyme-linked immunosorbent assay (ELISA; Bethyl Laboratories Inc.; Montgomery, TX) was used. IgG ELISA was performed by diluting the serum with assay buffer to a concentration of 1:100,000. The IgA ELISA was also

performed with assay buffer a dilution of 1:1,000. The ELISA's for both IgA and IgG were then carried out according to the manufacture's instructions.

Biomarkers of Intestinal Integrity Measurements

An ELISA specific for porcine was utilized to quantify intestinal integrity biomarker concentrations of Glucagon-like Peptide 2 (GLP-2; Assaypro; St. Charles, MO); Haptoglobin (Hp;Alpco; Salem, NH); and C-reactive protein (CRP; DuoSet; Minneapolis, MN). Serum was applied to the provided pre-coated plate (undiluted) for GLP-2 and at a 1:10,000 dilution with assay buffer for Hp and analyzed as directed in the provided instructions. For the analysis of CRP, serum was diluted to a concentration of 1:50,000 with assay buffer and also performed according to manufacture's protocol.

Statistical Analysis

Each pen was treated as an experimental unit and a random effect for growth performance, immune parameters, and intestinal integrity biomarker data. Treatment was considered a fixed effect. The model to analyze the data included a treatment and treatment × time (d) interaction, and all data were analyzed as a completely randomized design using the GLIMMIX procedure (SAS Inst. Inc., Cary, NC). All means were presented as least-squares means (± SEM).

RESULTS AND DISCUSSION

Growth Performance

There have not been many studies conducted investigating the value of egg yolk as a protein source for nursery pigs. However, the few studies that have been completed have mixed results. A series of studies performed by Song et al. (2012) concluded that spray-dried egg improved the growth rate of the pigs in comparison to a control diet, and

2 additional studies performed showed that growth rate was unaffected with the addition of spray-dried egg when compared to the performance of pigs given diets with spray-dried plasma. Another study performed by Zhang et al. (2015) evaluated the effects of replacing conventional animal proteins such as SDAP with unconventional ones, such as SDE. The results showed that during the course of the experiment, growth performance (ADG, ADFI, G:F) of pigs was not affected by protein source.

This study is consistent with previous reports, which showed that overall, pigs receiving the spray-dried egg yolk diet performed similarly to pigs receiving the spray-dried plasma diet. As can be seen in Figure 2.6, it was also shown that there were no effects of dietary treatment on pig BW at any time point during the experiment and there were no differences observed in ADG, ADFI, or G:F between pigs fed the plasma or the egg yolk treatments during Phase I or Phase II. However, there were certain instances where pigs fed the egg yolk diet performed superiorly to the plasma diet. For example, during Phase III, when the ADG of the pigs who consumed the egg yolk diet (648 g) tended to be greater ($P < 0.10$) than pigs that consumed the plasma and control diets respectively (539 g; 598 g). Which could be a result of the significantly greater concentrations of GLP-2 overall ($P < 0.10$) in the pigs consuming the SDEY treatment because GLP-2 is known to help with nutrient absorption resulting in greater ADG values.

Immune parameters: IgA and IgG

According to Werhahn et al. (1981) it is critical for pig survival that immunoglobulins such as IgA and IgG are absorbed post farrowing; however, after gut closer occurs which is within the first 12-24 hours postnatally columnar enterocytes cease

to allow the absorption of intact proteins (Butler et al., 2006; Danielsen et al., 2006; Kielland et al., 2015). This means that the pig has passive immunity, but due to the short survival time of colostral IgA (approximately 7 d) and colostral IgG (between 12 to 14 d), the pig is producing its own antibodies by the time weaning occurs (Thompson et al., 2008; Ogawa et al., 2016). This observation that piglets can not absorb large proteins post gut closure and are in fact creating their own immunoglobulins is the reason why several studies such as Zhang et al. (2016) have concluded that there must be another mechanism for dietary treatments including blood plasma and other proteins containing immunoglobulins such as egg yolk to affect growth performance and immune health of pigs.

There were no observed treatment × time (d) interactions for IgA or IgG, and circulating immunoglobulins affected by dietary treatment during the course of the experiment, which can be seen in Figure 2.7. This observation is similar to Weaver et al. (2014) who showed that the newly weaned pigs IgG serum concentrations were also not affected by dietary treatment (0% SDPP diet = 41.32 ng/mL; 6% SDPP diet = 37.33 ng/mL).

The concentration of IgA increased over the course of the experiment (d 0 the concentration was 0.17 mg/mL and by d 28, the concentration was 0.56 mg/mL). These results for IgA concentrations correlate well with the findings of Tran et al. (2014), which showed that over the course of the experiment, nursery pig serum IgA concentrations increased from 0.14 mg/mL at weaning to 0.41 mg/mL by d 28. A possible explanation for the increased IgA concentration may be maturation of the piglet's immune system,

because as was previously discussed, around the time of weaning the piglet becomes responsible for creating its own antibodies.

It was also observed that on d 14, pigs consuming the plasma diet tended to have the greatest concentration of IgA ($P < 0.09$). A potential reason for this could be that by d 14 pigs consuming the plasma treatment were having the greatest success relative to producing IgA and by d 21 pigs consuming the other dietary treatments had caught up relative to producing IgA.

The concentration of IgG in this study did not change over time, its concentration remained constant ($P > 0.10$) over the course of the 28-d experiment. A possible explanation for the IgG concentration remaining constant could be a result of a slow adaption to developing IgG. Perhaps the pig is maintaining the concentration of IgG it obtained from the colostrum and milk it consumed and has yet to develop its own antibodies (Hunter, 1986).

Biomarkers of Intestinal Integrity: GLP-2, Hp, and CRP

The biomarkers that were investigated in this study were glucagon-like peptide-2 (GLP-2), haptoglobin (Hp), and C-reactive protein (CRP). All 3 increase and decrease in concentration in pig blood in response to various physiological needs of the pig and to potential challenges.

It can be observed in Figure 2.10 that there were no differences among treatments for GLP-2 on d 0 or d 14 when analyzed separately and a potential reason why treatment did not have an effect on GLP-2 concentration could be related to feed intake. According to a study by Burrin et al. (2006), the secretion of GLP-2 is stimulated by feed intake and because there were no differences in ADFI between treatments on d 0 or d 14, it would be

plausible that lack of differences in feed intake contributed to the lack in differences in GLP-2 concentration.

There are numerous reasons why it is desirable for animals to have an elevated concentration of GLP-2. GLP-2 reduces inflammation, maintains anti-oxidative properties, provides protection against gut ischemia, enhances intestinal transport, increases absorption of nutrients, and reduces inflammation-induced oxidative stress in intestinal tissues (Chai et al., 2015; Connor et al., 2016). The aforementioned statement supports the observation that the concentration of GLP-2 was affected by treatment ($P < 0.10$) and the pigs with the greatest concentration were the pigs fed the egg yolk dietary treatment (1.191 ng/mL), while pigs fed the plasma and control treatments had decreased concentrations (0.717 ng/mL; 0.506 ng/mL respectively). A possible explanation for this observation is that GLP-2 helps with nutrient absorption and maintains the intestinal system guarded from disease, it could translate to the observation that the pigs consuming the egg yolk treatment had the greatest ADG ($P < 0.10$) at the end of the experiment. The pigs receiving the egg yolk treatment would not be capable of exhibiting the greatest growth rate if they did not have a healthy gastrointestinal tract and the ability to absorb nutrients efficiently both of which are contributed to by GLP-2.

Haptoglobin and CRP are both acute phase proteins (APP) and important components of the innate immune system (Saco et al., 2016). These APP both react in different ways and to different diseases; however, both can be used to determine the health status of an animal at any given time (Heegaard et al., 2011). Through measuring the concentration of CRP or Hp in the blood it can be deduced how immunocompromised the animal is, CRP and Hp both increase in response to inflammation, trauma, and

infection (Mold et al., 2002; Gutiérrez et al., 2012). It can be observed in Figures 2.8 and 2.9 respectively; that the concentrations of Hp and CRP remained constant over the course of the experiment ($P > 0.10$) and were not affected by dietary treatment, with the exception of individual time points (d 0 and 14) there were no differences among treatments for either APP. The piglets in this experiment were not challenged with a disease and were kept in a clean environment and because both CRP and Hp are excellent indicators of a disease compromised animal, we conclude that APP did not increase in concentration because the pigs were healthy.

CONCLUSION

The objective of this study was to determine whether a spray-dried egg yolk diet could be used to replace spray-dried plasma diet for nursery piglets. Based upon the results of this experiment, both protein sources could be used with similar effects on growth performance. Pigs maintained similar growth performance, biomarkers of intestinal integrity values, and immune parameter values. Thus, we recommend the use of the protein source that is more economically available at the time of purchase. In this case, the most cost efficient diet during Phase I was the plasma diet; however, during Phase II the egg yolk diet was the most cost effective. During Phase III a common diet was used, but the carry over effects on the pig performance indicated that the egg yolk was the least expensive. Thus, based on these results it would be most cost efficient to use egg yolk during Phase II and the plasma diet during the Phase I.

LITERATURE CITED

Andersen, C. B. et al. 2012. Structure of the haptoglobin-haemoglobin complex. Nature 489: 456-459.

Butler, J. E., J. Sun, N. Wertz, and M. Sinkora. 2006. Antibody repertoire development in swine. Dev. Comp. Immunol. 30: 199-221.

Coffey, R. D., and G. L. Cromwell. 1995. The impact of environment and antimicrobial agents on the growth response of early-weaned pigs to spray-dried porcine plasma. J. Anim. Sci. 73: 2532-2539.

Connor, E. E. et al. 2016. Glucagon-like peptide 2 and its beneficial effects on gut function and health in production animals. Domest. Anim. Endocrinol. 56 Suppl: S56-65.

Danielsen, M. et al. 2006. Proteome profiles of mucosal immunoglobulin uptake in inflamed porcine gut. Proteomics 6: 6588-6596.

Hicks, T. A., J. J. McGlone, C. S. Whisnant, H. G. Kattesh, and R. L. Norman. 1998. Behavioral, endocrine, immune, and performance measures for pigs exposed to acute stress. J. Anim. Sci. 76: 474-483.

Hong, J.W., O.S. Kwon, B.J. Min, W.B. Lee, K.S. Shon, I.H. Kim and J.W. Kim. 2004. Evaluation effects of spray-dried egg protein containing specific egg yolk antibodies as a substitute for spray-dried plasma protein or antibiotics in weaned pigs. Asian-Aust. J. Anim. Sci. 17(8): 1139-1144.

Horn, N., F. Ruch, G. Miller, K. M. Ajuwon, and O. Adeola. 2014. Impact of acute water and feed deprivation events on growth performance, intestinal characteristics, and serum stress markers in weaned pigs. J. Anim. Sci. 92: 4407-4416.

Hötzel, M. J., G.P.P. de Souza, O. A. Dalla Costa, L.C.P. Machado Filho. 2011. Disentangling the effects of weaning stressors on piglets' behavior and feed intake: Changing the housing and social environment. Applied Animal Behaviour Science. 135:44-50.

Hunter, P. 1986. The immune system of the neonatal and weaner piglet: A review. J S Afr Vet Assoc 57: 243-245.

Jin, L. Z., S. K. Baidoo, R. R. Marquardt, and A. A. Frohlich. 1998. In vitro inhibition of adhesion of enterotoxigenic Escherichia coli K88 to piglet intestinal mucus by egg-yolk antibodies. FEMS Immunol. Med. Microbiol. 21: 313-321.

Kielland, C., V. Rootwelt, O. Reksen, and T. Framstad. 2015. The association between immunoglobulin G in sow colostrum and piglet plasma. J. Anim. Sci. 93: 4453-

4462.

Lallés, J. P., G. Boudry, C. Favier, N. Le Floch, I. Luron, L. Montagne, I. P. Oswald, S. Pié, C. Piel, B. Séve. 2004. Gut function and dysfunction in young pigs: physiology. Anim. Res. 53: 301-316

Lallés, J.P., P. Bosi, H. Smidt, C.R. Stokes. 2007. Nutritional management of gut health in pigs around weaning. Proc Nutr Soc 66: 260-268. DOI:10.1017/S0029665107005484

Lowe, J., P. Gauger, K. Harmon, J. Zhang, J. Connor, P. Yeske, T. Loula, I. Levis, L. Dufresne, and R. Main. 2014. Role of transportation in spread of porcine epidemic diarrhea virus infection, United States. Emerg Infect Dis 20: 872-874.

Ogawa, S. et al. 2016. The effect of colostrum ingestion during the first 24 hours of life on early postnatal development of piglet immune systems. J. Anim. Sci.

Owen, K. Q., J. L. Nelssen, M. D. Tokah, R. D., Goodband, S. S. Dritz, and L. J. Kats. 1993. Spray-dried egg protein diets for early-weaned starter pigs. Swine Day. SRP695. pp. 50-53.

Patterson, A. R. 2010. Efficacy of experimentally produced spray-dried plasma on infectivity of porcine circovirus type 2[1] No. 88. p 4078–4085 J. Anim. Sci.

Polo, J. et al. 2013. Neutralizing antibodies against porcine circovirus type 2 in liquid pooled plasma contribute to the biosafety of commercially manufactured spray-dried porcine plasma. J. Anim. Sci. 91: 2192-2198.

Sangild, P. T. 2003. Uptake of colostral immunoglobulins by the compromised newborn farm animal. Acta Vet Scand Suppl 98: 105-122.

Song, M. et al. 2012. Effects of dietary spray-dried egg on growth performance and health of weaned pigs. J. Anim. Sci. 90: 3080-3087.

Tang, S. G. et al. 2015. Chemical Compositions of Egg Yolks and Egg Quality of Laying Hens Fed Prebiotic, Probiotic, and Synbiotic Diets. J. Food. Sci. 80: C1686-1695.

Thompson, C. L., B. Wang, and A. J. Holmes. 2008. The immediate environment during postnatal development has long-term impact on gut community structure in pigs. ISME J 2: 739-748.

Werhahn, E., F. Klobasa, and J. E. Butler. 1981. Investigation of some factors which influence the absorption of IgG by the neonatal piglet. Vet. Immunol. Immunopathol. 2: 35-51.

Zhang, S. et al. 2015. Comparison of spray-dried egg and albumen powder with conventional animal protein sources as feed ingredients in diets fed to weaned pigs. J. Anim. Sci. 86: 772-781.

Table 2.1 Composition of experimental diets (as-fed basis)

	Phase I			Phase II			Phase III
Treatments[1]	A	B	C	A	B	C	C
Ingredient, %							
corn	42.55	42.55	42.55	47.28	47.28	47.28	62.69
soybean meal, % CP	19	19	19	25	25	25	30
milk, whey powder	22.5	22.5	22.5	15	15	15	-
fish meal	7	7	7	4	4	4	
blood plasma	2.36	-	-	2.36	-	-	
egg yolk	-	6	-	-	6	-	
corn oil	3.3	-	3.3	3.3	0	3.3	1.5
dicalcium phosphate	0.35	0.35	0.5	0.4	0.4	0.6	0.7
limestone	0.7	0.7	0.7	0.94	0.94	0.94	0.8
sodium chloride	0.3	0.3	0.3	0.3	0.3	0.3	0.3
swine vitamin premix[2]	0.25	0.25	0.25	0.25	0.25	0.25	0.25
swine trace mineral premix[3]	0.15	0.15	0.15	0.15	0.15	0.15	0.15
zinc oxide	0.4	0.4	0.4	0.3	0.3	0.3	0.2
DL-Met	0.18	0.18	0.18	0.1	0.1	0.1	0.08
L-Lys-HCL	0.4	0.4	0.58	0.23	0.23	0.4	0.26
L-Thr	0.12	0.12	0.2	0.05	0.05	0.12	0.07
corn starch	0.34	-	2.19	0.34	0	2.21	-
L-Val	0.05	0.05	0.15	-	-	0.05	-
L-Trp	0.05	0.05	0.05	-	-	-	-

[1] Dietary treatments include: (A; Blood plasma diet) (B; Egg yolk diet) (C; Control diet)

[2] Vitamin premix containing: vitamin A as retinyl acetate, 5,500 IU; vitamin D_3 as cholecalciferol, 550 IU; vitamin E as alpha-tolcopherol acetate, 30 IU; vitamin K as menadione dimethylprymidinol bisulfide, 4.4 mg/kg; niacin, 33 mg/kg; pantothenic acid as d-Calcium patothenate, 22.05 mg/kg; riboflavin, 11 mg/kg; vitamin B12 as cyanocobalamin, 33 mg/kg.

[3] Trace mineral premix containing: copper (as $CuSO_4$ $5H_2O$), 10 mg/kg, iodine (as Ca (IO_3) H_2O), 0.25 mg/kg; iron ($FeSO_4$ $2H_2O$), 125 mg/kg; manganese (MnO), 15 mg/kg; selenium (Na_2SeO_3), 0.3 mg/kg; zinc ($ZnSO_4H_2O$), 125 mg/kg.

Table 2.2 Calculated and Determined Diet Composition

Calculated analysis	Phase I			Phase II			Phase III
Treatment[1]	A	B	C	A	B	C	C
ME[2], kcal/kg	3513	3511	3497	3489	3489	3470	3369
CP[3], %	18.60	18.60	17.11	18.90	18.90	17.42	18.21
STTD[4] P, %	0.5	0.5	0.49	0.41	0.41	0.41	0.34
Total P, %	0.7	0.7	0.7	0.63	0.63	0.63	0.60
Ca, %	0.85	0.85	0.89	0.80	0.80	0.84	0.70
Lys, %	1.5	1.5	1.5	1.35	1.35	1.35	1.23
Determined Analysis							
Crude Protein, %	21.31	21.38	19.62	21.09	21.67	19.79	20.48
Moisture, %	10.25	10.27	10.38	11.20	12.76	11.31	12.82
Crude Fat,%	5.95	5.74	6.60	4.92	2.65	4.34	2.47
Calcium, %	1.20	1.26	1.12	1.05	1.03	1.01	0.94
ADF, %	5.67	4.47	3.43	5.01	4.09	4.10	4.29
NDF, %	6.39	5.55	6.21	6.26	6.35	6.02	7.31
Phosphorus, %	0.83	0.85	0.80	0.71	0.74	0.68	0.65
Lys, %	1.56	1.57	1.44	1.39	1.47	1.41	1.40

[1] Dietary treatments include: (A; Blood plasma diet) (B; Egg yolk diet) (C; Control diet)
[2] Metabolizable Energy
[3] Crude Protein
[4] Standardized Total Tract Digestible

Table 2.3 Calculated AA composition in diet

Calculated Analysis (SID basis)	**Phase I**			**Phase II**			**Phase III**
Treatments[1]	A	B	C	A	B	C	C
Arg	1.14	1.14	1.04	1.23	1.23	1.13	1.27
His	0.49	0.49	0.43	0.51	0.51	0.46	0.51
Ile	0.80	0.80	0.75	0.82	0.82	0.76	0.78
Leu	1.62	1.62	1.47	1.65	1.65	1.50	1.59
Lys	1.50	1.50	1.50	1.35	1.35	1.35	1.23
Met	0.51	0.51	0.50	0.42	0.42	0.40	0.40
Met + Cys	0.82	0.82	0.76	0.74	0.74	0.67	0.68
Phe	0.84	0.84	0.75	0.90	0.90	0.81	0.90
Phe + Tyr	1.42	1.42	1.26	1.51	1.51	1.35	1.49
Thr	0.88	0.88	0.88	0.81	0.81	0.79	0.74
Trp	0.29	0.29	0.26	0.25	0.25	0.22	0.22
Val	0.96	0.96	0.96	0.92	0.92	0.87	1.00

[1]Dietary treatments include (A; Blood plasma diet) (B; Egg yolk diet) (C; Control diet)

Table 2.4 Determined Composition of Egg Yolk Sources

	Nepco Egg Yolk	Sparboe Egg Yolk	Mixed Egg Yolk
CP[1], %	37.20	34.65	35.86
Moisture, %	2.69	3.15	2.87
Crude Fat,%	46.22	47.23	46.70
Calcium, %	0.28	0.28	0.27
ADF, %	0.00	0.00	0.00
NDF, %	0.00	0.00	0.00
Phosphorus, %	1.06	1.09	1.08

[1] Crude protein

Table 2.5 AA Composition of Different Egg Yolk Sources

	Nepco Egg Yolk	**Sparboe Egg Yolk**	**Mixed Egg Yolk**
Taurine	0.02	0.02	0.02
Hydroxyproline	0.00	0.00	0.00
Aspartic Acid	3.56	3.24	3.40
Threonine	1.77	1.65	1.70
Serine	2.75	2.59	2.66
Glutamic Acid	4.61	4.26	4.42
Proline	1.44	1.41	1.46
Lanthionine	0.00	0.02	0.02
Glycine	1.18	1.07	1.11
Alanine	1.96	1.77	1.85
Cysteine	0.71	0.65	0.69
Valine	2.27	2.06	2.14
Methionine	1.03	0.95	1.01
Isoleucine	1.92	1.78	1.84
Leucine	3.19	2.96	3.05
Tyrosine	1.46	1.36	1.43
Phenylalanine	1.71	1.54	1.61
Hydroxylysine	0.00	0.03	0.03
Ornithine	0.00	0.00	0.00
Lysine	3.14	3.05	3.08
Histidine	0.91	0.85	0.87
Arginine	2.50	2.35	2.41
Tryptophan	0.53	0.49	0.55
Total	**36.66**	**34.10**	**35.35**

Table 2.6 Effects of feeding spray-dried blood plasma and spray-dried egg yolk on pig performance

Item	Treatment[1]				
	A	B	C	SEM[2]	P-Value
BW, kg					
d 0	7.15	7.14	7.15	0.12	0.10
d 7	7.93	7.81	7.63	0.18	0.51
d 14	10.47	10.06	9.80	0.33	0.36
d 21	14.32	14.07	13.68	0.5	0.67
d 28	18.08	18.61	17.86	0.62	0.69
Phase I (d 0 - 7)					
ADG, g	108.46	93.33	68.81	23.70	0.50
ADFI, g	169.29	146.56	132.5	16.20	0.30
G:F, g/kg	0.64	0.58	0.44	0.12	0.50
Phase II (d 7 - 14)					
ADG, g	361.73	322.34	308.09	27.23	0.38
ADFI, g	440	400.55	383.33	30.01	0.41
G:F, g/kg	0.82	0.81	0.80	0.03	0.77
Phase II (d 14 - 21)					
ADG, g	550.77	575.99	555.12	28.46	0.80
ADFI, g	787.02	806.88	798.93	38.87	0.93
G:F, g/kg	0.70	0.71	0.70	0.01	0.72
Phase II (d 7 - 21)					
ADG, g	456.25	422.1	431.61	26.49	0.80
ADFI, g	642.44	650.02	641.36	32.59	0.89
G:F, g/kg	0.71	0.66	0.67	0.02	0.82
Phase III (d 21 - 28)					
ADG, g	538.69	647.46	597.86	32.23	0.09
ADFI, g	888.45	983.17	992.14	40.96	0.17
G:F, g/kg	0.61	0.66	0.61	0.02	0.22

[1] Dietary treatments include:(A: Blood plasma; B: Egg yolk; C: Control diet).

[2] Standard error of the mean.

Table 2.6 (cont.) Effects of feeding spray-dried blood plasma and spray-dried egg yolk on pig performance

Item	Treatment[1]				
	A	B	C	SEM[2]	*P*-Value
Repeated Measures					
ADG, g	392.1	405.35	385.55	15.43	x
Trt	x	x	x	x	0.6479
Time	x	x	x	x	<.0001
Trt*Time	x	x	x	x	0.285
ADFI, g	572.85	581.4	578.66	17.28	x
Trt	x	x	x	x	0.9378
Time	x	x	x	x	<0.0001
Trt*Time	x	x	x	x	0.3057
G:F, g/kg	0.6918	0.6923	0.6329	0.03	x
Trt	x	x	x	x	0.2819
Time	x	x	x	x	<0.0001
Trt*Time	x	x	x	x	0.6497

[1] Dietary treatments include: (A: Blood plasma; B: Egg yolk diet; C: Control diet).
[2] Standard error of the mean.

Table 2.7 Concentrations of IgA and IgG

	Treatment[1]				
	A	B	C		
Item	LSM[2]	LSM[2]	LSM[2]	SEM[3]	*P*-Value
IgA	0.332	0.315	0.318	0.29	x
Trt	x	x	x	x	0.908
Time	x	x	x	x	<.0001
Trt*Time	x	x	x	x	1
IgG	4.13	4.70	4.84	28.25	x
Trt	x	x	x	x	0.150
Time	x	x	x	x	1
Trt*Time	x	x	x	x	0.994
d 0					
IgA, mg/ml	0.173	0.164	0.159	0.015	0.814
IgG, mg/ml	7.49	6.73	6.95	0.59	0.657
d 7					
IgA, mg/ml	0.173	0.170	0.171	0.013	0.990
IgG, mg/ml	3.77	3.86	3.95	0.22	0.846
d 14					
IgA, mg/ml	0.275	0.248	0.273	0.032	0.087
IgG, mg/ml	4.58	4.81	4.73	0.22	0.772
d 21					
IgA, mg/ml	0.291	0.309	0.300	0.066	0.983
IgG, mg/ml	3.98	4.06	3.84	0.16	0.633
d 28					
IgA, mg/ml	0.559	0.512	0.500	0.04	0.545
IgG, mg/ml	5.32	5.28	4.77	0.38	0.528

[1] Dietary treatments include: (A: Blood plasma; B: Egg yolk diet; C: Control diet).
[2] Least square mean
[3] Standard error of the mean

Table 2.8 Concentration of Haptoglobin

	Treatment[1]				
Response	A LSM[2]	B LSM[2]	C LSM[2]	SEM[3]	*P*-value
Repeated Measures					
Haptoglobin, ug/ml	32.38	32.22	32.68	30.05	x
Trt	x	x	x	x	1.000
Time	x	x	x	x	0.171
Trt*Time	x	x	x	x	1.000
d 0					
Haptoglobin, ug/ml	92.55	46.02	48.14	29.9	0.482
d 14					
Haptoglobin, ug/ml	0.26	0.20	0.31	0.07	0.539

[1] Dietary treatments include: (A: Blood plasma; B: Egg yolk diet; C: Control diet).
[2] Least square mean
[3] Standard error of the mean

Table 2.9 Concentration of C-Reactive Protein

Response	Treatment[1] A LSM[2]	B LSM[2]	C LSM[2]	SEM[3]	*P*-value
Repeated Measures					
CRP, ug/ml	25.64	21.22	19.84	2.17	x
Trt	x	x	x	x	0.150
Time	x	x	x	x	<.0001
Trt*Time	x	x	x	x	0.226
d 0					
CRP, ug/ml	8.16	8.26	8.46	0.95	0.976
d 7					
CRP, ug/ml	8.01	6.30	6.00	1.04	0.363
d 14					
CRP, ug/ml	9.38	6.45	7.47	1.03	0.162
d 21					
CRP, ug/ml	9.22	8.73	9.51	0.96	0.846
d 28					
CRP, ug/ml	93.43	76.43	67.76	10.72	0.258

[1] Dietary treatments include: (A: Blood plasma; B: Egg yolk diet; C: Control diet).
[2] Least square mean
[3] Standard error of the mean

Table 2.10 Concentration of Glucagon-like Peptide-2

	Treatment[1]				
	A	B	C		
Response	LSM[2]	LSM[2]	LSM[2]	SEM[3]	*P*-value
Repeated Measures					
GLP-2, ng/ml	0.717	1.191	0.506	0.2	x
Trt	x	x	x	x	0.092
Time	x	x	x	x	0.320
Trt*Time	x	x	x	x	0.633
d 0					
GLP-2, ng/ml	0.760	0.957	0.315	0.3	0.386
d 14					
GLP-2, ng/ml	0.673	1.424	0.698	0.3	0.145

[1] Dietary treatments include: (A: Blood plasma; B: Egg yolk diet; C: Control diet).
[2] Least square mean
[3] Standard error of the mean

Table 2.11 Phase III Cost Breakdown

	Control Diet			
Ingredient	kg	lb	Price/lb	Price in diet
Corn	112.84	248.25	0.06	16.09
Soybean Meal	54.00	118.80	0.19	22.89
Fish Meal	5.40	11.88	0.93	11.10
Corn Oil	2.70	5.94	0.77	4.56
Dicalcium phosphate	1.26	2.77	0.33	0.92
sodium chloride	0.54	1.19	0.13	0.15
Swine Vitamin Premix	0.45	0.99	0.93	0.92
Swine TM premix	0.27	0.59	0.23	0.14
zinc oxide	0.36	0.79	0.98	0.78
DL-Met	0.14	0.32	2.16	0.68
Limestone	1.44	3.17	0.01	0.04
L-Lys-HCL	0.47	1.03	0.94	0.97
L-Thr	0.13	0.28	1.01	0.28
Total (s)		396.00		59.54
Price of ingredients in diet				0.15

Table 2.12 Growth Performance g conversion to lb

Conversion	ADFI, (g/pig/d)	ADFI, (lb/pig/d)	ADG, g	ADG, lb	Feed Efficiency, gain:feed lb
Phase I					
plasma	169.29	0.37	108.46	0.24	0.64
yolk	146.56	0.32	93.33	0.21	0.64
control	132.50	0.29	68.81	0.15	0.52
Phase II					
plasma	642.44	1.41	456.25	1.00	0.71
yolk	650.02	1.43	422.10	0.93	0.65
control	641.36	1.41	431.61	0.95	0.67
Phase III					
plasma	888.45	1.95	538.69	1.19	0.61
yolk	983.17	2.16	647.46	1.42	0.66
control	992.14	2.18	597.86	1.32	0.60

Table 2.13 Cost of Feed per lb of gain by phase

	Cost of diet per lb	Feed Efficiency gain:feed lb	Cost of feed per lb of gain
Phase I			
Plasma	0.31	0.64	0.48
Yolk	0.37	0.64	0.58
Control	0.27	0.52	0.52
Phase II			
Plasma	0.36	0.71	0.51
Yolk	0.32	0.65	0.49
Control	0.23	0.67	0.34
Phase III			
Plasma	0.15	0.61	0.25
Yolk	0.15	0.66	0.23
Control	0.15	0.60	0.25

CHAPTER 3: THE EFFECTS OF SPRAY-DRIED EGG YOLK ON NURSERY PIG MICROBIOTA

ABSTRACT

A 4-wk study was conducted to evaluate the effects of egg yolk compared to spray-dried plasma on the microbiome in the pigs' intestinal tract. Seventy-two pigs (age 24 d; initial body BW (7 ± 0.54 kg) were randomly allocated to18 pens (4 pigs/pen; 2 barrows 2 gilts; 6 pens/treatment). In Phase I and II (wk 1 through 3) pigs were fed 1 of 3 dietary treatments, which were standard nursery diets (control) supplemented with spray-dried plasma (2.36%), or egg yolk (6%). In Phase III (wk 4), all pigs were fed the same diet. Fecal samples were collected from every pig (n = 72) weekly from d 0 to d 28 of the experiment to evaluate fecal microbiota. The OTUs were similar among treatments ($P > 0.8$). There were changes among the phases; from d 0 to Phase I and II there was a significant increase in diversity, ($P < 0.01$) which was followed by a decrease in diversity ($P < 0.01$) in Phase III. Dominance was observed to be similar among treatments; however, the dominance was greater ($P < 0.01$) in Phase III when compared to the other phases. It was observed that treatments had no effect ($P = 0.33$) on overall bacterial community composition, but phase did have an effect ($P < 0.001$) on overall bacterial community composition.

Key words: egg yolk, microbiome, spray-dried plasma, weanling pig

INTRODUCTION

When a pig is born its gastrointestinal tract is sterile; however, quickly after parturition the pig's gut becomes colonized with bacterial species from both its mother and its environment. These microbes make up the host's microbiome which is a term used by Joshua Lederberg, a Nobel prize winner, to describe the extensive collection of indigenous microbes (Bian et al., 2016; Gomez de Agüero et al., 2016; Dou et al., 2017). It is both remarkable and critical that the pig's gastrointestinal tract (GIT) become populated with between 1×10^{13} and 1×10^{15} microbial cells, because they have a critical impact on the pig's health and performance which are important to the swine industry (Koh et al., 2015).

These microbes, which are mostly bacteria, belong to numerous different phyla, the majority of which are not able to be cultured ex vivo. Therefore, in order for a better and more comprehensive understanding of the complex microbiome present in pigs and other mammals, a new type of technology used known as broad-range sequencing of 16S ribosomal RNA (rRNA) genes is used (Hooper et al., 2001; Leser et al., 2002). By identifying the complexity of the pig's microbiome, scientists have come to know the true impact they have on a pig because these microorganisms help a pig to absorb and metabolize nutrients as well as protect them against pathogens which is accomplished through microbial fermentation where the main product are volatile fatty acids which inhibit pathogens in the GIT (Lallés et al., 2007; Looft et al., 2007; Zhao et al., 2015).

This study evaluated how effectively spray-dried egg yolk in comparison to spray-dried plasma establishes the healthy microbiome in a newly weaned piglet. The abundance of microbial community structure based on protein source at each time point

and in each individual pig was evaluated to investigate the effects of the 2 dietary treatments.

MATERIALS AND METHODS

Experimental Design and Dietary Treatments

A 4-wk study was conducted with 72 weaned pigs, which were sorted by sex and initial BW and randomly allocated dietary treatments. The dietary treatments were formulated to contain concentrations of the following protein sources: 1) Plasma (2.36%); 2) Egg yolk (6%); and 3) Control (CTL; no egg yolk or spray-dried plasma). The diets were formulated to meet or exceed the NRC (2012) requirements. For details concerning the experimental design and treatments see Chapter 2.

Data and Sample Collection

Fecal samples were taken weekly on d 0, 7, 14, 21, and 28 from all 72 pigs. The samples were obtained directly from individual pigs to prevent any possible contamination. The samples were stored in a -20°C freezer for future analysis.

DNA Extraction and PCR Amplification

Frozen fecal samples were removed from the -20°C freezer and DNA extraction was performed on each individual fecal sample (71 pigs x 5 time points) using 1-2 g of feces utilizing the PowerMag™ Soil DNA Isolation Kit (MO BIO Laboratories, Carlsbad, CA). According to the manufacture's instructions with several modifications; Briefly, 1 to 2 g of fecal sample were added to sterile 2.0 mL Safe-Lock tubes (Eppendorf, North America, Inc. USA) containing 0.5 g silica beads (Scientific Asset Management, Basking Ridge, NJ) and 60 μL of lysis buffer; bead-beating was completed in a Tissue Lyser (QIAGEN Inc., Valencia, CA) at full speed (30 beats/s) for two 10 min

intervals, with incubation of the samples in a water bath (95°C) between the 2 bead-beating steps. Centrifugation at (4500 × G) was performed on the samples and the supernatant was removed and placed into sterilized 1.5 mL tubes (Fisherbrand, Fischer Scientific, USA). The remaining protocol was performed according to the manufacture's instructions. Finally, 130 µL of elution buffer was added to each sample to elute the DNA. After the quality of the DNA was determined using gel electrophoresis the remaining DNA was stored at -20°C and later used for further analysis.

Preparation of Amplicon Libraries and DNA Sequencing

To PCR amplify the hypervariable V4 region of the bacterial 16S rRNA gene barcoded universal primers were used as described by Kozich et al. (2013). A 15 µL reaction volume was used to amplify the V4 region of the 16s rRNA gene. The PCR reaction was composed of 10 µL of a 1x Terra PCR mix (Clontech Laboratories Inc., Mountain View, CA), 0.5 µL of Taq-polymerase, 1 µL of 10 mM of each primer, and ~50 ng of DNA. A Veriti 96-well thermocycler (Life Technologies™, Carlsbad, CA) was used to perform the amplifications. The PCR conditions for the reaction were 3 min at 98°C followed by 25 cycles of 30 s at 98°C, 30 s at 55°C, and 45 s at 68°C, with a final elongation step of 4 min at 68°C.

After the amplification, the PCR products were resolved in a 2% agarose gel. The samples were run through gel electrophoresis (QD LE Agarose, Green Bio Research, Baton Rouge, LA) at 120 V for 60 min to verify size and that amplification had occurred. Next, through the use of Invitrogen Sequel Prep™ Normalization Plate kit (Frederick, Maryland) samples were normalized to 1 to 2 ng according to the manufacture's protocol. Following normalization up to 96 samples were combined or 'pooled' together utilizing

an epMotion M5073 liquid handler (Eppendorf AG, Hamburg, Germany) to guarantee equal representation of amplicon DNA from each sample. Each of the pooled libraries were size selected using a 1.5% gels using the Pippin Prep (Sage Science, Inc., USA). The size selected amplicons were quantified using an Agilent Bioanalyzer 2100 high sensitivity chip (Agilent Technologies, Santa Clara, CA) and sequenced using the MiSeq Illumina Sequencing platform using a 500 cycle V2 kit (Illumina, Inc., USA) according to the manufacture's protocol.

Bioinformatic Analysis

Raw reads from the Miseq were processed utilizing the quality filter and analysis pipeline developed by the Fernando lab (Turnbaugh et al., 2009; 2010; Paz et al., 2016). Next, the processed data were used for microbial community analysis as described by Paz et al. (2016). After removing the low-quality reads, the resulting reads were analyzed using an Operational Taxonomic Units (OTU) based approach by clustering sequences into OTUs based on 97% sequence similarity. Using the platform Quantitative Insights Into Microbial Ecology (QIIME) (Caporaso et al., 2010) the pre-processed reads were clustered into OTUs at 97% similarity to generate OTUs after screening for chimeric sequences using UCHIME (Edgar et al., 2009). Using QIIME (Caporaso et al., 2010) based on the Mersenne Twister pseudorandom number generator, the OTU table was rarefied across samples to the lowest sample depth (5059 reads). All statistical analyses were performed with samples at an even depth of 5059 as described above. Alpha metrics were used to describe bacterial richness (observed OTUs), diversity (Shannon-Wiener index), and dominance (1-Simpson index) through the use of QIIME (Caporaso et al., 2010). Rarefaction curves were constructed through the use of observed OTUs and the

Shannon-Wiener index. A core measurable microbiome was created based on the standard that an OTU must be present in at least 80% of the samples within both treatments and phases. Core bacterial community composition differences were evaluated using the weighted UniFrac distance matrix (Lozupone et al., 2011) as an input for a permutational multivariate analysis of variance (PERMANOVA) using R as described previously (Paz et al., 2016). The weighted UniFrac distance matrix was used in the principal coordinate analysis (PCoA) to visualize relationships between treatments and phases. The bioinformatics tool Linear Discriminate Analysis Effect Size (LEfse) (Segata et al., 2011) was used with default parameters to identify OTU's that are significantly different between treatments and phases. Statistical significance was determined to be at a *P*-value less than 0.05.

RESULTS

Alpha Diversity

Figures 3.8 and 3.9 depict alpha rarefaction curves. The diversity of OTUs can be observed between phases in Figure 3.8, indicating significant diversity and the diversity of OTUs between treatments in Figure 3.9 shows that there is virtually no difference in diversity at all. Figures 3.10 and 3.11 depict the results of box plots where the sequencing depth in this study was able to characterize approximately 95% of the bacterial communities across treatments and approximately 93% of the bacterial communities among phases. Through analysis of microbial communities, there were detectable differences in OTUs across phases, where an increase in diversity was observed from d 0 to Phase I and II ($P < 0.01$) which was followed by a decrease in

diversity in Phase III ($P < 0.01$). There were no differences in OTUs observed between dietary treatments ($P > 0.80$).

Beta Diversity

Phylum level distribution of the bacterial community present for the entire study is represented in Figure 3.1. The 3 most abundant phyla were Firmicutes at 35.6%, Bacteroidetes at 41.6%, and Proteobacteria at 11%, which make up 88.2% of the taxonomic distribution. There were 27 other phyla identified, some of which were Tenericutes, Actinobacteria, Verrucomicrobia, Spirochaetes, Plantomycetes, and Fibrobacteres, in addition to some unknown, which make up the other 11.8% of the bacterial community.

Figures 3.2 and 3.3 depict the Principle Coordinate Analysis results, which shows 43.06% of the variation of the bacterial community. In Figure 3.2 the phase results showed that for d 0 and Phase III there is clustering while for Phase I and II there was no clustering, but an even mixture. This was supported by PERMANOVA with a *P*-value of 0.001. In Figure 3.5, the LEFse diagram showed a selection of the top 10 most significantly different OTU's among phases, but overall it was found that there were 306 OTUs identified to be different among phases. While in Figure 3.7, a heat map depicted the distribution of the major OTU's across phases of which there were numerous.

In Figure 3.3 the treatment results showed significant clustering indicating no differences among treatments. This was supported by PERMANOVA with a *P*-value of 0.705. In Figure 3.4 the LEFse diagram provided observations that support that there were 11 OTUs identified as different among treatments and 4 OTUs that could not be assigned to a specific family. However, there were 5 OTUs, which belonged to the

following families: Spirochaetaceae, Fibrobacteraceae, Ruminococcaceae, Lachnospiraceae, and Clostridiaceae that were observed to be different among the 3 treatments. It is also significant to observe in Figure 3.4 that the majority of the families/OTU's that were identified were different between the SDEY and SDP treatment groups. The heat map in Figure 3.6 depicted the distribution of the major OTU's across treatments, which there are few of which supports the previous observations.

DISCUSSION

As with a neonate's acquisition and development of antibodies, the newly born pig also obtains its microbiota from the sow and is left to develop the rest of its microbiome on its own, which is acquired from its environment and solid feed diet (Mach et al., 2015). This microbiome is made up of approximately 500-1,000 different species of bacteria (Pajarillo et al., 2014).

Alpha Diversity

The results for this study are also similar to those by Frese et al. (2015) where it was demonstrated that the major phyla of a pig's microbiome are established from the beginning, and minor phyla populations develop over time; gradually increasing diversity. The diversity of the bacterial communities can be visualized and therefore verified by examining the alpha rarefaction curves in Figures 3.8 and 3.9. These plots show that the sequencing depth was in fact adequate to characterize the microbial communities. In addition, the box plots in Figures 3.10 and 3.11 are visual aids for demonstrating how different the microbial communities are between phases and how similar they were between treatments. These results concerning phase agree with those proposed by Pluske et al. (2013), that the microbial population changes the most

immediately post weaning. This observation correlates with Figure 3.10 which shows a high level of diversity on d 0, which could be due to the piglet still having the well established and diversified microbial population it received from its dam and immediately following this high diversity a significant drop is observed in Phase I and Phase II where the pig's microbiota changes from being well diversified when consuming its mother's milk to being poorly diversified once it has begun consuming solid feed. According to Thompson et al. (2008), the piglet microbial population becomes much more stable and therefore less varied around 28 d of age, which would coincide with Phase III in this study. The animal having adapted to a solid feed diet resulted in a more stable and well-diversified microbial population, which could explain this increase in diversity. The lack of diversity between dietary treatments, which can be seen in Figure 3.11, can potentially be explained by the observation that dietary treatments were formulated to be as similar as possible, which may have resulted in no significant differences between treatments.

Beta Diversity

The results of the taxonomic distribution of bacteria that can be observed in Figure 3.1, of this study agree with a study completed by Dou et al. (2017) and Leser et al. (2002) where it was discovered that the dominant phyla in weanling pigs were Firmicutes and Bacteroidetes, which is consistent with this study (Firmicutes 35.6%; Bacteroidetes 41.6%). The following Phyla that were significant in abundance after the 2 previously mentioned were Proteobacteria, Spirochaetes, and Acinobacteria, which agrees with a study conducted by Koh et al. (2015) where these phyla were found in the gut of healthy pigs.

In Figure 3.6 as well as Figure 3.3 it is shown that overall the OTU's are not significantly different among treatments, a possible rational for this is because once again, all of the treatments were formulated to be as similar as possible. Which would result in the animals having minimal differences in their microbial populations, because as we have previously demonstrated, pigs consuming the dietary treatments exhibited similar growth performance, circulating immunoglobulins, and biomarkers of intestinal integrity. Therefore, it would follow that microbial populations were similar as well. However, it is reasonable to conclude that although the value was not statistically significant ($P > 0.8$) the largest differences in abundance of families were between pigs that consumed the SDP and SDEY treatments because these treatments had the most different ingredient compositions of the three dietary treatments due to the protein source (SDEY vs. SDP).

Figure 3.5 and Figure 3.2 provide a representation of some shifts in bacterial OTU's among phases. It is shown that during d 0 diversity is high followed by a great decrease in microbial diversity during Phases I and II, which is then followed by an increase during Phase III. These observations agree with previous studies done by Pajarillo et al. (2014) and Frese et al. (2015). A potential rational for why microbial populations shifted extensively between phases and that a total of 306 OTU's were significantly different among phases could be explained by the observation by Bian et al. (2016) that changing the diet of a pig to solid feed from sow milk will have the most significant effect on gut microbial population. Therefore, the microbial populations between phases will have far more changes than that of the microbial populations between treatments. The clustering in Figure 3.2 and lack of clustering in Figure 3.3

illustrates this conclusion. In Figure 3.2, the clustering at d 0 and Phase III and lack of clustering at Phase I and II are also significant. This clustering is consistent with the previous conclusion; because at d 0 the pigs had just been weaned and had not begun consuming solid feed (resulting in their microbiota being very similar). Significant variation would occur as they began eating solid feed, which can be seen by the lack of clustering during Phase I and II. The clustering at Phase III could potentially be explained by the observation that the pigs had reached an age where their microbiota had been established and the pigs had adapted to their solid feed diet, and therefore had few microbial differences.

The significant differences in microbial populations between phases can also be seen in the LEFse diagrams in Figure 3.5. Although these only show the top 10 most significantly different OTU's among phases, we have seen that there are in fact 180 significantly different OTU's between d 0 and Phase I, 226 between d 0 and Phase II, 298 between d 0 and Phase III, 131 between Phase I and II, 300 between Phase I and III and finally 287 between Phase II and III. These results are reasonable because the microbial populations would be drastically different between both Phase I and III and d 0 and Phase III since as previously discussed the microbial populations are most different at those time points. In addition, the microbial populations were most similar during Phase I and II as was also previously determined.

The LEFse diagrams in Figure 3.4 depict the differences in microbial populations between treatments, the majority of which while not statistically significant ($P > 0.10$) were between the yolk and plasma treatment groups. As previously mentioned of the 11 significantly different OTU's, 5 could be characterized into the families Spirochaetaceae,

Fibrobacteraceae, Ruminococcaceae, Lachnospiraceae, and Clostridiaceae. Based upon a study by Unno et al. (2014) according to taxonomic abundance analysis both Spirochaetaceae and Clostridiaceae families were associated with host weight loss. These results correlate with this experiment because the piglets that consumed the CTR and SDEY treatments both contained Clostridiaceae in their microbiota and the piglets that consumed the SDP treatment contained Spirochaetaceae in their microbiota and both can result in weight loss which could be a factor in their being no significant differences in their ADG values since all 3 dietary treatments contained a microbial family that has been shown to contribute to weight loss. In contrast, in a study performed by He et al. (2016) investigating the contribution of porcine gut microbiota to fatness determined that 1 of the major families was Ruminococcaceae, which would apply to this experiment because pigs consuming the SDEY treatment had the greatest ADG value during Phase III ($P = 0.09$). This could be due to the SDEY treatment containing 3 OTU's, which were classified as Ruminococcaceae while pigs that consumed the CTR treatment only had 1 OTU in this family and SDP treatment did not have any OTU's from this family, which may have resulted in the pigs that consumed the SDEY treatment having had a slight gain advantage over the other 2 treatments. The family, Lachnospiraceae could potentially be a factor in preventing diarrhea because it was found in great abundance in the microbiome of healthy piglets vs. the piglets experiencing post-weaning diarrhea according to a study by Dou et al. (2017). This result could explain why pigs that consumed the SDP treatment performed just as well as the other two treatments. While the other 2 treatments contained Ruminococcaceae which may have helped in fat development, SDP pigs did not, however SDP pigs did also possess potentially harmful

microbes (Spirochaetaceae) who's harmful effects could potentially be mitigated with the assistance of Lachnospiraceae species which the pigs consuming the other 2 treatments did not have. Finally, the effect of the family Fibrobacteraceae cannot be determined because further investigation is required, as there is currently not sufficient information to determine the significance of this family to the swine microbiota.

A variety of OTU's were determined to be different between phases while there were very few differences between treatments; however, these OTU's were presented at the family level because longer read lengths are required for more specificity and several of the OTU's were classified as unknown. In the future, these limitations could be mitigated if longer read lengths are used. This should help to classify these microbes more specifically and provide more insight into the effects different dietary treatments may have on piglet microbial populations.

CONCLUSION

In this study it was important to evaluate the microbial populations in the 72 individual pigs. By evaluating their microbiota it was discovered that there were no changes among dietary treatment groups, but there were changes among phases. It can be concluded that the 2 protein sources were more similar than originally predicted. It appears that changes in phase play a more important role in establishing microbial populations than dietary treatments.

LITERATURE CITED

Bian, G., S. Ma, Z. Zhu, Y. Su, E. G. Zoetendal, R. Mackie, J. Liu, C. Mu, R. Huang, H. Smidt, and W. Zhu. 2016. Age, introduction of solid feed and weaning are more important determinants of gut bacterial succession in piglets than breed and nursing mother as revealed by a reciprocal cross-fostering model. Environ Microbiol 18: 1566-1577.

Dai, Z. L., G. Wu, and W. Y. Zhu. 2011. Amino acid metabolism in intestinal bacteria: links between gut ecology and host health. Front. Biosci. (Landmark Ed) 16: 1768-1786.

Dou, S., P. Gadonna-Widehem, V. Rome, D. Hamoudi, L. Rhazi, L. Lakhal, T. Larcher, N. Bahi-Jaber, A. Pinon-Quintana, A. Guyonvarch, I. L. Huërou-Luron, and L. Abdennebi-Najar. 2017. Characterisation of Early-Life Fecal Microbiota in Susceptible and Healthy Pigs to Post-Weaning Diarrhoea.

Frese, S. A., K. Parker, C. C. Calvert, and D. A. Mills. 2015. Diet shapes the gut microbiome of pigs during nursing and weaning. Microbiome 3: 28.

Gomez de Agüero, M., S. C. Ganal-Vonarburg, T. Fuhrer, S. Rupp, Y. Uchimura, H. Li, A. Steinert, M. Heikenwalder, S. Hapfelmeier, U. Sauer, K. D. McCoy, and A. J. Macpherson. 2016. The maternal microbiota drives early postnatal innate immune development. Science 351: 1296-1302.

He, M., S. Fang, X. Huang, Y. Zhao, S. Ke, H. Yang, Z. Li, J. Gao, C. Chen, and L. Huang. 2016. Evaluating the Contribution of Gut Microbiota to the Variation of Porcine Fatness with the Cecum and Fecal Samples. Front Microbiol 7: 2108.

Hooper, L. V., and J. I. Gordon. 2001. Commensal host-bacterial relationships in the gut. Science 292: 1115-1118.

Kim, H. B., and R. E. Isaacson. 2015. The pig gut microbial diversity: Understanding the pig gut microbial ecology through the next generation high throughput sequencing. Vet. Microbiol. 177: 242-251.

Koh, H. W., M. S. Kim, J. S. Lee, H. Kim, and S. J. Park. 2015. Changes in the Swine Gut Microbiota in Response to Porcine Epidemic Diarrhea Infection. Microbes. Environ. 30: 284-287.

Leser T. D. et. al. 2000. Changes in bacterial community structure in the colon of pigs fed different experimental diets and after infection with *Brachyspira hyodsenteriae.* Appl. Environ. Microbiol. 66: 3290-3296.

Leser, T. D. et al. 2002. Culture-independent analysis of gut bacteria: the pig

gastrointestinal tract microbiota revisited. Appl. Environ. Microbiol. 68: 673-690.

Looft, T. et al. 2012. In-feed antibiotic effects on the swine intestinal microbiome. Proc. Natl. Acad. Sci. U S A 109: 1691-1696.

Mach, N. et al. 2015. Early-life establishment of the swine gut microbiome and impact on host phenotypes. Environ. Microbiol. Rep 7: 554-569.

Pajarillo, A.B., E., J. P. Chae, M. P. Balolong, H. Bum Kim, and D. K. Kang. 2014. Assessment of fecal bacterial diversity among healthy piglets during the weaning transition. J. Gen. Appl. Microbiol. 60: 140-146.

Paz HA, Anderson CL, Muller MJ, Kononoff PJ, Fernando SC. 2016. Rumen Bacterial Community Composition in Holstein and Jersey Cows Is Different under Same Dietary Condition and Is Not Affected by Sampling Method. Front Microbiol.7:1206. Epub 2016/08/19. doi: 10.3389/fmicb.2016.01206. PubMed PMID: 27536291; PMCID: 4971436.

Pluske, J. R. 2013. Feed- and feed additives-related aspects of gut health and development in weanling pigs. J Anim Sci Biotechnol 4: 1.

Unno, T. et al. 2015. Effects of antibiotic growth promoter and characterization of ecological succession in swine gut microbiota. J. Microbiol. Biotechnol. 25(4): 431-438.

Zhao, W. et al. 2015. The dynamic distribution of porcine microbiota across different ages and gastrointestinal tract segments. PLoS One 10: e0117441.

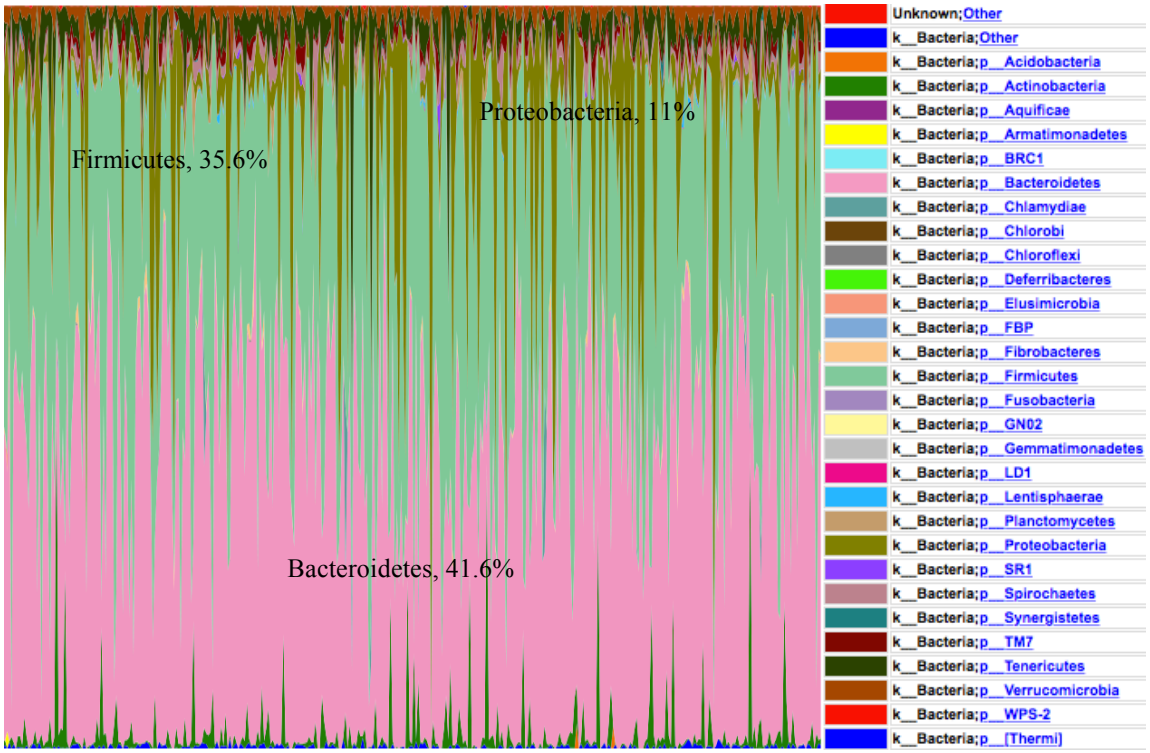

Figure 3.1 Porcine bacterial community taxonomic distribution at the phylum level with the abundance of the top 3 genera present.

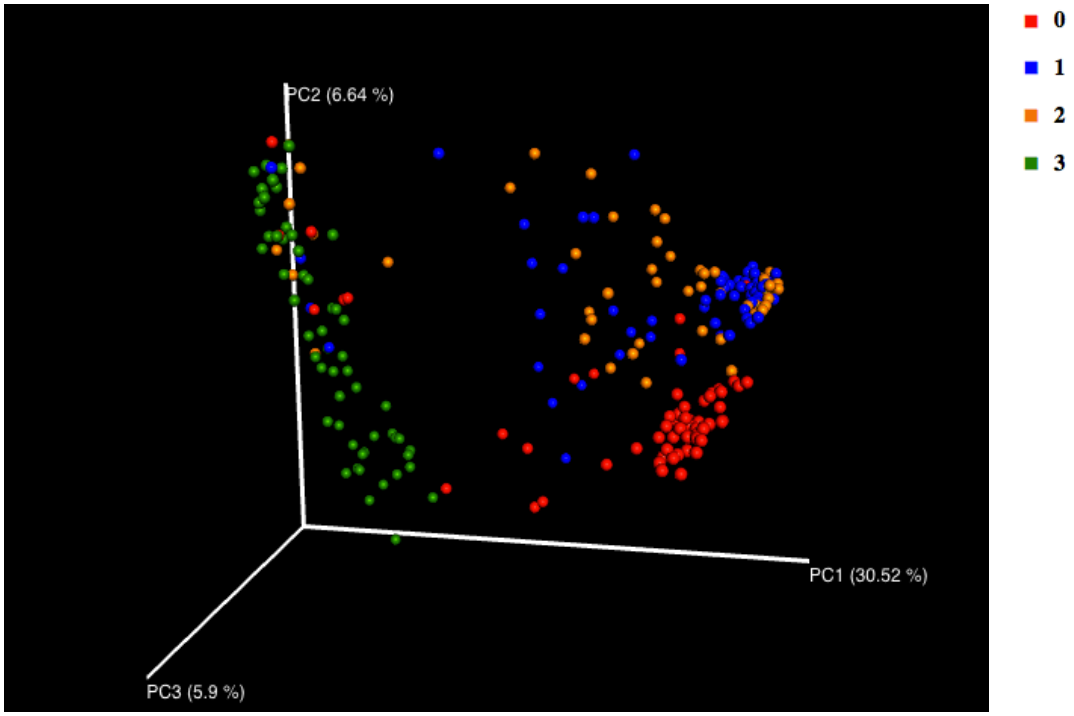

Figure 3.2 Principle Coordinate Analysis of the porcine bacterial community organized by phase.

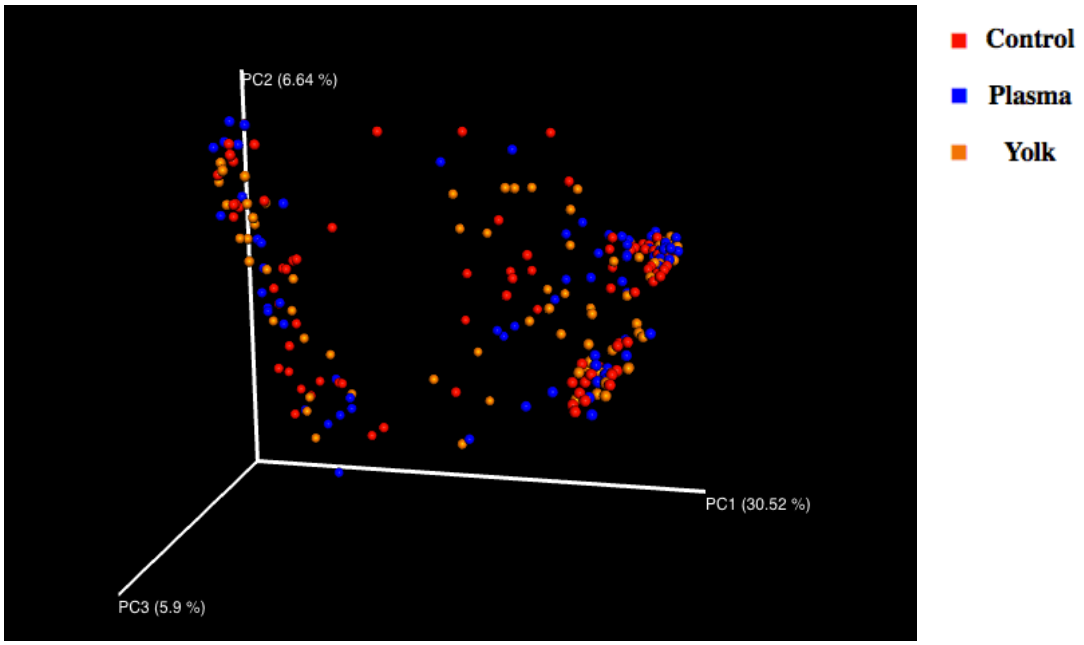

Figure 3.3 Principle Coordinate Analysis of the porcine bacterial community organized by treatment.

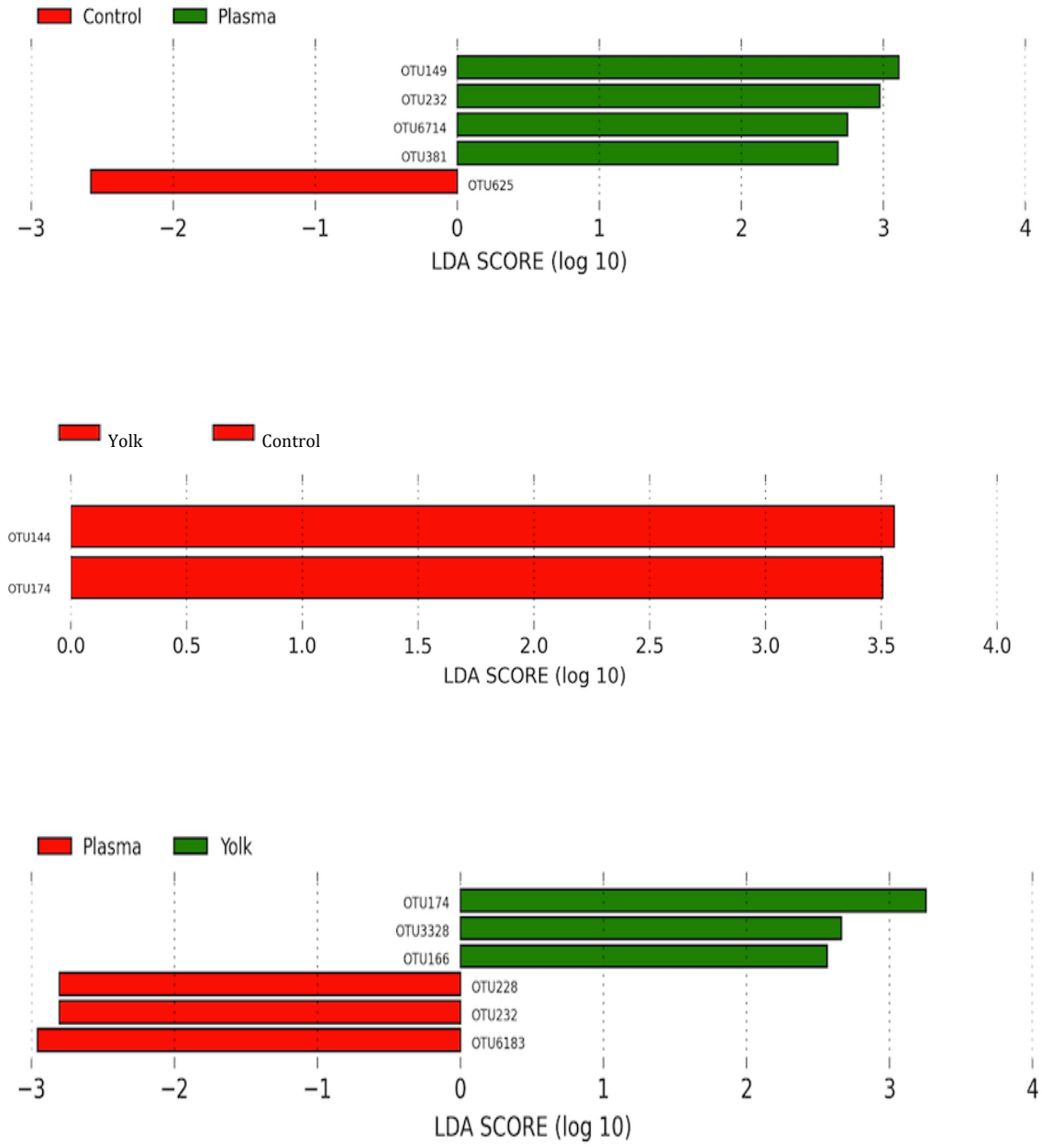

Figure 3.4 Lefse diagrams depicting pairwise comparisons in microbial populations among treatments.

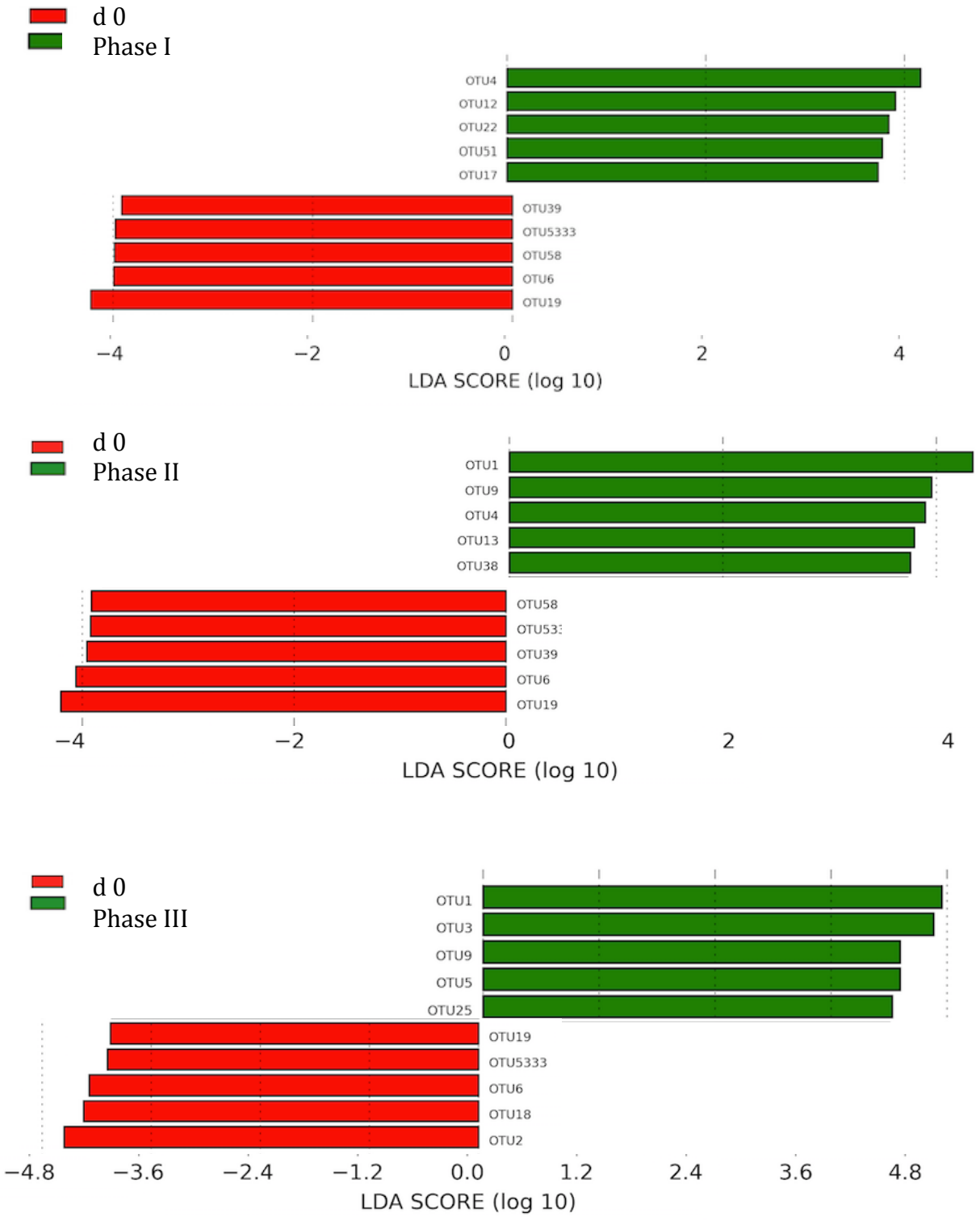

Figure 3.5 Lefse diagrams depicting pairwise comparisons in microbial populations among phases.

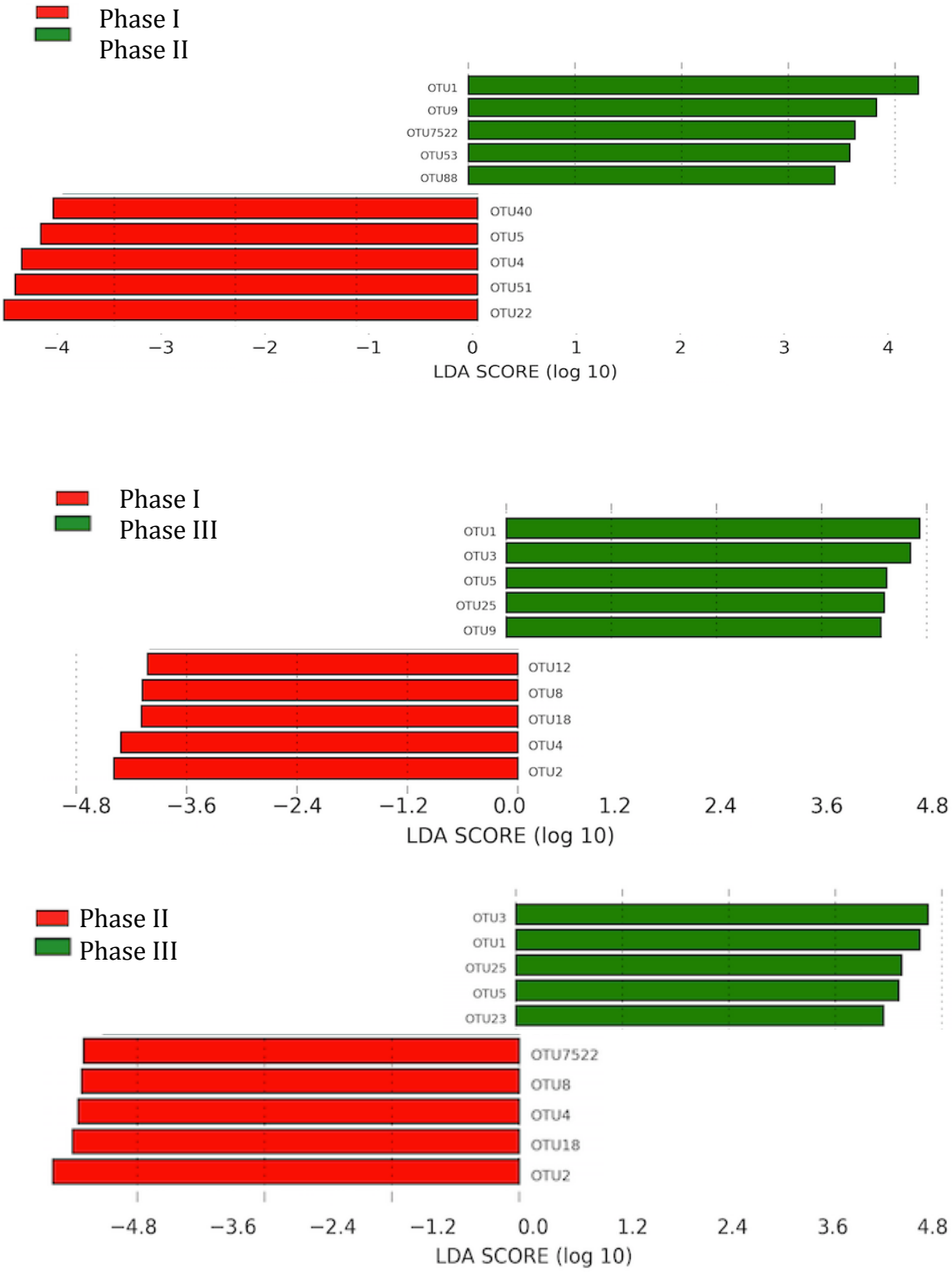

Figure 3.5 (cont.) Lefse diagrams depicting pairwise comparisons in microbial populations among phases.

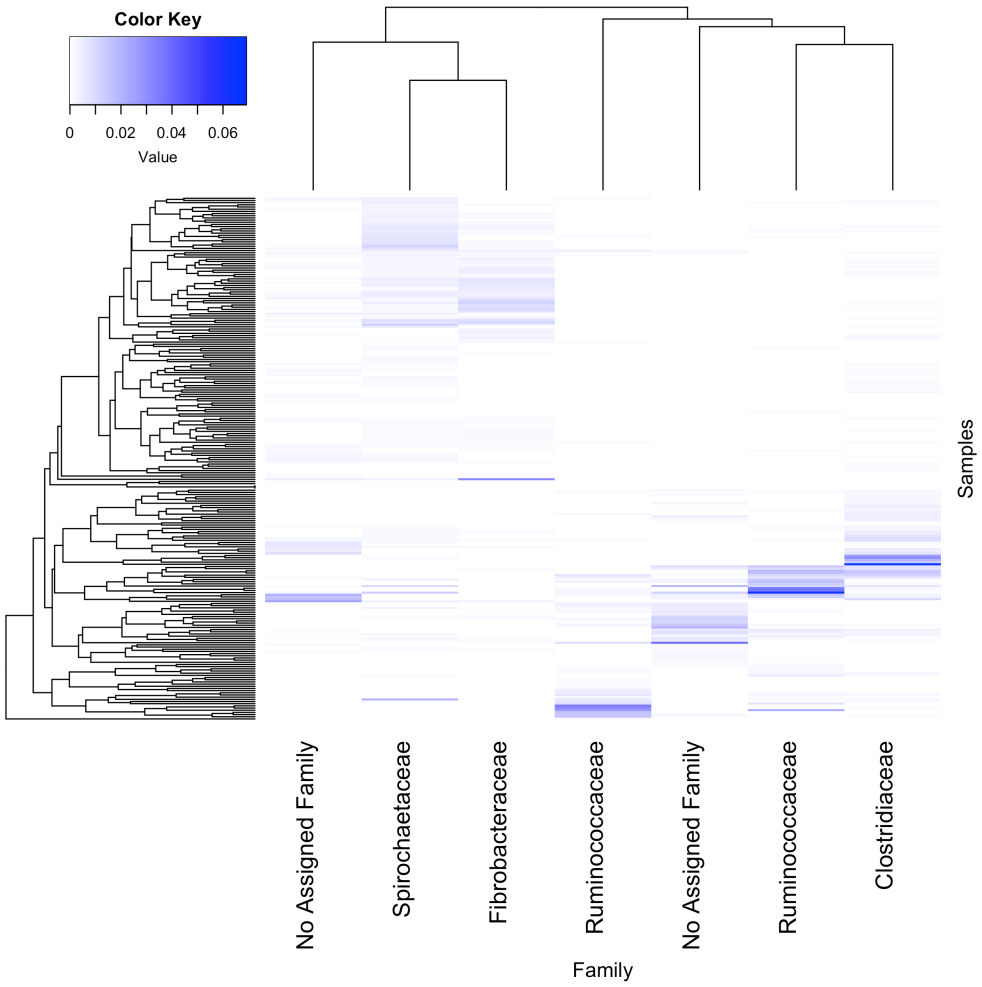

Figure 3.6 Heat Map of the Most Abundant Families Across Treatments

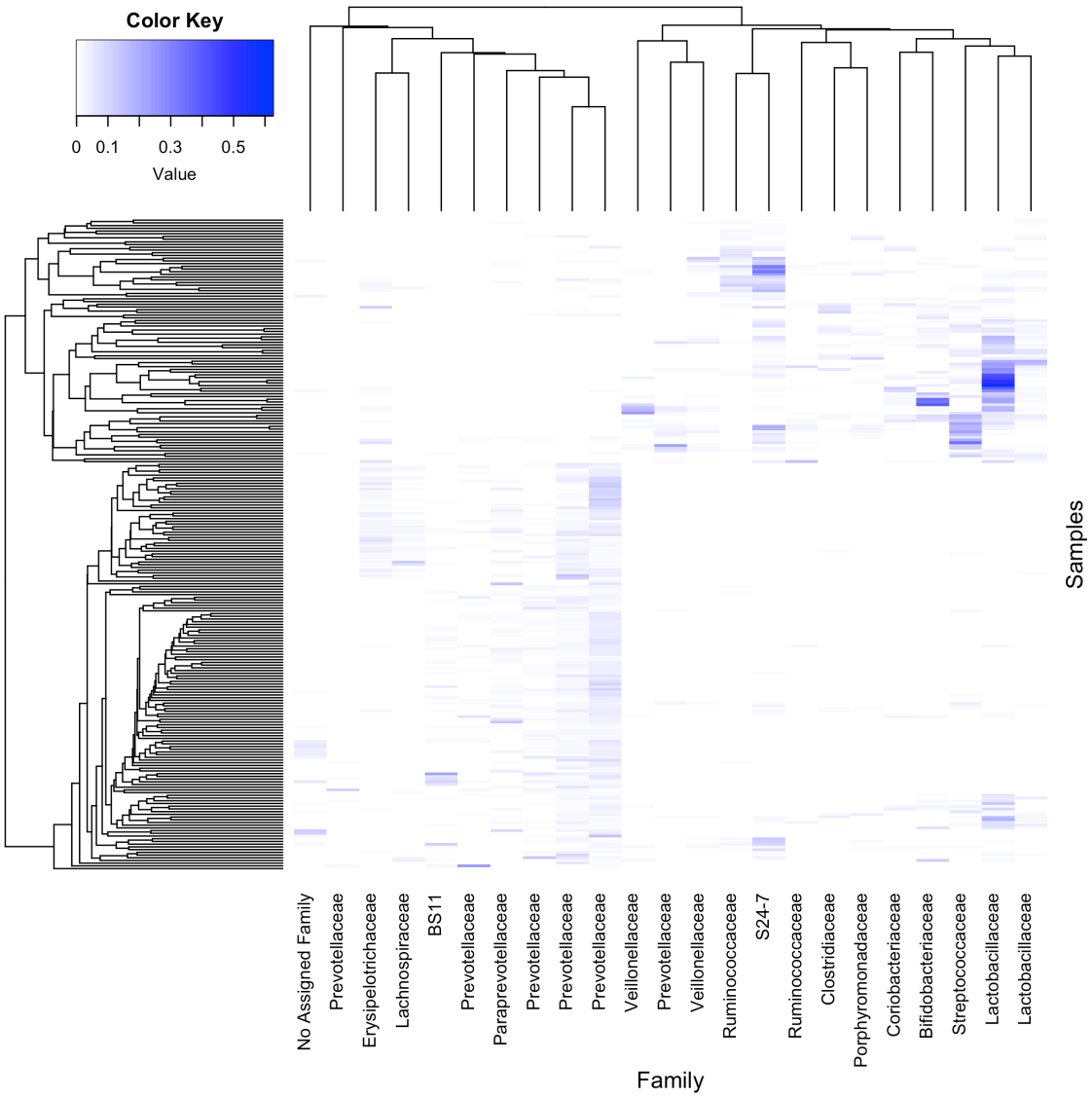

Figure 3.7 Heat Map of Significantly Abundant Families Across phases

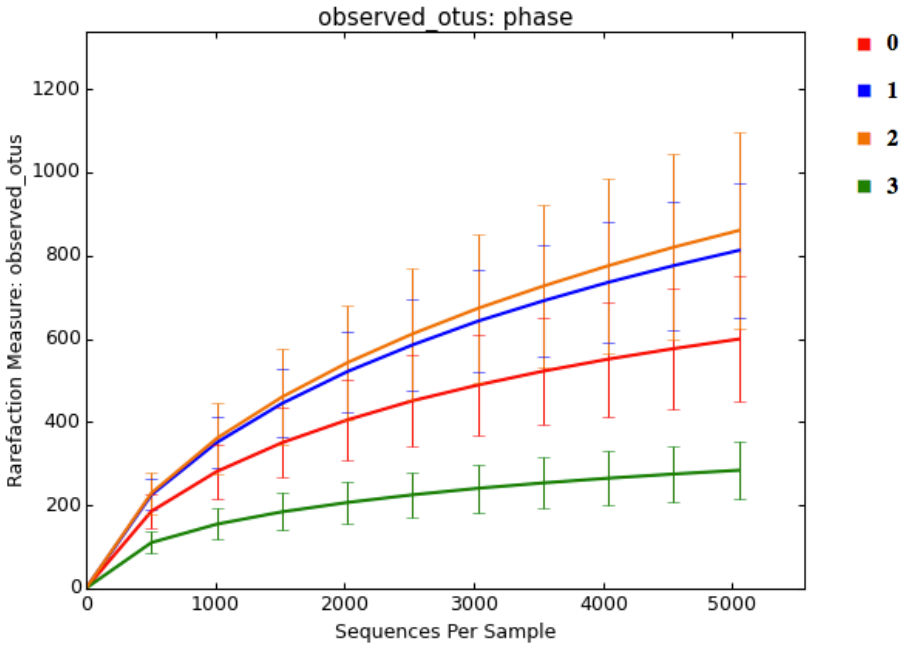

Figure 3.8 Bacterial alpha rarefaction curves showing significant diversity among phases. There is an increase in diversity from d 0 to Phases I and II ($P < 0.01$) and a decrease in diversity in Phase III ($P < 0.01$).

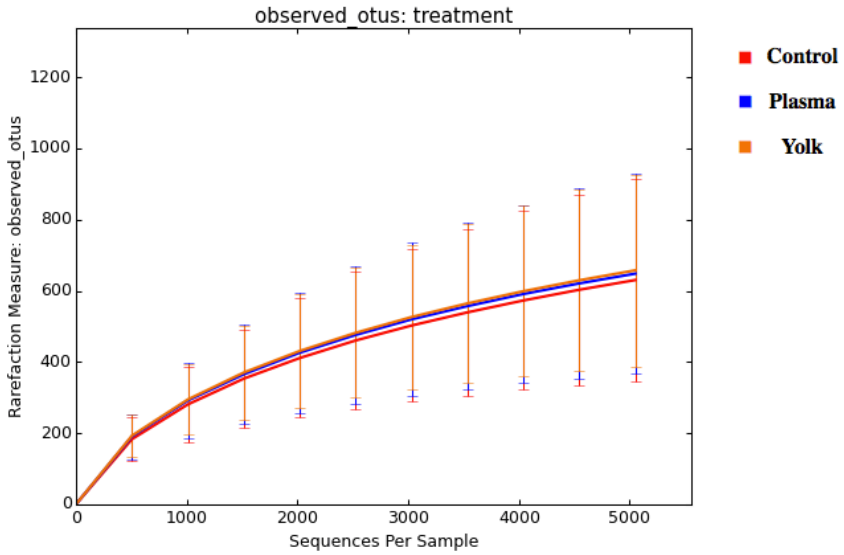

Figure 3.9 Bacterial alpha rarefaction curve showing no diversity among treatments ($P >$ 0.80).

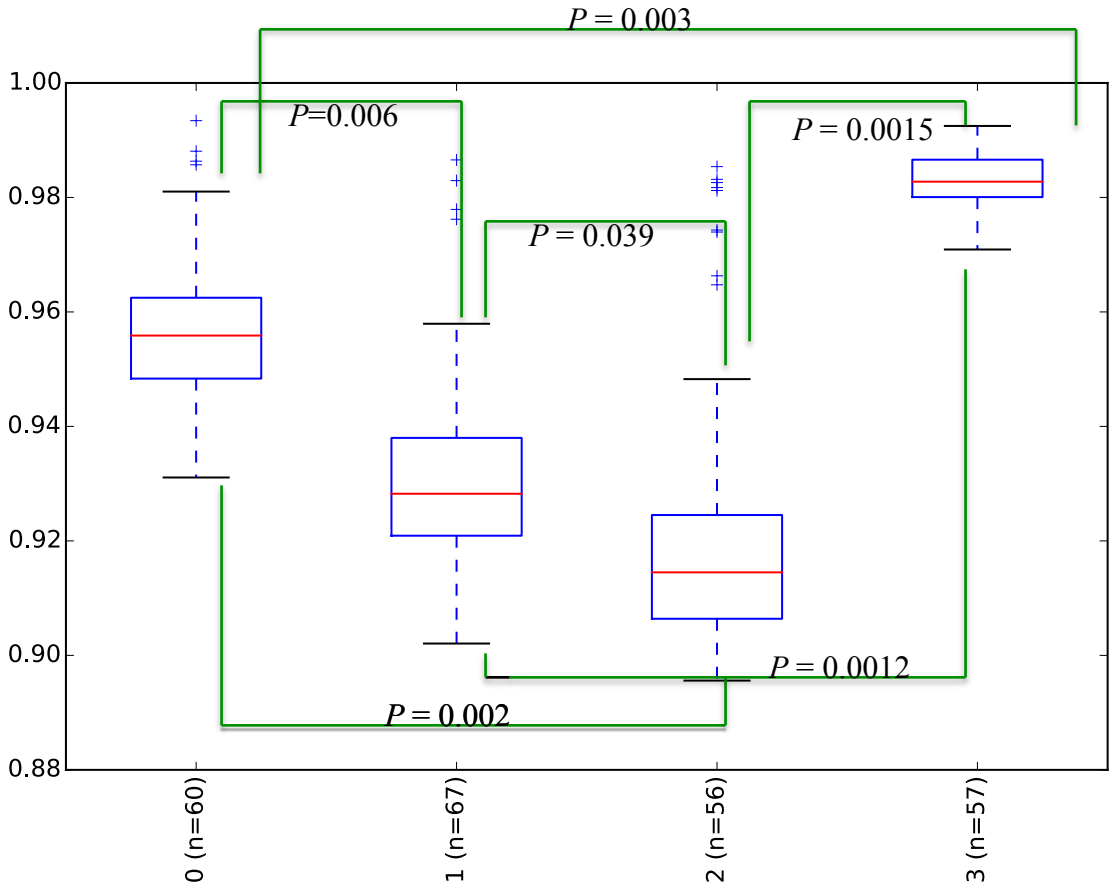

Figure 3.10 Box plot characterizing approximately 93% of the bacterial communities among phases.

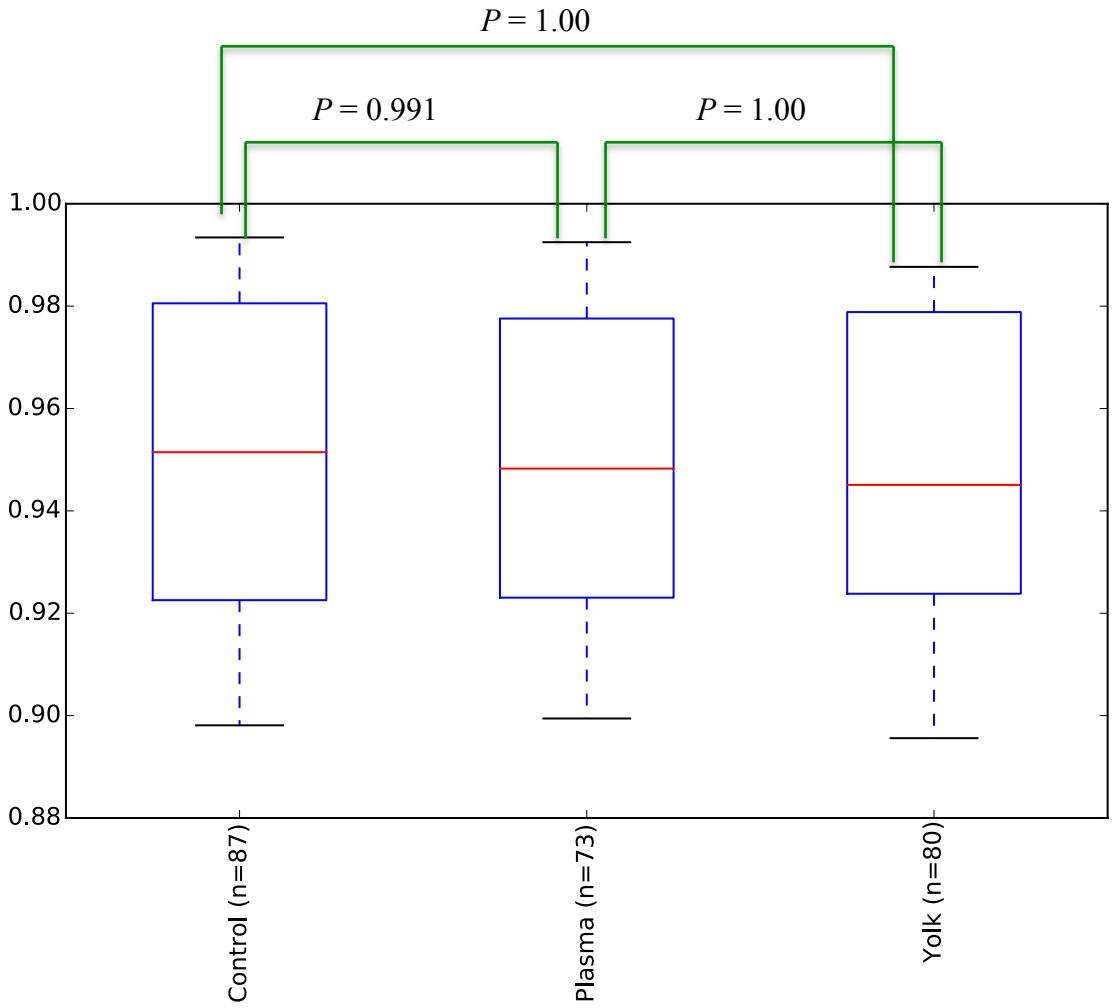

Figure 3.11 Box plot characterizing approximately 95% of bacterial communities among treatments.

Appendix

Table 4.1 Ingredient Cost Breakdown

Ingredient	**Cost per lb**
corn	0.06
soybean meal	0.19
milk, whey powder	0.36
fish meal	0.93
blood plasma	2.16
egg yolk	2.30
corn oil	0.77
dicalcium phosphate	0.33
limestone	0.01
sodium chloride	0.13
swine vitamin premix	0.93
swine TM premix	0.23
zinc oxide	0.98
DL-Met	2.16
L-Lys-HCL	0.94
L-Thr	1.01
corn stach	0.01
L-Val	4.30
L-Trp	9.05

Table 4.2 Phase I Cost Breakdown of Plasma Diet

Plasma				
Ingredient	kg	lb	Price/lb	Price in diet
corn	19.12	42.06	0.06	2.73
soybean meal	8.60	18.92	0.19	3.65
milk, whey powder	10.14	22.31	0.36	8.13
fish meal	3.20	7.04	0.93	6.58
blood plasma	1.06	2.33	2.16	5.03
egg yolk	0.00	0.00	2.30	0.00
corn oil	1.48	3.26	0.77	2.50
dicalcium phosphate	0.16	0.35	0.33	0.12
limestone	0.32	0.69	0.01	0.01
sodium chloride	0.14	0.30	0.13	0.04
swine vitamin premix	0.11	0.25	0.93	0.23
swine TM premix	0.07	0.15	0.23	0.03
zinc oxide	0.18	0.40	0.98	0.39
DL-Met	0.08	0.18	2.16	0.38
L-Lys-HCL	0.18	0.40	0.94	0.37
L-Thr	0.05	0.12	1.01	0.12
corn stach	0.15	0.34	0.01	0.00
L-Val	0.02	0.05	4.30	0.21
L-Trp	0.02	0.05	9.05	0.45
Total (s)		99.18		30.97
Price of ingredients in diet/lb				0.31

Table 4.3 Phase I Cost Breakdown of Egg Yolk Diet

Egg Yolk				
Ingredient	kg	lb	Price/lb	Price in diet
corn	19.12	42.06	0.06	2.73
soybean meal	8.60	18.92	0.19	3.65
milk, whey powder	10.14	22.31	0.36	8.13
fish meal	3.20	7.04	0.93	6.58
blood plasma	0.00	0.00	2.16	0.00
egg yolk	2.70	5.94	2.30	13.66
corn oil	0.00	0.00	0.77	0.00
dicalcium phosphate	0.16	0.35	0.33	0.12
limestone	0.32	0.69	0.01	0.01
sodium chloride	0.14	0.30	0.13	0.04
swine vitamin premix	0.11	0.25	0.93	0.23
swine TM premix	0.07	0.15	0.23	0.03
zinc oxide	0.18	0.40	0.98	0.39
DL-Met	0.08	0.18	2.16	0.38
L-Lys-HCL	0.18	0.40	0.94	0.37
L-Thr	0.05	0.12	1.01	0.12
corn stach	0.15	0.34	0.01	0.00
L-Val	0.02	0.05	4.30	0.21
L-Trp	0.02	0.05	9.05	0.45
Total (s)		99.53		37.10
Price of ingredients in diet/lb				0.37

Table 4.4 Phase I Cost Breakdown of Control Diet

Control				
Ingredient	kg	lb	Price/lb	Price in diet
corn	22.60	49.72	0.06	3.22
soybean meal	10.00	22.00	0.19	4.24
milk, whey powder	12.00	26.40	0.36	9.62
fish meal	3.80	8.36	0.93	7.81
blood plasma	0.00	0.00	2.16	0.00
egg yolk	0.00	0.00	2.30	0.00
corn oil	1.80	3.96	0.77	3.04
dicalcium phosphate	0.25	0.55	0.33	0.18
limestone	0.35	0.77	0.01	0.01
sodium chloride	0.15	0.33	0.13	0.04
swine vitamin premix	0.13	0.28	0.93	0.26
swine TM premix	0.08	0.17	0.23	0.04
zinc oxide	0.20	0.44	0.98	0.43
DL-Met	0.09	0.20	2.16	0.43
L-Lys-HCL	0.29	0.64	0.94	0.60
L-Thr	0.10	0.22	1.01	0.22
corn stach	1.10	2.41	0.01	0.03
L-Val	0.08	0.17	4.30	0.71
L-Trp	0.03	0.06	9.05	0.50
Total (s)		116.66		31.39
Price of ingredients in diet/lb				0.27

Table 4.5 Phase II Cost Breakdown of Plasma Diet

Plasma

Ingredient	kg	lb	Price	Price in diet
corn	56.74	124.83	0.06	8.09
soybean meal	30.00	66.00	0.19	12.72
milk, whey powder	18.00	39.60	0.36	14.43
fish meal	4.80	10.56	0.93	9.87
blood plasma	2.84	6.25	2.16	13.48
egg yolk	0.00	0.00	2.30	0.00
corn oil	3.96	8.71	0.77	6.69
dicalcium phosphate	0.48	1.06	0.33	0.35
limestone	0.36	0.79	0.01	0.01
sodium chloride	1.13	2.48	0.13	0.32
swine vitamin premix	0.30	0.66	0.93	0.62
swine TM premix	0.18	0.40	0.23	0.09
zinc oxide	0.36	0.79	0.98	0.78
DL-Met	0.12	0.26	2.16	0.57
L-Lys-HCL	0.28	0.61	0.94	0.57
L-Thr	0.41	0.90	1.01	0.91
corn stach	0.06	0.13	0.01	0.00
L-Val	0.00	0.00	4.30	0.00
Total (s)		264.03		69.50
Price of ingredients in diet/lb				0.26

Table 4.6 Phase II Cost Breakdown of Egg Yolk Diet

Egg Yolk				
Ingredient	kg	lb	Price	Price in diet
corn	56.74	124.83	0.06	8.09
soybean meal	30.00	66.00	0.19	12.72
milk, whey powder	18.00	39.60	0.36	14.43
fish meal	4.80	10.56	0.93	9.87
blood plasma	0.00	0.00	2.16	0.00
egg yolk	7.20	15.84	2.30	36.43
corn oil	0.00	0.00	0.77	0.00
dicalcium phosphate	0.48	1.06	0.33	0.35
limestone	0.36	0.79	0.01	0.01
sodium chloride	1.13	2.48	0.13	0.32
swine vitamin premix	0.30	0.66	0.93	0.62
swine TM premix	0.18	0.40	0.23	0.09
zinc oxide	0.36	0.79	0.98	0.78
DL-Met	0.12	0.26	2.16	0.57
L-Lys-HCL	0.28	0.61	0.94	0.57
L-Thr	0.00	0.00	1.01	0.00
corn stach	0.06	0.13	0.01	0.00
L-Val	0.00	0.00	4.30	0.00
Total (s)		264.01		84.85
Price of ingredients in diet/lb				0.32

Table 4.7 Phase I Cost Breakdown of Control Diet

Control				
Ingredient	kg	lb	Price	Price in diet
corn	66.20	145.64	0.06	9.44
soybean meal	35.00	77.00	0.19	14.84
milk, whey powder	21.00	46.20	0.36	16.84
fish meal	5.60	12.32	0.93	11.51
blood plasma	0.00	0.00	2.16	0.00
egg yolk	0.00	0.00	2.30	0.00
corn oil	4.20	9.24	0.77	7.10
dicalcium phosphate	0.84	1.85	0.33	0.61
limestone	0.42	0.92	0.01	0.01
sodium chloride	1.32	2.90	0.13	0.37
swine vitamin premix	0.35	0.77	0.93	0.72
swine TM premix	0.21	0.46	0.23	0.11
zinc oxide	0.42	0.92	0.98	0.91
DL-Met	0.14	0.31	2.16	0.66
L-Lys-HCL	0.56	1.23	0.94	1.16
L-Thr	3.09	6.81	1.01	6.90
corn stach	0.17	0.37	0.01	0.00
L-Val	0.07	0.15	4.30	0.66
Total (s)		307.09		71.85
Price of ingredients in diet/lb				0.23