Effect of Diet Complexity and Dietary Fish Peptide Supplementation on Weanling Pigs

by

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A thesis submitted to the Graduate Faculty of
Auburn University
in partial fulfillment of the
requirements for the Degree of
Master of Science

Auburn, Alabama
August 8, 2020

Keywords: Weanling pig, Complex diet, Simple corn-soybean meal diet, Fish peptides

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ABSTRACT

Young pigs are subjected to various stressors at weaning, and providing a highly palatable and digestible diet is, obviously, important in preventing or alleviating growth check soon after weaning and also optimizing growth performance thereafter. Although a corn-soybean meal (SBM) diet is the golden standard for feeding pigs, such a diet may not be appropriate for weanling pigs because of many digestive, metabolic, and immunological challenges. For instance, their enzyme profile is designed to utilize milk protein, carbohydrate, and fat, i.e., not nutrients in corn and SBM. For those reasons, weanling pigs have been fed complex diets that contain many special ingredients such as dried whey, soy protein concentrate, plasma protein, fish meal, blood meal, oat groats, and others. Providing such diets with highly-palatable and highly-digestible ingredients to weanling pigs can be rather costly. However, a small amount of some alternative feed supplement, such as fish peptides, can be included as an alternative source of nutrients for weanling pigs. In addition, such a feed additive may have some bioactive or functional properties than can be beneficial for their health and growth performance. By using such a feed additive, along with a rapidly increasing enzyme technologies in recent years, it might be possible for weanling pigs to utilize corn-SBM diets more efficiency.

As part of the project to explore the possibility of replacing complex diets with semi simple or simple corn-SBM diets for weanling pigs, a study was conducted to assess the effect of fish peptides on their growth performance, serum metabolite profile, and serum cytokines. Forty-eight gilts and 48 castrated males (initial body weight, 7.87 ± 0.71 kg) weaned at 3 to 4 wk of age were randomly assigned to 6 dietary treatments with 4 replicate pens (2 gilts and 2 castrated males/pen) per treatment. Because of the availability of pigs and facility at one time, the study was conducted in 2 trials, and each trial used 24 gilts and 24 castrated males. Two trials were

approximately 8 wk apart. After weaning, pigs were fed a common pre-starter diet for 4 d before beginning of the study, and a 2-phase feeding program was used. Each phase consisted of 2 wk. Two typical complex, positive control (POS) diets containing various special ingredients were formulated and used as the positive control (POS) diets. Two simple, corn-SBM-based negative control (NEG) diets were formulated to be iso-lysinic to the POS diets, and the NEG diets for both phase 1 and 2 were supplemented with 0, 0.5, 1.0, 1.5, or 2.0% fish peptides. A small amount of dried whey (5%) was included in the phase 1 NEG and fish peptide diets to ensure that pigs were growing. Dried whey was not include in the phase 2 NEG and fish peptide diets. Fish peptides were included in the diet by replacing the part of corn and SBM. All 6 diets were formulated to meet or exceed the 2012 NRC nutrient requirements. Pigs were weighed and feed intake was recorded weekly. During the fourth week of the study, approximately 5 mL of blood was collected from each pig via vena cava puncture using a sterile needle and evacuated tube. Serum was separated, and an aliquot was stored at -20ºC. Serum sample from each pig was analyzed for metabolites, whereas serum samples were pooled by pen and analyzed for serum cytokines.

During the last 2 wk of the study, the intake of feed, Lys, and DE increased cubically (P = 0.025, 0.025, and 0.026, respectively) as fish peptide supplementation increased from 0 to 2%. Overall, pigs fed the diets supplemented with 1.5% fish peptides had greater feed, Lys, and DE intakes (approximately 8%) than those fed other diets, but those differences were not statistically significant (cubic, P = 0.135, 0.136, and 0.167 respectively). Overall weight gain of pigs fed the diets containing fish peptides seemed to be greater (6.7%) than those fed the POS diet, but, again, it was not statistically significant (P = 0.137). There were no clear trends in the efficiency of overall utilization of feed, Lys, or DE intake for weight gain. Pigs tended to respond linearly

and quadratically (P = 0.092 and 0.106, respectively) to the increase in fish peptide supplementation in serum total protein. Serum urea N concentration was greater in pigs fed the diets supplemented with fish peptides compared with those fed the POS diets (P = 0.005), whereas it increased linearly (P = 0.017) as fish peptide supplementation increased from 0 to 2%. Although pigs tended to respond quadratically (P = 0.093) to fish peptide supplementation in serum triglyceride, there was no clear effect of dietary treatments on serum albumin, globulin, glucose, or cholesterol concentration, or albumin to globulin ratio. Similarly, there was no clear effect of dietary treatments on any of the serum cytokine concentrations.

In conclusion, the response patterns of weanling pigs to dietary treatments were rather inconsistent. Pigs did not respond to the complex diets and simple corn-SBM-based diets as expected, and their response to dietary supplementation of 0 to 2% fish peptides to simple corn-SBM-based diets were not consistent. Because of the considerable variations in the data, the effort to estimate the optimum inclusion rate by fitting selected response criteria against various regression models was not successful. Based on the subjective evaluation, however, the greatest values in many response criteria seemed to be observed with pigs fed the diets containing 1.5% fish peptides, even though, again, the response patterns to fish peptide supplementation were rather inconsistent.

ACKNOWLEDGMENTS

I would like to express my sincere gratitude and appreciation to my major professor, Dr. Lee I. Chiba, for his teaching, supervision, and guidance throughout the graduate program. I would like to express my gratitude to Dr. Werner G. Bergen and Dr. Nar K. Gurung for their willingness to serve on the advisory committee and their support, encouragement, and constructive advice. I would like to thank all the faculty and staff of the Department of Animal Sciences and Swine Research and Education Center for their support and inputs throughout the program. I would also like to expresses deep appreciation to the fellow graduate students. I would like to thank my family for their continued support and encouragement throughout my graduate program.

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I. INTRODUCTION

The most important period in the pig's life is weaning. During this period, pigs are exposed to various weaning stressors, including the separation from the sow, adaptation to new environment, change in the diet, and establishment of the new hierarchy. These stressors can lead to so called growth check, i.e., not gaining any weight for a week or so after weaning. As far as the nutritional stress is concerned, pigs are forced to switch from milk or a liquid feed to a solid diet. Their digestive system is immature and their enzymes are geared toward utilizing milk lipids and proteins. The activities of their digestive enzymes are not sufficient to breakdown a corn-soybean meal (SBM)-based diet, which has been the golden standard in feeding pigs. Therefore, feeding a simple corn-SBM diet to weanling pigs is not appropriate because of, again, their immature digestive system.

Providing weanling pigs some highly palatable and digestible complex diets containing many special ingredients, such as dried whey, soy protein concentrate, plasma protein, fish meal, blood meal, and others, can be one of the ways to alleviate the problem. Research has been shown that pigs fed the complex diets had a better growth performance during the early post-weaning period compared with pigs fed the simple diets. However, feeding complex diets to weanling pigs can be rather costly, and also it is rather difficult to procure a small amount of all those ingredients necessary to prepare complex diets and keep those ingredients fresh.

In addition, weanling pigs have not developed sufficient own immunity yet. Because of that, over the years, it has been a common practice to include appropriate antibiotics in the complex diets to help them defend themselves against pathogenic microorganisms, thus, various diseases. However, a continuous use of antibiotics in the diet may have led to the development of antibiotics resistance, which can be passed on to humans; thus, potentially threatening the public

health. Considering the cost and resistance problem, it is necessary to explore the possibility of using some alternative approaches to the use of antibiotics. Some recent research has demonstrated that various compounds, such as essential oils, zinc oxide, and some bioactive peptides, may have similar antimicrobial properties as traditional antibiotics.

The ultimate research goal for our growing pig research program is to make contributions to the development of environmentally friendly, optimum feeding strategies for successful and sustainable pig production. The effort to replace rather costly complex diets with semi-simple or simple corn-SBM diets for weanling pigs may contribute greatly to our overall objective. As part of the overall objective to replace the complex diets with simple corn-SBM diets, this study was conducted to assess the effectiveness or optimum dose of dietary fish peptides on weanling pigs. Specific objectives were to assess the effect of dietary fish peptides on growth performance and serum metabolite and cytokine concentrations in weanling pigs. The effect of fish peptides, along with some other feed additives, such as various exogenous enzymes, in our effort to replace complex diets completely with simple diets will be addressed in the future.

II. LITERATURE REVIEW

Introduction

Over the years, a corn-soybean meal (SBM) diet has been the golden standard for feeding pigs because it can effectively satisfy the energy needs and provide an excellent balance of indispensable amino acids. However, feeding a corn-SBM simple diet to young pigs, especially the pigs in the starter period, may cause some problems. Pigs in the weanling period do not have a mature digestive system. The enzymes in weanling pigs are geared toward using milk carbohydrates and lipids, and the enzymes necessary to utilize simple corn-soy diets are increasing but still insufficient until, at least, 7 to 8 wk of age. Therefore, feeding a simple corn-SBM diet to weanling pigs may lead to, e.g., diarrhea, loss of appetite, and loss of weight, and consequently, reduced growth performance. Feeding a highly palatable and digestible complex diet containing many special ingredients to weanling pigs can be one of the ways to alleviate the problems. However, feeding a complex diets containing special ingredients to weanling pigs can be rather costly. It is also rather difficult to procure and store a small amount of all those necessary ingredients to prepare a complex diet. For successful and sustainable pig production, it is rather important to use a corn-SMB-based diet for all classes of pigs. With the increasing development of various feed additives in recent years, such as various exogenous enzymes and bioactive or functional feed additives, it is possible that weanling pigs may be able to extract energy and nutrients from corn and SBM more efficiently.

Pigs in the starter period have not yet developed sufficient immunity. For that reason, it has been a common practice over the years to include appropriate antibiotics in their diets to help weanling pigs defend themselves against pathogenic microorganisms, thus, improving their health status and, consequently, their growth performance. However, with the continuous use of

the therapeutic dose of antibiotics in the feed can lead to the development of antibiotic resistance, which can reduce the effectiveness of the antibiotics. Furthermore, resistant factor or resistant bacteria can be passed on to humans, threatening the public health. Of course, many factors are responsible for the development of resistance, but antibiotics used in animals for preventive, therapeutic, and other purposes play, certainly, some roles. Considering the antibiotic resistance problems and also the cost of supplementation, it is necessary to find some viable alternatives to the use of antibiotics in weanling pig diets.

To make contributions to the development of environmentally friendly, optimum feeding strategies for successful and sustainable pig production, which is our ultimate goal for our growing pig research program, increasing the efficiency of utilization of a corn-SBM diet/diets by weanling pigs is, obviously, important. As pointed out previously, with the recent development in the feed additives, such exogenous enzymes and bioactive or functional feed additives, it might be possible to enhance weanling pigs' ability of extract more energy and nutrients from corn and SBM. In this review, some subjects or issues associated with the possibility of using simple corn-SBM diets for weanling pigs are discussed briefly.

Diet Complexity

Problems Associated with Weanling Pigs

The most important period in the pig's life is weaning. In this period, pigs are exposed to various weaning stressors, including environmental, nutritional, and social stresses, and also the separation from the sow. All these stressors can lead to so called growth check, and pigs are not gaining any weight for a week or so after weaning (Tokach et al., 1992; Azain, 1993). Feeding weanling pigs a simple corn-SBM diet can aggravate the situation, and, obviously, such a diet is

not appropriate because of their immature digestive system (Le Dividich and Sève, 2000). Their digestive enzymes are geared toward utilizing milk lipids and carbohydrates, and the activities of various digestive enzymes necessary for utilizing corn-SBM diets are not sufficient (Jensen et al., 1997) until 7 to 8 wk of age. Therefore, it is necessary to include some milk products and other highly digestible and palatable ingredient in their diet, i.e., weanling pigs need good "complex diets."

Simple and Complex Diets

Although, again, a corn-SBM diet has been the golden standard for feeding pigs over the years, weanling pigs just cannot utilize such a diet efficiently. Weanling pigs cannot breakdown corn and SBM efficiently and feeding such diets may lead to a low feed intake and poor nutrient absorption, and eventually a poor growth performance. It has been shown that pigs commonly lose about 100 to 250 g body weight (BW) during the first day after weaning and may recover in 4 d or so (Le Dividich and Sève, 2000). Providing weanling pigs some highly palatable and digestible complex diets containing many special ingredients, such as dried whey, soy protein concentrate, plasma protein, fish meal, blood meal, and others, can be one of the ways to alleviate the problems (Whang et al., 2000). Mavromichalis et al. (2001) showed that pigs fed complex diets had a better growth performance during the early post-weaning period compared with pigs fed the simple corn-SBM-based diets.

Antibiotics in Weanling Pig Diets

In the past 5 decades, antibiotics have been widely used in the livestock and poultry industries. Inclusion of dietary antibiotics in the pig diets has been shown to improve growth rate and efficiency of feed utilization, reduce mortality and morbidity, and enhance reproductive performance, especially effective for the young pigs (Cromwell, 2002). Cromwell (2002) also

indicated that antibiotics were capable of preventing and treating diseases with greater doses. The results of 67 field trials conducted over 22 yr period (H.M. Maddock, unpublished data) indicated that antibiotics reduced mortality by one-half (2.0 vs. 4.3%) in young pigs. Moreover, the reduction in mortality was even greater under high-disease conditions and environmental stress (3.1 vs. 15.6%).

However, it has been pointed out that the continuous use of antibiotics in feed can lead to the antibiotic resistance, and the resistant factors can be transferred to pathogenic bacteria in both animals and humans, eventually, threatening the public health (Van der Fels-Klerx et al., 2011). In addition, drug residues in the meat products have become a concern by the consumers (Vondruskova et al., 2010). Because of these concerns, the use of antibiotics as a growth promoter has been either banned or its use has been regulated in many countries.

Alternatives to Antibiotics

General

Because of the aforementioned potential problems, antibiotics are no longer allowed to be used in pig diets as a growth promoter in most countries. The European Union banned the use of antibiotics as a growth promoter in animal feed, which became effective on January 1, 2006 (Wenk, 2003). In addition, new regulations by the US Food and Drug Administration, which went into effect on January 1, 2017, restrict the use of antibiotics in animal feed. Thus, it is necessary to explore some potential alternatives in maintaining the animal's health status and enhance their growth performance (Windisch et al., 2008). Some of the most widely researched alternatives include probiotics, prebiotics, enzymes, and acidifiers, and also some plant extracts such as essential oils (Thacker, 2013). In addition, recent research demonstrated that various

compounds such as Zn oxide and some bioactive peptides may have similar antimicrobial properties as traditional antibiotics (Jensen-Waern et al., 1998; Langhout, 2000; Khattak et al., 2014; Zamora-Sillero et al., 2018).

Assessing Intestinal and Overall Health of Pigs and Other Animals

The effect of antibiotics in animal feed can be assessed by many different ways. For instance, determining the rate and efficiency of growth rate, morbidity, and mortality rate can be, certainly, beneficial in assessing their effectiveness. In addition, determining some aspects of the immunity and microbiome, such as cytokines and microbiota, can be useful and pertinent for the discussion of this particular issue.

Cytokines. Cytokines are small secreted proteins released by cells that have some specific effects on the interactions and communications between cells, and they are primarily involved in the host response to diseases or infections (Dinarello, 2000; Zhang et al., 2007), which can be used partly to assess the immune status. Cytokines include chemokines, interferons, interleukins (IL), lymphokines, and tumor necrosis factor (TNF), and they can be classified into 2 general categories, pro-inflammatory and anti-inflammatory cytokines (Zhang et al., 2007). Pro-inflammatory cytokines such as IL-1β, IL-6, and TNF-α can be involved in promoting cellular immune response, and they are produced predominantly by activated macrophages (Lucey et al., 1996; Zhang et al., 2007). Pro-inflammatory cytokines can induce fever and activate B and T lymphocytes and endothelial cells, therefore, plasma concentration of pro-inflammatory cytokines can be an indicator for determining infections, trauma, or ischemia (Dinarello, 2000). Anti-inflammatory cytokines, including IL-1 receptor antagonist, IL-4, IL-10, IL-11, and IL-13, can inhibit the activity of pro-inflammatory cytokines (Lucey et al., 1996; Zhang et al., 2007).

Interactions between pro-inflammatory and anti-inflammatory cytokines can regulate the ultimate effect of pathogens on the animal.

Intestinal Microbiota. Intestinal microbiota can be classified as the entire population of microorganisms that colonizes the gastrointestinal tract, and it can be used partly to assess the intestinal health and overall immune status of animals. It includes not just bacteria, but also other microbes such as fungi, archaea, viruses, and protozoa (Sekirov et al., 2010). The primary function of some intestinal microbes is to help in nutrient metabolism, especially to breakdown non-digestible fiber. Other functions include their involvement in the drug metabolism, prevention of colonization of pathogenic microorganisms, and providing the intestinal barrier (Jandhyala et al., 2015). Nonruminant species lack certain enzymes to digest certain polysaccharides in cereal or grain and others, while some microbes within the gut microflora are capable of fermenting polysaccharides into short chain fatty acids (SCFA). Those SCFA can be used as a source of energy and have also been shown to have a direct inhibitory activity towards some gastrointestinal pathogens (Gibson, 2004). At the same time, some healthy microbiota can resist some invasive pathogenic microorganisms (Jandhyala et al., 2015).

Essential Oils

General. Essential oils can be defined as plant essences obtained from plant material by steam or water distillation, and usually have the characteristic odor or flavor of the plant in question (Stein et al., 2006; Cross et al., 2007). They are typically mixtures of secondary plant metabolites and may contain phenolic compounds, terpenes, alkaloids, lectins, aldehydes, polypeptides, or polyacetylenes (Gatnau, 2009). Essential oils may exhibit a range of potentially beneficial properties, including antimicrobial (Deans and Ritchie, 1987; Paster et al., 1990; Hammer et al., 1999), antioxidant (Vichi et al., 2001), and antiviral (Bishop, 1995) activities.

Because of those beneficial properties, essential oils can be one of the alternatives to replace antibiotics in pig diets.

Effect of Essential oils on Cytokines. Essential oils are capable of acting as, again, antimicrobials, antioxidants, and immune enhancers to produce animals in an antibiotic-free production system (Kettunen et al., 2006; Tiihonen et al., 2010). Li et al., (2012) indicated that inclusion of essential oils in pig diets decreased cytokine, IL-6. Mao et al. (2005) found a decreased plasma IL-1β and increase in lymphocyte proliferation with supplementation of pig diets with plant extracts. Kong et al. (2007) indicated that dietary supplementation of plant extract decreased serum IL-1β and increased serum TNF concentration. Those results indicated that essential oils or some plant extracts can suppress some pro-inflammatory cytokines, thus, can affect the pig's immune response.

Effect of Essential Oils on Regulation of Intestinal Microbes. In recent years, the results of in vitro studies have shown that essential oils can have antimicrobial activity against microflora commonly present in the intestinal tract of pigs. Michiels et al. (2009) indicated that essential oils were capable of inhibiting the pig's intestinal microbes in in vitro incubation model. Prabuseenivasan et al. (2006) demonstrated that cinnamon, clove, geranium, lemon, lime, orange, and rosemary oils exhibited strong activity against select bacterial strains. Matan et al. (2006) and Aureli et al. (1992) also reported cinnamon, clove, and rosemary oils had strong in vitro antimicrobial activities to inhibit various pathogens.

Although there are a considerable body of literatures providing in vitro evidence that essential oils do have consistent effects on the gut microflora regulation (Aureli et al., 1992; Matan et al., 2006; Prabuseenivasan et al., 2006; Michiels et al., 2009), the results of in vivo studies have been rather inconsistent. Ohno et al. (2003) found that the density of *H. pylori* in the

stomach of mice treated with lemongrass oil was reduced compared with untreated mice. Sudjana et al. (2009) also indicated that olive leaf extract may have a role in regulating the composition of the gastric flora by selectively reducing *H. pylori* and *C. jejuni*. However, Sudjana et al. (2009) demonstrated that administering carrot seed oil to mice did not result in decreases in the bacteria counts compared with those without carrot seed oil. Similarly, Bergonzelli et al. (2003) indicated that essential oils were unlikely to be an efficient anti-Helicobacter agents in in vivo.

Similar results have been reported in pigs and poultry. Li et al. (2012) indicated that adding essential oils to the pig diet reduced the occurrence of diarrhea and decreased *E. coli* counts in feces. In addition, feeding essential oils increased lymphocyte transformation and leukocyte phagocytosis rates. Numerous in vivo studies demonstrated that supplementing poultry diets with essential oils, either individually or in some combination, resulted in a clear growth inhibition of *Clostridium perfringens* and *E. coli* in the hindgut and ameliorated intestinal lesions and weight loss (Mitsch et al., 2004; Jamroz et al., 2006; Jerzsele et al., 2012). Manzanilla et al. (2006) and Castillo et al. (2006) indicated that cinnamaldehyde and capsicum oleoresin increased the population of *Lactobacilli* and the ratio of *Lactobacilli* to *Enterobacteria* in the jejunum and cecum of early-weaned piglets, but there were no effects on the inhibition of pathogenic bacteria. Similarly, Si et al. (2006) showed that the selected essential oils and their components (carvacrol, thymol, and cinnamaldehyde) were not effective in reducing Salmonella serotype Typhimurium DT104 shedding.

Effect of Essential Oils on Growth Performance. In recent years, researchers have been interested in whether essential oils can affect animals' growth performance; however, the results have been rather inconsistent. In poultry, Lee et al. (2003, 2004) found that inclusion of essential

oils in the diet can increase secretions of endogenous digestive enzymes, enhance nutrient digestion and gut passage rate and improve growth performance. Amad et al. (2011) reported that supplementation of chicken diets with plant extracts could improve apparent ileal digestibility of nutrients. In addition, synergistic effects of feeding either individual essential oil or the combination of essential oils on growth rate have been well documented (Bassett, 2000; Langhout, 2000; Alcicek et al., 2003; Denli et al., 2004). On the other hand, there were studies that showed adding essential oils in chicken diets did not affect performance (Case et al., 1995; Botsoglou et al., 2002). The reasons for these inconsistent results in different studies have not yet been elucidated, but various factors may have been responsible, such as differences in the inclusion rates, sources of herbs used to produce a blend of essential oils, composition of the basal diet, and environmental conditions (Khattak et al., 2014).

In pigs, Li et al. (2012) compared the growth performance of pigs fed a normal control diet without essential oil supplementation and those fed a diet supplemented with antibiotics or a combination of thymol and cinnamaldehyde. They found that including essential oils in diets could enhance nutrient digestibility and improve growth rate. Similar studies showed that the addition of dietary phytogenic feed additives to weanling pig diets could affect growth performance (Holden and McKean, 2002; Kamel, 2002; Silvia, 2002; Hong et al., 2004; Cho et al., 2006). However, adding essential oils to pig diets has not always affected feed intake of pigs, which led to the inconsistent growth responses. In their review, Zeng et al. (2015) indicated that the change in feed intake, thus, their response was associated with the range of dietary supplementation of essential oils in the pig diets. In addition, Zhai et al. (2018) indicated that the growth response to essential oils may be dependent the chemical composition of essential oils, as well as on the dietary characteristics and experimental conditions, and the direct effects on gut

microflora and indirect effects through the gut-associated immune system should be explored further to elucidate fully the effects of essential oils.

Zinc Oxide

Zinc has been reported to have many biological functions, including anti-inflammation and anti-diarrheal effects and also the maintenance of the epithelial barrier integrity (Roselli et al., 2003, Patel et al., 2010). It has been suggested that ZnO can be used as an antimicrobial agent (Roselli et al., 2003, Patel et al., 2010) to resist some intestinal diseases. Zinc oxide is currently listed as a generally recognized as safe material to be used as food additive by the Food and Drug Administration in the United States. There is a considerable body of literature showing that feeding pharmacological doses of Zn (2,000 to 4,000 mg/kg of Zn as ZnO) may reduce the risk of diarrhea and improve growth performance of animals (Hahn and Baker, 1993; Ou et al., 2007; Zhang and Guo, 2009). Therefore, ZnO has a potential to be used as one of the potential alternatives to antibiotics. However, there has been some concerns on the use of high doses of Zn in animal diets because of its potential negative environmental impact (Poulsen and Larsen, 1995; Carlson et al., 2004). The ban on the use of ZnO in the pig diet will be in effect in June of 2022 among the EU countries, and other countries may follow suit.

Effect of Zinc Oxide on Cytokines. There is some evidence that ZnO or Zn products have anti-inflammatory effects to modulate animal inflammatory responses (Patel et al., 2010). Hu et al. (2013) found that, 7 d after weaning, zeolite ZnO (Z-ZnO) decreased mRNA for TNF-α and IFN-γ in weanling pigs. Moreover, inclusion of the Z-ZnO resulted in downregulation of proinflammatory cytokines, indicating that weaning-induced inflammation was, in fact, alleviated. Hu et al. (2013) also indicated that Z-ZnO and ZnO increased mRNA levels of TGF-β1 and IL-10 at d 7 postweaning, which was consistent with the result of Roselli et al. (2003)

who showed that ZnO could upregulate the mRNA level of TGF-β in enterotoxigenic E. coli infected cells.

In humans, Zn is capable of mediating positively the gene expression of IL-2 and IFN-γ in the Th1 cell line and negatively the expression of TNF-α, IL-1β, and IL-8 in the monocyte-macrophage cell line (Bao et al., 2003). Prasad et al. (2004) conducted a study to evaluate the effect of zinc supplementation on immune response of healthy human subjects (20- to 50- yr-old), and they found that supplementing Zn reduced the concentrations of the oxidative stress–related byproducts, malondialdehyde (MDA), 4-hydroxyalkenals (HAE), and 8-hydroxydeoxyguanosine in the plasma. In addition, the result showed that supplementation with Zn inhibited the ex vivo induction of TNF-α and IL-1β mRNA in mononuclear cells (MNC), and provided protection against TNF-α–induced nuclear factor–κβ activation in isolated MNC (Prasad et al., 2004). Other studies indicated that providing elderly the therapeutic dose of zinc (> 50 mg elemental Zn/d for > 12 wk) helped preventing many chronic disorders that have been related to oxidative stress and chronic inflammatory cytokines such as TNF-α, IL-1β, and IL-8 (Pennington, 1993; Elliott et al., 1994; Beutler, 1995; Steven et al., 2002). It should be kept in mind that there may be considerable differences in the administration dose between animal and humans studies.

Effect of Zinc Oxide on Regulation of Intestinal Microbes. The reports on the effect of ZnO on the intestinal microbes have been rather inconsistent. Roselli et al. (2003) reported that ZnO had antibacterial activity in regulating the animal's gut microflora. Similarly, Sawai (2003) found that ZnO was capable of inhibiting the growth of *Staphylococcus aureus* and *E. coli*. In young pigs, high doses of ZnO reduced bacterial translocation from the small intestine to the ileal mesenteric lymph node (Huang et al., 1999). On the other hand, Jensen-Waern et al. (1998)

indicated that supplementation with 2,500 mg Zn/kg as ZnO had no effect on the number of *E. coli* and *Enterococcus spp.* in fecal samples during the first 2 wk after weaning. In addition, although Hu et al. (2013) reported that dietary supplementation with ZnO (2,250 mg Zn/kg) decreased the viable count of *Clostridium*, ZnO did not affect the number of *E. coli*. It is possible that gram-positive bacteria are more susceptible to ZnO than gram-negative bacteria (Sawai, 2003). Katouli et al. (1999) reported that inclusion of ZnO in weanling pig diets reduced post-weaning diarrhea only during the first 2 weeks. Accordingly to poVellenga et al. (1992), weanling pigs cannot absorb high doses of ZnO and excreted ZnO into the feces, which may lead to the environmental problem as mentioned previously.

Similarly, the effects of ZnO on gut intestinal microflora in humans have not been consistent. Reselli et al. (2003) reported that ZnO reduced bacterial adhesion and inhibited enterotoxigenic *E. coli* internalization. Recent research indicated that ZnO nanoparticle could be used externally to control the spreading of bacterial infections, and as the concentration of ZnO nanoparticle increased, the growth of *K. pneumoniae* decreased (Reddy et al., 2014). On the other hand, other studies indicated that ZnO did not have a positive effects on gut microbes. Although some studies reported that ZnO nanoparticle can be used to treat mild bacterial infections, Zn is also an essential trace element for some viruses and it can increase enzymatic activity of viral integrase in humans (Elster et al., 1994; Lee et al., 1997; Baum et al., 2000). Hiller and Perlmutter (1971) have also indicated that patients treated with Zn increased the infectious pancreatic necrosis virus by 69.6%. Again, the administration doses are different between animal and human studies.

Effect of Zinc Oxide on Growth Performance of Weanling Pigs. As pointed out before, the weaning period is the most stressful phase for newly weaned pigs, and the stressors could

induce intestinal barrier dysfunction and digestive disorders, and impair their growth performance (Smith et al., 2010, Peace et al., 2011, Kim et al., 2012). Some studies have shown that supplementation of the diet with Zn affected post-weaning pigs' growth performance positively (Hahn and Baker, 1993; Hill et al, 2000). Zinc oxide was the only inorganic form of Zn that produced those beneficial effects (Hahn and Baker, 1993; Schell and Kornegay, 1996). Carlson et al. (2004) demonstrated that pigs fed 2,000 ppm Zn as ZnO had greater weight gain than pigs fed the basal diet.

In general, inclusion of high doses of ZnO in the weanling pig diet improved growth performance and decreased post-weaning diarrhea as reported in several reviews (Hahn and Baker, 1993, Ou et al., 2007, Zhang and Guo, 2009). Hu et al. (2012) reported that supplementing the diet with 500 mg/kg of Zn as ZnO resulted in no growth benefit, which was consistent with some previous investigations that used less than 1,000 mg Zn/kg (Davis et al., 2004, Hollis et al., 2005), indicating a high dose/doses of ZnO may be necessary to observe some beneficial effects. However, there might be some problem associated with supplementing the diet with high doses of ZnO. It may result in the excretion of large quantities of Zn in the feces, which can lead to environmental problems (Poulsen and Larsen, 1995, Carlson et al., 2004), as pointed out before.

Bioactive Peptides

Bioactive peptides can be defined as small amino acid sequences derived from food or feed proteins that possess some potential physiological properties beyond providing normal and adequate nutrition. Within the precursor proteins, the amino acid sequence of bioactive peptides is inactive, but it can display diverse biological activities after being released (Udenigwe and Aluko, 2012).

To produce bioactive peptides, proteolysis is needed, which may occur through enzymatic digestion of the precursor protein, either in in vitro or in the digestive tract (in vivo). Moreover, food or feed processing and enzymes from microorganisms or plants can trigger proteolysis and release potential bioactive peptides (López-Barrios et al., 2014). Research showed that all food or feed proteins can release bioactive peptides, but animal protein sources, especially dairy products, have been extensively researched for their bioactive peptides. Bioactive properties of bioactive peptides derived from milk have been demonstrated to have effects on the digestive, endocrine, cardiovascular, immune, and nervous systems in both in vitro and in vivo studies (Haque et al., 2008; Choi et al., 2012).

Ryan et al. (2011) indicated that meat and fish bioactive peptides possessed antioxidant, antimicrobial, and antiproliferative activities. It seems that fish-derived bioactive peptides contain a great potential for their use in the production of drugs and functional food or feed (Sila and Bougatef, 2016). In the process of protein hydrolysis, bioactive peptides released from fish protein can have various bioactive capabilities such as antioxidative, antihypertensive, antithrombotic, immunomodulatory, and antimicrobial properties (Kim and Wijesekara, 2010). Because of some of their properties, bioactive peptides may have a potential as an alternative to antibiotics.

Effect of Bioactive Peptides on Cytokines. In recent years, researchers have been interested in discovering the potential of bioactive peptides derived from various dietary proteins to reduce the risk of chronic diseases or boosting natural immune protection to promote human health (Ma et al., 2006). Yang et al. (2009) indicated that IL-2, IFN-γ, IL-5, and IL-6 were increased in the serum of mice that were exposed to marine oligopeptide preparation (MOP). Yang et al. (2009) concluded that MOP has the capability of modulating the immune system

positively because it can stimulate the T helper (Th) cells, causing secretion of Th1 and Th2 cytokines. The IL-2, IFN-γ, TNF-α, and granulocyte-macrophage colony-stimulating factor belong to Th1 cells, whereas IL-4, IL-5, IL-6, and IL-10 belong to Th2 cells. The MOP, thus, may have a beneficial effect on the immune function of people or other animals. Moreover, studies showed that other bioactive peptides, e.g., constituents from soybean (Cheng et al., 2007) and fish protein concentrate (Duarte et al., 2006), could also enhance Th cells to increase the production of various cytokines.

Because the effects of bioactive peptides in mice or humans have been well established, researchers have also been interested in evaluating the effects of such peptides on pigs. Rong et al. (2015) evaluated the effects of casein glycomacropeptide (CGMP) derived from milk as a potential bioactive feed additive on growth performance, intestinal morphology, intestinal barrier permeability, and inflammatory responses in pigs challenged with *E. coli* K88 (*E. coli* K88). They found that CGMP can reduce the inflammatory response caused by an *E. coli* K88 challenge.

Young et al. (2012) evaluated the anti-inflammatory activity of soy-derived di- and tripeptides on colitis pigs treated with dextran sodium sulfate. The results indicated that TNF-α concentrations were reduced in soy peptide-treated pigs, which were similar to the concentration observed in negative control pigs (i.e., pigs without colitis). Although the expression of IL-6 mRNA did not decrease in the pigs treated with soy peptides, local colonic IL-6 concentrations in soy peptide-treated pigs were dropped to the concentrations observed in the negative control group. The reduced expression of interferon gamma and IL-1b and the slightly decreased mRNA expression of IL-12b in pigs treated with soy peptides showed less inflammation in the colon (Young et al., 2012). Inflammation of colitis is regulated by Th1 cytokines; thus, an increase of

IFN-γ and TNF-α in serum can be an indirect indicator of inflammation. It can also stimulate the release of other proinflammatory cytokines, including IL1-B, IL6, and IL12-B, and lead to the perpetuation of the condition (Bouma and Strober, 2003).

Although the positive effects of bioactive peptides on cytokine in pigs were well demonstrated (e.g., Zhang et al., 2019), there were other studies that showed no effects. Zhao et al. (2008) indicated that the cytokine mRNA responses observed in their study did not support an intestinal anti-inflammatory effect of spray-dried plasma protein and hydrolyzed marine plant protein. Therefore, the effects of bioactive peptides on animal's cytokine have been rather inconsistent.

Fish Peptides or Hydrolysates. The fish industry is a pillar of the economy in many countries worldwide, and in recent years, the production of fish protein peptides or hydrolysates has increased worldwide (Zamora-Sillero, 2018). The use of fish peptides or hydrolysates as a source of protein for young pigs is not really a new idea (e.g., Stoner et al., 1985), even though the literature on the use of such products for pig nutrition has been rather scarce. Some relatively recent data indicated that fish peptides or hydrolysates can be effective in improving the growth performance of young pigs (Thuy et al., 2016), or they can be an effective replacement for some commonly used protein sources for young pigs (Tucker et al., 2011; Nørgaard et al., 2012). Fish peptides or hydrolysates are available worldwide as a supplement and also as a functional dietary ingredient, and various biological activities have been demonstrated in animals and humans (Gevaert et al., 2016), including antioxidative, antimicrobial, antihypertensive, and other activities (Rajabzadeh et al., 2018; Zamora-Sillero, 2018). Thus, fish peptides or hydrolysates can be used as a possible viable alternative to antibiotics.

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III. EFFECT OF DIET COMPLEXITY AND DIETARY FISH PEPTIDE SUPPLEMENTATION ON WEANLING PIGS

Effect of Diet Complexity and Dietary Fish Peptide Supplementation on Weanling Pigs

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A B S T R A C T

The objective of this study was to investigate the effect of supplementing a simple corn-soybean meal (SBM) diet with dietary fish peptides on growth performance, serum metabolite profile, and serum cytokines in weanling pigs. Forty-eight gilts and 48 castrated males (initial body weight, 7.87 ± 0.71 kg) weaned at 3 to 4 wk of age were randomly assigned to 6 dietary treatments with 4 replicate pens (2 gilts and 2 castrated males/pen) per treatment. After weaning, pigs were fed a common pre-starter diet for 4 d before beginning of the study, and a 2-phase feeding program was used with each phase consisting of 2 wk. Two typical complex diets containing various special ingredients were formulated and used as a positive control (POS) diet. Two simple corn-SBM, negative control (NEG) diets were formulated to be iso-lysinic to the POS diets, and the NEG diets for phase 1 and 2 were supplemented with 0, 0.5, 1.0, 1.5, or 2.0% fish peptides. A small amount of dried whey (5%) was included in the phase 1 NEG and fish peptide diets to ensure that pigs were growing. Dried whey was not include in the phase 2 NEG and fish peptide diets. Fish peptides were included in the diet by replacing the part of corn and SBM. All 6 diets were formulated to meet or exceed the 2012 NRC nutrient requirements. During the fourth week of the study, approximately 5 mL of blood was collected via vena cava puncture using a sterile needle and evacuated tube. Serum was separated, and an aliquot was stored at -20ºC. Serum sample from each pig was analyzed for metabolites, whereas serum samples were pooled by pen and analyzed for serum cytokines. During the last 2 wk of the study, the intake of feed, Lys, and DE increased cubically (P = 0.025, 0.025, and 0.026, respectively) as fish peptide supplementation increased from 0 to 2%. Overall, pigs fed the diets supplemented with 1.5% fish peptides had greater feed, Lys, and DE intakes (approximately 8%) than those fed other diets, but those differences were not statistically significant (cubic, P = 0.135, 0.136, and

0.167 respectively). Overall weight gain of pigs fed the diets containing fish peptides seemed to be greater (6.7%) than those fed the POS diet, but it was not statistically significant (P = 0.137). There were no clear trends in the efficiency of overall utilization of feed, Lys, or DE intake for weight gain. Pigs tended to respond linearly and quadratically (P = 0.092 and 0.106, respectively) to the increase in fish peptide supplementation in serum total protein. Serum urea N concentration was greater in pigs fed the diets supplemented with fish peptides compared with those fed the POS diets (P = 0.005), whereas it increased linearly (P = 0.017) as fish peptide supplementation increased from 0 to 2%. Although pigs tended to respond quadratically (P = 0.093) to fish peptide supplementation in serum triglycerides, there was no clear effect of dietary treatments on serum albumin, globulin, glucose, or cholesterol concentration, or albumin to globulin ratio. Similarly, there was no clear effect of dietary treatments on any of the serum cytokine concentrations. In conclusion, the response patterns of weanling pigs to dietary treatments were rather inconsistent. Pigs did not respond to the complex diets and simple corn-SBM-based diets as expected, and their response to dietary supplementation of 0 to 2% fish peptides to simple corn-SBM-based diets were not consistent. Because of the considerable variations in the data, the effort to estimate the optimum inclusion rate by fitting selected response criteria against various regression models was not successful. Based on the subjective evaluation, however, the greatest values in many response criteria seemed to be observed with pigs fed the diets containing 1.5% fish peptides, even though, again, the response patterns to fish peptide supplementation were rather inconsistent.

Keywords: Weanling pig, Diet complexity, Dietary Fish Peptides

1. Introduction

Young pigs are subjected to various stressors at weaning, and providing a highly palatable and digestible diet is, obviously, important in preventing or alleviating so called growth check soon after weaning and optimizing growth performance thereafter. Although a corn-soybean meal (SBM) diet is the gold standard for feeding pigs, such a diet may not be appropriate for weanling pigs because of many digestive, metabolic, and immunological challenges. For instance, their digestive enzyme profile is designed to utilize milk protein, carbohydrate, and lipids and not corn and SBM (e.g., Lindemann et al., 1986; Le Dividich and Sève, 2000). For those reasons, weanling pigs have been fed complex diets that contain many special ingredients, such as dried whey, soy protein concentrate, plasma protein, fish meal, blood meal, oat groats, and others (Himmelberg et al., 1985; Mahan et al., 2004; Tran et al., 2014).

Providing such diets with highly-palatable and digestible ingredients to weanling pigs can be, however, a costly proposition. It is possible that there might be some viable alternative supplements such as fish peptides or hydrolysates that can be not only an excellent source of nutrients, but also a bioactive or functional feed additive (Zamora-Sillero, 2018). Such an additive or supplement may enhance not only the efficiency of energy and nutrient utilization but also animal health, thus, improving the growth performance of pigs.

In addition, with the development and availability of various enzymes (e.g., Olukosi et al., 2007; Zhang et al., 2014), it might be possible to use a corn-SBM-based diet more efficiently for weanling pigs. Enzymes such as carbohydrases, proteases, phytase, and lipase can be used to extract more energy and nutrients from a simple corn-SBM-based diet. With an increased competition between humans and food producing animals for quality sources of energy and nutrients in recent years, it is imperative to utilize, e.g., corn and SBM efficiently and also

minimize the use of other quality sources of energy and nutrients for successful and sustainable pig and other food animal production in the future.

The ultimate research goal for our growing pig research program is to make contributions to the development of environmentally friendly, optimum feeding strategies for successful and sustainable pig production. In this proposed research, the effort will be made to replace a complex diet with a semi simple or simple corn-SBM diet for weanling pigs by dietary supplementation with a high-available source of nutrients, such as fish peptides, and also various exogenous enzymes.

As an initial part of the project, a study was conducted to determine the optimal inclusion rate of fish peptides in weanling pig diets. Two sets of diets (i.e., diets for 2 phases, each consisting of 2 wk) were used during the 4-wk starter phase. Specific objective were to investigate various inclusion rates of fish peptides on: a) growth performance, b) Serum metabolite profile, i.e., total protein, albumin, globulin, albumin to globulin ratio, blood urea N, glucose, cholesterol, and triglyceride concentrations, and c) serum cytokine concentrations.

2. Materials and methods

2.1. Animals and facilities

The protocol for animal care was approved by the Institutional Animal Care and Use Committee of Auburn University (Auburn, AL, US). A total of 96 piglets weaned at 3 to 4 wk of age (initial body weight, 7.87 ± 0.71 kg) were placed in pens (1.5 m^2) in an environmentally controlled nursery with slotted floors based on their sex and body weight. Pigs were randomly assigned to 6 dietary treatments with 4 replicate pens per treatment and 4 pigs per pen. Because of the availability of pigs and facility at one time, the study was conducted in 2 trials. Each trial

used 24 gilts and 24 castrated males, and 2 trials were approximately 8 wk apart. Pigs were allowed ad libitum access to feed and water throughout each 4-wk trial period, and the weights and feed consumption data were collected weekly.

2.2. Dietary treatments

After weaning, pigs were fed a common pre-starter diet for 4 d before beginning of the study, and a 2-phase feeding program was used with each phase consisting 2 wk. Two typical complex, positive control (POS) diet were formulated to contain 12.15 and 11.07 g/kg standard ileal digestible (SID) Lys/kg for pigs weighing 7 to 11 (Phase 1) and 11 to 25 kg (Phase 2), respectively (Tables 1 and 2; NRC, 2012). Those SID concentrations used in the current study were 90% of the NRC (2012) recommendations for those respective weight classes. In addition to corn and SBM, the complex diet for phase 1 contained spray dried whey (Honeyville, Brigham City, UT), soy protein concentrate, poultry fat, plasma protein (Appetein, APC Inc., Ankey, IA), and Zn oxide. The phase 2 POS diet contained reduced amount of spray dried whey and soy protein concentrate. Two simple, corn-SBM-based negative control (NEG) diets were formulated to be iso-lysinic to the POS diets for the phase 1 and 2. The NEG diets for phase 1 and 2 were supplemented with 0, 0.5, 1.0, 1.5, or 2.0% fish peptides (Vitech Bio-Chem Corporation, Orange, CA, US). A small amount of dried whey (5%) was included in the phase 1 NEG and fish peptide diets to ensure that pigs were growing. Fish peptides were included in the diets by replacing the part of corn and SBM. Minerals and vitamins for all diets were provided in amounts calculated to meet or exceed the NRC (2012) recommendations. Feed samples were collected from each batch of feed mixed, and pooled sub-samples were analyzed for crude protein (AOAC, 2000).

2.3. Collection of blood

During the fourth week of the study, approximately 5 mL of blood was collected via vena cava puncture using a sterile needle and evacuated tube. Blood samples were allowed to clot and serum samples were separated by centrifugation at 1,500 × *g* for 15 min at room temperature to obtain clean serum samples. An aliquot was stored at -20ºC until analyzed for serum metabolites and cytokines.

2.4. Analysis of serum samples

After thawing, serum samples were analyzed for metabolites, including total protein, albumin, globulin, albumin to globulin ratio, blood urea N, glucose, cholesterol, and triglyceride concentrations, by using an automated analyzer at Auburn University Clinical Pathology Laboratory (Auburn, AL). In addition, serum samples pooled by pen were subjected to the cytokine assay, which was used as an indicator of the health status of pigs. The multiplex assay consisted of granulocyte-macrophage colony-stimulating factor (GM-CSF), interferon-γ (IFNγ), interleukin (IL)-1α, IL-1Rα, IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12, IL-18, and tumor necrosis factor-α (Eve Technology Corp., Calgary, AB, Canada).

2.5. Optimum inclusion rate of fish peptides

The effort was made to estimate the optimum dietary inclusion rate of fish peptides for weanling pigs. Several approaches, including broken-line regression analyses (Mercer et al., 1989; Robbins et al., 2006; Salze et al., 2019), and selected response criteria (e.g., growth performance during the first 2 wk and overall and some serum metabolites) were used for the purpose. In addition, the effort was made to determine the optimum dose of fish peptides using some subjective approaches. For instance, the greatest and lowest values for each selected response criteria were expressed in a scale of 6 (0 being the lowest value and 6 being the greatest

value), and various response criteria were presented in a single figure. The averages for each inclusion rate for the selected response criteria were also included in the figure.

2.6. Statistical analysis

Data were analyzed using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC, US). The pen was considered as the experimental unit. The trial and treatment, along with appropriate body weight as a covariate, were included in the initial statistical model. Covariates considered for the analysis were initial body weight for growth performance data and third week body weight for serum metabolite and cytokine data. The results of the initial statistical analyses indicated that the trial and trial × treatment interactions were not important source of variation, thus, the data for the 2 trials were combined and analyzed accordingly. Preplanned contrasts consisted of POS vs. NEG diets, POS vs. fish peptide diets, and linear, quadratic, and cubic effects of fish peptides. The results are considered statistically significant if $P \leq 0.05$ and trends if $P \leq 0.10$.

3. Results

3.1. Growth performance

From d 0 to 7 of the study, pigs fed the diet containing 1.5% fish peptides seemed to consume more feed, Lys, and digestible energy (DE) and gain more weight than those fed other diets, but those differences were not statistically significant (Table 3). Similarly, from d 7 to 14, pigs fed the diet containing 1.5% fish peptides seemed to consume more feed, Lys, and DE, but those were, again, not statistically significant. Pigs fed the POS diet tended to have ($P = 0.071$) and lower weight gain ($P = 0.039$) than those fed the NEG and fish peptide diets, respectively. Those tendencies in the intake and reduced weight gain were reflected in the tendencies for pigs

fed the POS diet to have lower gain:DE intake than those fed the NEG (P = 0.089) or fish peptide diets (P = 0.083).

From d 14 to 21, the intake of feed, Lys, and DE tended to increase linearly (P = 0.062, 0.048, and 0.069, respectively) and increased cubically (P = 0.040, 0.039, and 0.045, respectively) as dietary supplementation of fish peptides increased from 0 to 2% (Table 4). Pigs fed the POS diets tended to consume less feed (P = 0.077) and Lys (P = 0.069) than those fed the fish peptide diets. Weight gain or the efficiency of feed, Lys, or DE utilization for weight gain was not affected by the dietary treatments. During d 21 to 28, there were tendencies for the cubic effect of fish peptides on feed (P = 0,066), Lys (P = 0.064), or DE (P = 0.94) intake, but the rate and efficiency of weight gain were not affected by the dietary treatments (P = 0.151, 0.147, and 0.146, respectively).

During the first 2 wk of the study, feed, Lys, and DE intakes of pigs fed the diet containing 1.5% fish peptides seemed to be greater than those fed other diets, but those differences were not statistically significant. Pigs fed the POS diet tended to have lower weight gain (P = 0.059) than those fed the diets supplemented with fish peptides. There were no clear effect of dietary treatments on the efficiency of utilization of feed, Lys, or DE intake for weight gain. The intake of feed, Lys, and DE during the last 2 wk of the study increased cubically (P = 0.025, 0.025, and 0.026, respectively) as fish peptide supplementation increased. Weight gain of pigs fed the diets containing 0.5% fish peptides was, numerically, 10% greater than those fed other diet, which was reflected in the cubic trends for the efficiency of feed, Lys, and DE utilization (P = 0.055, 0.056, and 0.056, respectively) with the increase in fish peptide supplementation from 0 to 2%.

Overall, pigs seemed to respond cubically to the increase in dietary fish peptide in feed, Lys, or DE intake, with those fed the diets supplemented with 1.5% fish peptides having a greater intake of feed, Lys, or DE (approximately 8%) than those fed other diets, but those differences were not statistically significant (cubic, $P = 0.135$, 0.136, and 0.167, respectively; Table 6). Weight gain of pigs fed the diets containing fish peptides seemed to be greater (6.7%) than those fed the POS diet, but, again, it was only numerically ($P = 0.137$). The cubic effects on feed, Lys, and DE intakes seemed to be reflected oppositely in the efficiency of feed, Lys, or DE intake as the dietary fish peptides increased from 0 to 2%, but the differences were not statistically significant (cubic, $P = 0.172$, 0.171, and 0.142, respectively).

3.2. Serum metabolites and cytokines

The effect of fish peptide supplementation on metabolite and cytokine concentrations in the serum of weanling pigs during the fourth week of the study is presented in Tables 7 and 8, respectively. Pigs fed the diets containing fish peptides tended to respond linearly and quadratically ($P = 0.092$ and 0.106, respectively) in total protein concentration to the increase in fish peptide supplementation (Table 7). Blood urea N concentration was greater in pigs fed the diets supplemented with fish peptides than those fed the POS diets ($P = 0.005$), whereas it increased linearly ($P = 0.017$) as fish peptide supplementation increased from 0 to 2%. Triglyceride concentration in pigs fed the NEG diet seemed to be greater than those fed the POS diet, even though it was not statistically significant ($P = 0.117$), and it was also responsible for the tendency for the quadratic response ($P = 0.093$) to the increase in fish peptide supplementation from, with the greater serum triglyceride concentrations in pigs fed the NEG diet and the diet containing 2% fish peptides. There was no clear effect of treatments on serum albumin, globulin, glucose, or cholesterol concentration or albumin to globulin ratio. Similarly,

there was no clear effect of dietary treatments on any of the cytokine concentrations (Table 8). The data on serum GM-CSF, IFNγ, or IL-6 were not presented because they were either not detected or observed values were rather aberrant.

3.3. Optimum inclusion rate

Because of considerable variations in the data set, the effort to estimate an optimum inclusion rate or inclusion rates by fitting selected response criteria against various regression models was not successful. No valid estimate was obtained. Based on the subjective evaluation, however, the greatest values seemed to be observed in pigs fed the phase 1 and 2 diets containing 1.5% fish peptides, even though, again, the response patterns of weanling pigs to fish peptide supplementation were rather inconsistent (Fig. 1).

4. Discussion

Weaning is one of the most critical period in the pig's life, and they are exposed to various digestive, metabolic, and immunological challenges, which can lead to so called growth check or a period of no growth soon after weaning. Among various weaning stressors, perhaps, the most important one is the digestive challenge (Lindemann et al., 1986; Jensen et al., 1997; Le Dividich and Sève, 2000). Young pig's digestive enzyme profile is geared toward the utilization of milk protein, carbohydrates, and lipids. Because of their immature digestive system, corn-SBM diets are not appropriate for weaning pigs (e.g., Li et al., 1991), even though corn-SBM diets are the gold standard for feeding pigs. For that reason, weanling pigs are typically fed complex diets containing various special ingredients, such as dried whey, plasma protein, fish meal, soy protein concentrate or isolates, blood meal, and others, to enhance their growth performance.

Providing such diets containing highly-palatable and highly-digestible special ingredients to weanling pigs can be very effective in promoting their growth performance (Himmelberg et al., 1985; Whang et al., 2000; Wolter et al., 2003; Mahan et al., 2004; Cromwell et al., 2008; Cervantes-Pham and Stein, 2010; Tran et al., 2014), but it can be rather costly. With the development and availability of various feed additives, such as enzymes or multi-enzyme complexes and bioactive compounds, such as fish peptides or hydrolysates, in recent years, it might be possible for weanling pigs to extract energy and nutrients from corn and SBM more efficiently and fully. This study was conducted as part of the effort to replace complex diets with simple corn-SBM-based diets by supplementing weanling pig diets with various feed additives, such as fish peptides, which can be not only a great source of nutrients and also considered as a bioactive or functional feed additive (Zamora-Sillero, 2018).

During the first 2 wk of the study, the growth performance of pigs fed the simple corn-SBM or NEG diet were similar to those fed the complex or POS diet, which was contrary to earlier findings (e.g., Himmelberg et al., 1985; Dritz et al., 1996; Whang et al., 2000). The reason for the lack of difference between pigs fed the complex and simple diets is not clear. Perhaps, using 90% of the NRC (2012) SID Lys requirements was not sufficiently low enough to magnify the response of dietary factor to the dietary restriction. It is also possible that feeding the pre-starter diet for 4 d immediately after weaning and before the initiation of the study and including 5% of dried whey to the phase 1 NEG diet may have neutralized the possible adverse effect of feeding a simple corn-SBM diet to newly weaned pigs.

The use of fish peptides or hydrolysates as a source of protein for young pigs is not really a new idea (Stoner et al., 1985), even though the literature on the use of such products for pig nutrition has been rather scarce. Some relatively recent data indicated that fish peptides or

hydrolysates can be effective in improving the growth performance of young pigs (Thuy et al., 2016), or they can effectively replace some commonly used protein sources for young pigs (Tucker et al., 2011; Nørgaard et al., 2012; Sun et al., 2016). Fish peptides or hydrolysates are available worldwide as a supplement and also a functional dietary ingredient, and various biological activities have been demonstrated in animals and humans (Gevaert et al., 2016), including antioxidative, antimicrobial, antihypertensive, and other activities (Rajabzadeh et al., 2018; Zamora-Sillero, 2018).

Supplementing a simple corn-SBM diet with 0 to 2% fish peptides seemed to result in a quadratic response in weight gain during the first week, with the weight gain being 16% greater in pigs fed the diet containing 1.5% fish peptides than those fed other fish peptide diets. Unfortunately, there were no other responses and the effect in weight gain was not statistically significant ($P = 0.147$). Feed, Lys, and DE intakes increased cubically during the second 2-wk of the study, but those increases were not reflected in the rate and efficiency of feed, Lys, or DE for weight gain. The current findings on the lack of response to fish peptides in weight gain or feed efficiency are rather consistent with the recent report by Poudel et al. (2020), even though they indicated that fish peptides affected the fecal microbiome composition of pigs during the first few weeks after weaning. Their results may indicate that, after all, fish peptides may have some biological activities or some roles as a bioactive or functional compound.

Blood total protein, albumin, and albumin to globulin ratio can be used as an indicator of adequate protein metabolism in animals (Lowrey et al., 1962). Positive correlations observed between serum albumin and growth performance in young pigs (Mule at al., 2006) may support their contention. In the current study, although serum total protein seemed to increase linearly and quadratically as dietary fish peptide supplementation increased, there were no effects of

dietary treatments on serum albumin or albumin to globulin ratio. On the other hand, serum urea N increased linearly with the increase in dietary fish peptides from 0 to 2%. Blood urea N can be an important indicator of protein and amino acid adequacy and efficiency of amino acid utilization (Coma et al., 1995; Whang and Easter, 2000), and it may decrease with the increased efficiency of feed or nutrient utilization (Chiba et al., 1991; Fabian et al., 2002). Therefore, it is rather difficult to make any definite conclusions on the effect of fish peptides on the protein metabolism. Although there was a tendency for the quadratic response in serum triglycerides with the increase in fish peptide supplementation, serum glucose or cholesterol concentration was not affected by the dietary treatments.

Cytokines are involved in a variety of biological processes, including cell activation, growth, and differentiation (Murtaugh and Foss, 2002). In addition, cytokines may play an important role in the immune and inflammatory responses (van der Meide and Schellekens, 1996; Zhang and An, 2007). The IL-1, IL-6, and TNF-α are typical pro-inflammatory cytokines (Dinarello, 1991, 2000), and weaning has been associated with the early and transient response in the expression of genes for inflammatory cytokines (Pié et al., 2004). Fish peptides has been reported to have some effect on relative mRNA abundance of TNF-α compared with some other dietary treatments, but they had no effect on IL-6 or IL-10 mRNA abundance in weanling pigs (Zhao et al., 2008). In the current study, the results on serum cytokines were rather erratic, and there were no consistent trends or patterns of response to the dietary treatments. Therefore, it was not possible to assess the effect of supplementing weanling pig diets with fish peptides on the health status of weanling pigs.

5. Conclusion

The response patterns of weanling pigs to dietary treatments were rather inconsistent. Pigs did not respond to the complex diets and simple corn-SBM-based diets as expected, and their response to dietary supplementation of 0 to 2% fish peptides to simple corn-SBM-based diets were not consistent. Because of the considerable variations in the data, the effort to estimate the optimum inclusion rate by fitting selected response criteria against various regression models was not successful. Based on the subjective evaluation, however, the greatest values in many response criteria seemed to be observed with pigs fed the diets containing 1.5% fish peptides, even though, again, the response patterns to fish peptide supplementation were rather inconsistent.

Declaration of Conflicting Interest

The authors certify that there is no financial or personal relationship with other individuals or organizations that can affect the current research project improperly, or no professional or personal interest of any nature or kind in any product, service, or organization that could be construed as influencing the current article.

Acknowledgements

This research project was funded in part by Alabama Agricultural Experiment Station (Auburn University, Auburn, AL, US) and Leveking Bio-Engineering Co., Ltd. (Shenzhen, China)/Vitech Bio-Chem Corporation (Orange, CA, US). Appreciation is extended to Nutra Blend LLC (Neosho, MO, US), APC, Inc. (Ankeny, IA, US), University of Illinois (Urbana-Champaign, IL), and Kansas State University (Manhattan, KS) for donating vitamin and trace mineral premix, plasma protein, soy protein concentrate, and Zn oxide, respectively. Technical

assistance of B. Anderson, R. Britton, the staff at the Auburn University Swine Research and Education Center, and J. Pate and his staff at the Auburn University Poultry Research Farm is gratefully acknowledged.

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Table 1
Composition of Phase 1 starter pig diets (as-fed basis) [1,2,3].

Item	POS	NEG	FP 0.5	FP 1.0	FP 1.5	FP 2.0
Ingredient (g/kg)						
Corn	456.2	509.1	508.4	507.7	507.0	506.3
Soybean meal (48% CP)	273.4	409.3	405.0	400.8	396.5	392.2
Sweet whey powder	150	50.0	50.0	50.0	50.0	50.0
Soy protein concentrate	40.0	-	-	-	-	-
Animal plasma	20.0	-	-	-	-	-
Fish peptides	-	-	5.0	10.0	15.0	20.0
Poultry fat	30.0	-	-	-	-	-
Dicalcium phosphate	12.0	12.9	12.8	12.7	12.6	12.4
Limestone	8.20	8.50	8.60	8.60	8.70	8.80
Salt	3.50	3.50	3.50	3.50	3.50	3.50
Zinc oxide (ZnO)	4.20	4.20	4.20	4.20	4.20	4.20
Vitamin-mineral premix[5]	2.50	2.50	2.50	2.50	2.50	2.50
Calculated composition						
DE (Mcal/kg)	3.67	3.41	3.41	3.41	3.41	3.41
CP (g/kg)	226.2	243.1	243.4	243.7	244.0	243.3
Ca (g/kg)	8.0	8.0	8.0	8.0	8.0	8.0
P (g/kg)	7.0	7.0	7.0	7.0	7.0	7.0
Ca:P	11.4	11.4	11.4	11.4	11.4	11.4
SID Lys (g/kg)	12.15	12.15	12.15	12.15	12.15	12.15
SID Lys:DE (g/Mcal)	3.31	3.56	3.56	3.56	3.56	3.56
Analyzed composition (g/kg)						
CP	211.4	236.2	244.7	242.8	238.0	233.0

[1] POS = positive complex diet, NEG = negative simple corn-soybean meal diet, and FP 0.5, 1.0, 1.5, and 2.0 = NEG diet supplemented with 0.5, 1.0, 1.5, and 2% fish peptides (FP).

[2] Fed Phase 1 starter diets from 7.87 ± 0.71 to 14.25 ± 1.05 kg.

[3] CP = crude protein, DE = digestible energy, and SID = standardized ileal digestible.

[4] Peptiva (Vitech Bio-Chem Corporation, Orange, CA, US).

[5] Provided the following (unit/kg diet; Nutra Blend, Neosho, MO, US): Fe (ferrous sulfate), 150 mg; Zn (zinc oxide), 150 mg; Mn (manganous oxide), 37.5 mg; Cu (copper sulfate), 150 ppm; I (ethylenediamine dihydroiodide), 5 ppm; Se (sodium selenite), 0.3 ppm; vitamin A, 6,614 IU; vitamin D_3, 1,102 IU; vitamin E, 26 IU; vitamin B_{12}, 0.03 mg; menadione (menadione Na bisulfite complex), 1 mg; riboflavin, 6 mg; D-pantothenic acid (D-Ca pantothenate), 45 mg; niacin, 28 mg; and choline (choline chloride), 110 mg.

Table 2
Composition of Phase 2 starter pig diets (as-fed basis) [1,2,3].

Item	POS	NEG	FP 0.5	FP 1.0	FP 1.5	FP 2.0
Ingredient (g/kg)						
Corn	562.8	589.2	588.5	587.9	587.2	586.5
Soybean meal (48% CP)	336.3	378.9	374.6	370.4	366.1	361.8
Sweet whey powder	50.0	-	-	-	-	-
Soy protein concentrate	20.0	-	-	-	-	-
Animal plasma	-	-	-	-	-	-
Fish peptides	-	-	5.0	10.0	15.0	20.0
Poultry fat	-	-	-	-	-	-
Dicalcium phosphate	14.0	14.8	14.7	14.5	14.4	14.3
Limestone	8.10	8.30	8.40	8.40	8.50	8.60
Salt	3.50	3.50	3.50	3.50	3.50	3.50
Zinc oxide (ZnO)	2.80	2.80	2.80	2.80	2.80	2.80
Vitamin-mineral premix[5]	2.50	2.50	2.50	2.50	2.50	2.50
Calculated composition						
DE (Mcal/kg)	3.41	3.40	3.40	3.40	3.40	3.40
CP (g/kg)	225.2	229.4	229.7	230.0	230.3	230.6
Ca (g/kg)	8.0	8.0	8.0	8.0	8.0	8.0
P (g/kg)	7.0	7.0	7.0	7.0	7.0	7.0
Ca:P	11.4	11.4	11.4	11.4	11.4	11.4
SID Lys (g/kg)	11.07	11.07	11.07	11.07	11.07	11.07
SID Lys:DE (g/Mcal)	3.25	3.26	3.26	3.26	3.26	3.26
Analyzed composition (g/kg)						
CP	211.4	236.2	244.7	242.8	238.0	233.0

[1] POS = positive complex diet, NEG = negative simple corn-soybean meal diet, and FP 0.5, 1.0, 1.5, and 2.0 = NEG diet supplemented with 0.5, 1.0, 1.5, and 2% fish peptides (FP).

[2] Fed Phase 2 starter diets from 14.25 ± 1.05 to 22.00 ± 1.52 kg.

[3] CP = crude protein, DE = digestible energy, and SID = standardized ileal digestible.

[4] Peptiva (Vitech Bio-Chem Corporation, Orange, CA, US).

[5] Provided the following (unit/kg diet; Nutra Blend, Neosho, MO, US): Fe (ferrous sulfate), 150 mg; Zn (zinc oxide), 150 mg; Mn (manganous oxide), 37.5 mg; Cu (copper sulfate), 150 ppm; I (ethylenediamine dihydroiodide), 5 ppm; Se (sodium selenite), 0.3 ppm; vitamin A, 6,614 IU; vitamin D_3, 1,102 IU; vitamin E, 26 IU; vitamin B_{12}, 0.03 mg; menadione (menadione Na bisulfite complex), 1 mg; riboflavin, 6 mg; D-pantothenic acid (D-Ca pantothenate), 45 mg; niacin, 28 mg; and choline (choline chloride), 110 mg.

Table 3

Effect of fish peptides on weekly growth performance of weanling pigs (first 2 wk) [1,2,3]

Item	ADFI (g/d)	LysI (g/d)	DEI (Mcal/d)	WG (g/d)	G:F (g/kg)	G:LysI (g/g)	G:DEI (g/Mcal)
d 0 to 7							
POS	495	5.77	1.76	383	780	67.5	221
NEG	493	5.72	1.69	366	746	64.3	218
FP 0.5	509	5.90	1.73	368	732	63.4	215
FP 1.0	493	5.73	1.68	378	773	66.7	227
FP 1.5	539	6.26	1.84	415	781	67.3	229
FP 2.0	496	5.77	1.69	324	659	57.6	193
SEM	9	0.13	0.04	13	29	2.7	9
P-value							
POS vs. NEG	0.972	0.905	0.613	0.668	0.736	0.711	0.934
POS vs. FP	0.617	0.654	0.845	0.704	0.577	0.581	0.833
FP, linear	0.669	0.626	0.692	0.684	0.575	0.626	0.582
FP, quadratic	0.579	0.556	0.628	0.147	0.353	0.381	0.356
FP, cubic	0.515	0.507	0.512	0.162	0.435	0.480	0.459
d 7 to 14							
POS	772	9.00	2.75	425	566	49.2	161
NEG	782	9.09	2.67	575	737	63.8	216
FP 0.5	816	9.47	2.78	495	608	52.9	179
FP 1.0	793	9.24	2.70	544	691	59.8	203
FP 1.5	851	9.85	2.90	598	709	61.1	208
FP 2.0	775	9.04	2.64	611	794	68.6	233
SEM	17	0.24	0.06	24	32	2.9	10
P-value							
POS vs. NEG	0.867	0.907	0.728	0.071	0.116	0.120	0.089
POS vs. FP	0.458	0.475	0.951	0.039	0.118	0.123	0.083
FP, linear	0.886	0.850	0.897	0.324	0.370	0.383	0.372
FP, quadratic	0.405	0.407	0.432	0.364	0.202	0.204	0.205
FP, cubic	0.581	0.607	0.603	0.342	0.543	0.570	0.547

[1] POS = positive complex diet, NEG = negative simple corn-soybean meal diet, and FP 0.5, 1.0, 1.5, and 2.0 = NEG diet supplemented with 0.5, 1.0, 1.5, and 2% fish peptides (FP). n = 4.

[2] Fed Phase 1 starter diets from 7.87 ± 0.71 to 14.25 ± 1.05 kg and Phase 2 starter diets from 14.25 ± 1.05 to 22.00 ± 1.52 kg.

[3] ADFI = average daily feed intake, LysI = standardized ileal digestible Lys intake, DEI = digestible energy intake, WG = weight gain, G:F = gain to feed, G:LysI = gain to LysI, G:DEI = gain to DEI, and SEM = pooled standard error of the mean.

Table 4
Effect of fish peptides on weekly growth performance of weanling pigs (last 2 wk) [1,2,3]

Item	ADFI (g/d)	LysI (g/d)	DEI (Mcl/d)	WG (g/d)	G:F (g/kg)	G:LysI (g/g)	G:DEI (g/Mcal)
d 14 to 21							
POS	942	10.9	3.34	600	629	53.8	176
NEG	1,000	11.6	3.41	479	474	40.9	139
FP 0.5	948	11.0	3.23	545	572	49.0	168
FP 1.0	978	11.4	3.33	571	582	50.1	171
FP 1.5	1,081	12.6	3.68	545	503	43.4	147
FP 2.0	1,037	12.1	3.53	550	527	45.1	155
SEM	16	0.2	0.05	31	29	2.3	8
P-value							
POS vs. NEG	0.224	0.242	0.672	0.276	0.124	0.140	0.191
POS vs. FP	0.077	0.069	0.423	0.583	0.288	0.313	0.465
FP, linear	0.062	0.048	0.069	0.566	0.868	0.878	0.863
FP, quadratic	0.472	0.506	0.484	0.547	0.363	0.362	0.353
FP, cubic	0.040	0.039	0.045	0.770	0.380	0.419	0.370
d 21 to 28							
POS	1,139	13.3	4.05	491	426	36.5	120
NEG	1,177	13.7	4.01	588	501	43.1	147
FP 0.5	1,100	12.8	3.75	664	628	54.6	184
FP 1.0	1,120	13.1	3.82	460	408	35.1	120
FP 1.5	1,232	14.3	4.20	565	457	39.3	134
FP 2.0	1,165	13.6	3.97	571	491	42.4	144
SEM	23	0.4	0.09	28	29	2.6	8
P-value							
POS vs. NEG	0.546	0.578	0.877	0.282	0.460	0.468	0.356
POS vs. FP	0.756	0.801	0.541	0.299	0.385	0.379	0.271
FP, linear	0.462	0.442	0.511	0.506	0.401	0.412	0.398
FP, quadratic	0.514	0.503	0.555	0.470	0.750	0.769	0.748
FP, cubic	0.066	0.064	0.094	0.364	0.151	0.147	0.146

[1] POS = positive complex diet, NEG = negative simple corn-soybean meal diet, and FP 0.5, 1.0, 1.5, and 2.0 = NEG diet supplemented with 0.5, 1.0, 1.5, and 2% fish peptides (FP). n = 4.

[2] Fed Phase 1 starter diets from 7.87 ± 0.71 to 14.25 ± 1.05 kg and Phase 2 starter diets from 14.25 ± 1.05 to 22.00 ± 1.52 kg.

[3] ADFI = average daily feed intake, LysI = standardized ileal digestible Lys intake, DEI = digestible energy intake, WG = weight gain, G:F = gain to feed, G:LysI = gain to LysI, G:DEI = gain to DEI, and SEM = pooled standard error of the mean.

Table 5
Effect of fish peptides on growth performance of weanling pigs during the first 2-wk and last 2-wk periods [1,2,3]

Item	ADFI (g/d)	LysI (g/d)	DEI (Mcal/d	WG (g/d)	G:F (g/kg)	G:LysI (g/g)	G:DEI (g/Mcal)
d 0 to 14							
POS	634	7.40	2.25	406	651	56.5	185
NEG	636	7.38	2.17	467	737	63.7	216
FP 0.5	666	7.73	2.26	439	663	57.5	195
FP 1.0	642	7.47	2.19	459	719	62.2	211
FP 1.5	694	8.04	2.37	505	734	63.3	215
FP 2.0	635	7.40	2.17	467	740	64.1	217
SEM	13	0.18	0.05	11	21	2.2	7
P-value							
POS vs. NEG	0.977	0.969	0.650	0.136	0.284	0.300	0.200
POS vs. FP	0.507	0.531	0.960	0.059	0.309	0.325	0.190
FP, linear	0.806	0.764	0.817	0.451	0.662	0.659	0.667
FP, quadratic	0.421	0.414	0.474	0.949	0.570	0.563	0.583
FP, cubic	0.619	0.633	0.601	0.161	0.455	0.486	0.478
d 14 to 28							
POS	1,103	12.21	3.76	545	494	44.6	145
NEG	1,110	12.29	3.78	534	480	43.4	164
FP 0.5	1,045	11.57	3.55	604	581	52.5	171
FP 1.0	1,076	11.91	3.66	515	477	43.1	140
FP 1.5	1,171	12.96	3.98	555	472	42.6	139
FP 2.0	1,124	12.45	3.82	568	504	45.5	148
SEM	14	0.16	0.05	23	25	2.2	7
P-value							
POS vs. NEG	0.860	0.865	0.931	0.850	0.792	0.794	0.817
POS vs. FP	0.964	0.972	0.943	0.740	0.729	0.731	0.703
FP, linear	0.123	0.123	0.125	0.888	0.594	0.593	0.593
FP, quadratic	0.376	0.376	0.380	0.935	0.783	0.784	0.785
FP, cubic	0.025	0.025	0.026	0.328	0.055	0.056	0.056

[1] POS = positive complex diet, NEG = negative simple corn-soybean meal diet, and FP 0.5, 1.0, 1.5, and 2.0 = NEG diet supplemented with 0.5, 1.0, 1.5, and 2% fish peptides (FP). n = 4.

[2] Fed Phase 1 starter diets from 7.87 ± 0.71 to 14.25 ± 1.05 kg and Phase 2 starter diets from 14.25 ± 1.05 to 22.00 ± 1.52 kg.

[3] ADFI = average daily feed intake, LysI = standardized ileal digestible Lys intake, DEI = digestible energy intake, WG = weight gain, G:F = gain to feed, G:LysI = gain to LysI, G:DEI = gain to DEI, and SEM = pooled standard error of the mean.

Table 6

Effect of fish peptides on growth performance of weanling pigs during the entire 4-wk study [1,2,3]

Item	ADFI (g/d)	LysI (g/d)	DEI (Mcal/d	WG (g/d)	G:F (g/kg)	G:LysI (g/g)	G:DEI (g/Mcal)
d 0 to 28							
POS	839	9.78	2.98	478	567	48.8	160
NEG	859	9.97	2.93	497	579	49.9	169
FP 0.5	852	9.89	2.89	528	626	54.1	184
FP 1.0	843	9.82	2.88	485	575	49.6	169
FP 1.5	923	10.72	3.15	528	572	49.3	168
FP 2.0	867	10.10	2.96	513	592	51.1	174
SEM	13	0.21	0.05	9	10	0.9	3
P-value							
POS vs. NEG	0.603	0.659	0.766	0.533	0.757	0.749	0.387
POS vs. FP	0.280	0.234	0.932	0.137	0.409	0.398	0.118
FP, linear	0.293	0.255	0.362	0.629	0.735	0.731	0.725
FP, quadratic	0.923	0.912	0.967	0.926	0.941	0.931	0.916
FP, cubic	0.135	0.136	0.167	0.796	0.172	0.171	0.142

[1] POS = positive complex diet, NEG = negative simple corn-soybean meal diet, and FP 0.5, 1.0, 1.5, and 2.0 = NEG diet supplemented with 0.5, 1.0, 1.5, and 2% fish peptides (FP). n = 4.

[2] Fed Phase 1 starter diets from 7.87 ± 0.71 to 14.25 ± 1.05 kg and Phase 2 starter diets from 14.25 ± 1.05 to 22.00 ± 1.52 kg.

[3] ADFI = average daily feed intake, LysI = standardized ileal digestible Lys intake, DEI = digestible energy intake, WG = weight gain, G:F = gain to feed, G:LysI = gain to LysI, G:DEI = gain to DEI, and SEM = pooled standard error of the mean.

Table 7

Effect of fish peptides on serum metabolites in weanling pigs (wk 4) [1,2,3]

Item	TP (g/dL)	Alb (g/dL)	Glob (g/dL)	Alb:Glob	BUN (mg/dL)	Gluc (mg/dL)	Chol (g/Mcal)	TG (mg/dL)
POS	5.36	4.08	1.38	2.95	14.38	130	67.0	47.8
NEG	5.34	4.02	1.28	3.38	15.90	123	69.3	59.8
FP 0.5	5.32	3.99	1.23	3.40	16.45	128	67.8	52.0
FP 1.0	5.40	4.02	1.28	3.23	15.38	131	63.5	44.5
FP 1.5	5.32	4.10	1.38	3.30	18.40	124	66.5	49.8
FP 2.0	5.05	3.90	1.15	3.35	18.03	128	62.5	52.8
SEM	0.04	0.05	0.04	0.12	0.40	2	1.4	2.1
P-value								
POS vs. NEG	0.877	0.712	0.441	0.266	0.163	0.358	0.653	0.117
POS vs. FP	0.438	0.579	0.253	0.224	0.005	0.695	0.624	0.732
FP, linear	0.092	0.751	0.729	0.858	0.017	0.689	0.197	0.332
FP, quadratic	0.106	0.508	0.384	0.763	0.427	0.568	0.865	0.093
FP, cubic	0.374	0.395	0.152	0.835	0.458	0.462	0.704	0.880

[1] POS = positive complex diet, NEG = negative simple corn-soybean meal diet, and FP 0.5, 1.0, 1.5, and 2.0 = NEG diet supplemented with 0.5, 1.0, 1.5, and 2% fish peptides (FP). n = 4.

[2] Fed Phase 1 starter diets from 7.87 ± 0.71 to 14.25 ± 1.05 kg and Phase 2 starter diets from 14.25 ± 1.05 to 22.00 ± 1.52 kg.

[3] TP = total protein, Alb = albumin, Glob = globulin, Alb:Glob = Alb to Glob ratio, Bun = blood urea N, Chol = cholesterol, and TG = triglyceride. Blood samples were collected during the fourth week of the study.

Table 8

Effect of fish peptides on serum cytokines (pg/mL) in weanling pigs (wk 4) [1,2,3]

Item	IL-1α	IL-1β	IL-1Ra	IL-2	IL-4	IL-8	IL-10	IL-12	IL-18	TNFα
POS	140	766	1,550	1,390	686	476	594	1,750	2,574	-250
NEG	203	2,342	1,789	1,568	20,015	557	5,434	1,592	9,813	176
FP 0.5	204	2,801	2,216	1,581	26,556	561	5,999	2,329	12,879	2,074
FP 1.0	268	3,358	1,694	1,978	20,364	1,125	8,350	2,069	8,490	2,592
FP 1.5	324	3,745	3,305	2,721	40,049	688	9,154	2,170	16,904	847
FP 2.0	165	960	447	1,388	14,788	824	5,077	815	2,968	1,800
SEM	58	740	662	445	9,677	71	2,155	296	3,686	476
P-value										
POS vs. NEG	0.741	0.508	0.918	0.905	0.581	0.730	0.535	0.872	0.588	0.775
POS vs. FP	0.513	0.338	0.847	0.660	0.368	0.101	0.287	0.905	0.464	0.102
FP, linear	0.922	0.748	0.806	0.824	0.971	0.243	0.894	0.490	0.766	0.548
FP, quadratic	0.512	0.285	0.504	0.554	0.677	0.247	0.591	0.172	0.536	0.275
FP, cubic	0.571	0.570	0.510	0.524	0.683	0.982	0.703	0.844	0.615	0.299

[1] POS = positive complex diet, NEG = negative simple corn-soybean meal diet, and FP 0.5, 1.0, 1.5, and 2.0 = NEG diet supplemented with 0.5, 1.0, 1.5, and 2% fish peptides (FP). n = 4.

[2] Fed Phase 1 starter diets from 7.87 ± 0.71 to 14.25 ± 1.05 kg and Phase 2 starter diets from 14.25 ± 1.05 to 22.00 ± 1.52 kg.

[3] IL-1α = interleukin-1α; IL-1β = interleukin-1β; IL-1Ra = interleukin-1 Ra; IL-2 = interleukin-2; IL-4 = interleukin-4; IL-8 = interleukin-8; IL-10 = interleukin-10; IL-12 = interleukin-12; IL-18 = interleukin-18; and TNFα = tumor necrosis factors α. Blood samples were collected during the fourth week of the study.

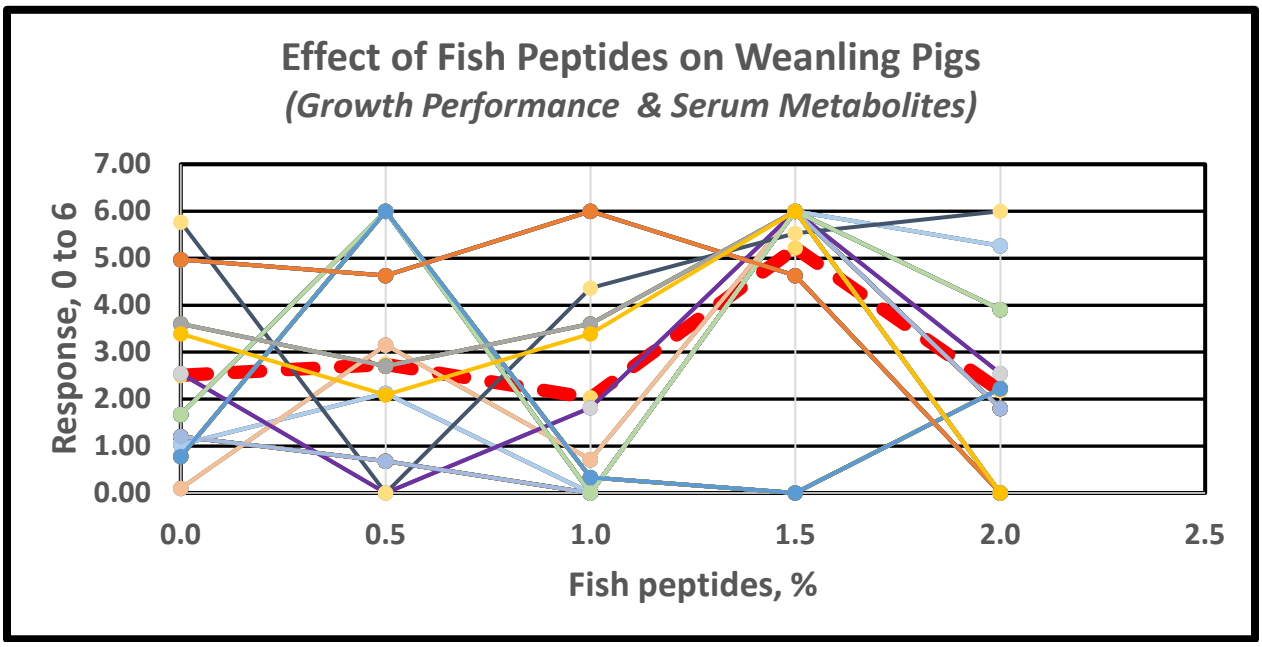

Fig. 1. Effect of fish peptide supplementation on growth performance of weanling pigs and their serum metabolites. Response criteria used to create this figure: feed intake, weight gain, and gain to feed ratio during the first 2 wk after weaning and also overall, and serum total protein, albumin, globulin, and urea N concentrations during the fourth week of the study. Each response criterion was expressed on the scale of 0 (lowest value) to 6 (greatest value) and the thick dashed line represents the average of 10 response criteria. Fed phase 1 starter diets from 7.87 ± 0.71 to 14.25 ± 1.05 kg and phase 2 starter diets from 14.25 ± 1.05 to 22.00 ± 1.52 kg.

IV. SUMMARY AND CONCLUSION

Young pigs are subjected to various stressors at weaning, and providing a highly palatable and digestible diet is important in preventing or alleviating growth check soon after weaning and optimizing growth performance thereafter. Although a corn-SBM diet is the gold standard for feeding pigs, such a diet may not be appropriate for weanling pigs because of many digestive, metabolic, and immunological challenges. Thus, weanling pigs have been fed complex diets that contain many special ingredients, which are highly-palatable and highly-digestible. Providing such diets can be rather costly. However, a small amount of some alternative feed supplement, such as fish peptides, can be included as a source of nutrients for weanling pigs. In addition, such a feed additive can have some bioactive or functional properties that can be beneficial for their health and growth performance. By using such a feed additive, along with a rapidly increasing enzyme technologies in recent years, it might be possible for weanling pigs to utilize corn-SBM diets more efficiency.

As part of the project to explore the possibility of replacing complex diets with semi simple or simple corn-SBM diets for weanling pigs, a study was conducted to assess the effect of fish peptides on their growth performance, serum metabolite profile, and serum cytokines. Forty-eight gilts and 48 castrated males (initial body weight, 7.87 ± 0.71 kg) weaned at 3 to 4 wk of age were randomly assigned to 6 dietary treatments with 4 replicate pens (2 gilts and 2 castrated males/pen) per treatment. Because of the availability of pigs and facility at one time, the study was conducted in 2 trials, and each trial used 24 gilts and 24 castrated males. Two trials were approximately 8 wk apart. After weaning, pigs were fed a common pre-starter diet for 4 d before beginning of the study, and a 2-phase feeding program was used. Each phase consisted of 2 wk. Two typical complex, positive control (POS) diets containing various special ingredients were

formulated and used as the positive control (POS) diets. Two simple, corn-SBM-based negative control (NEG) diets were formulated to be iso-lysinic to the POS diets, and the NEG diets for both phase 1 and 2 were supplemented with 0, 0.5, 1.0, 1.5, or 2.0% fish peptides . A small amount of dried whey (5%) was included in the phase 1 NEG and fish peptide diets to ensure that pigs were growing. Dried whey was not included in the phase 2 NEG and fish peptide diets. Fish peptides were included in the diet by replacing the part of corn and SBM. All 6 diets were formulated to meet or exceed the 2012 NRC nutrient requirements. Pigs were weighed and feed intake was recorded weekly. During the fourth week of the study, approximately 5 mL of blood was collected from each pig via vena cava puncture using a sterile needle and evacuated tube. Serum was separated, and an aliquot was stored at -20ºC. Serum sample from each pig was analyzed for metabolites, whereas serum samples were pooled by pen and analyzed for serum cytokines.

During the last 2 wk of the study, the intake of feed, Lys, and DE increased cubically as fish peptide supplementation increased from 0 to 2%. Overall, pigs fed the diets supplemented with 1.5% fish peptides had greater feed, Lys, and DE intakes (approximately 8%) than those fed other diets, but those differences were not statistically significant. Overall weight gain of pigs fed the diets containing fish peptides seemed to be greater (6.7%) than those fed the POS diet, but, again, it was not statistically significant. There were no clear trends in the efficiency of overall utilization of feed, Lys, or DE intake for weight gain. Pigs tended to respond linearly and quadratically to the increase in fish peptide supplementation in serum total protein. Serum urea N concentration was greater in pigs fed the diets supplemented with fish peptides compared with those fed the POS diets, it increased linearly as fish peptide supplementation increased from 0 to 2%. Although pigs tended to respond quadratically to fish peptide supplementation in serum

triglyceride, there was no clear effect of dietary treatments on serum albumin, globulin, glucose, or cholesterol concentration, or albumin to globulin ratio. Similarly, there was no clear effect of dietary treatments on any of the cytokines.

In conclusion, the response patterns of weanling pigs to dietary treatments were rather inconsistent. Pigs did not respond to the complex diets and simple corn-SBM- based diets as expected, and their response to dietary supplementation of 0 to 2% fish peptides to simple corn-SBM-based diets were not consistent. Because of the considerable variations in the data, the effort to estimate the optimum inclusion rate by fitting selected response criteria against various regression models was not successful. Based on the subjective evaluation, however, the greatest values in many response criteria seemed to be observed with pigs fed the diets containing 1.5% fish peptides, even though, again, the response patterns to fish peptide supplementation were rather inconsistent.

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VI. APPENDICES

Appendix A: Principle of the Total protein Analysis (Roche Diagnostics, Indianapolis, IN)

Under alkaline conditions, divalent copper in the biuret reagent reacts with protein peptide bonds to form the characteristic purple-colored biuret complex:

$$\text{Protein} + Cu^{2+} \xrightarrow{\text{Alkaline solution}} \text{Cu-protein complex}$$

The color intensity is directly proportional to the protein concentration, which can be measured photometrically.

Appendix B: Principle of the Albumin Analysis (Roche Diagnostics, Indianapolis, IN)

It is a colorimetric assay with endpoint method. At a pH of 4.1, albumin displays a sufficiently cationic character to be able to bind with bromocresol green (BCG), an anionic dyestuff to form a blue-green complex:

$$\text{Albumin} + \text{BCG} \xrightarrow{\text{pH 4.1}} \text{Albumin BCG complex}$$

The color intensity of the blue-green color is directly proportional to the albumin concentration and can be measured photometrically.

Appendix C: Principle of the Urea nitrogen Analysis (Roche Diagnostics, Indianpolis, IN)

Urea is hydrolyzed by urease to form CO_2 and ammonia:

$$\text{Urea} + H_2O \xrightarrow{\text{Urease}} 2\,NH_4^+ + CO_2$$

The ammonia formed then reacts with α-ketoglutarate and NADH in the presence of GLDH to yield glutamate and NAD^+:

$$\alpha\text{-ketoglutarate} + NH_4^+ + \text{NADH} \xrightarrow{\text{GLDH}} \text{L-glutamate} + NAD^+ + H_2O$$

The decrease in absorbance due to consumption of NADH is measured kinetically.

Appendix D: Principle of Glucose Analysis (Diagnostic Chemicals Ltd)

Glucose is phosphorylated to hexokinase in the presence of adenosine triphosphate (ATP) and magnesium to form glucose-6-phosphate (G-6-P) and adenosine diphosphate (ADP):

$$\text{Glucose} + \text{ATP} \xrightarrow{\text{Hexokinase, } Mg^{2+}} \text{Glucose-6-phosphate} + \text{ADP}$$

G-6-P is then oxidized by glucose-6-phosphate dehydrogenase (G-6-PDH) in the presence of nicotinamide adenine dinucleotide (NAD^+) producing 6-phosphogluconate and NADH:

$$\text{Glucose-6-phosphate} + NAD^+ \xrightarrow{\text{G-6-PDH}} \text{6-phosphogluconate} + \text{NADH} + H^+$$

The formation of NADH causes an increase in absorbance at 340 nm which is directly proportional to the concentration of glucose in the sample.

Appendix E: Principle of Cholesterol Analysis (Roche Diagnostics, Indianpolis, IN)

Cholesterol is determined enzymatically using cholesterol esterase and cholesterol oxidase as follows. Cholesterol esters are cleaved by the action of cholesterol esterase to yield free cholesterol and fatty acids:

$$\text{Cholesterol esters} + H_2O_2 \xrightarrow{\text{Cholesterolesterase}} \text{Cholesterol} + \text{RCOOH}$$

Cholesterol is converted by oxygen with the aid of cholesterol oxidase to cholest-4-en-3-one and hydrogen peroxide:

$$\text{Cholesterol} + O_2 \xrightarrow{\text{Cholesterol oxidase}} \text{Cholest-4-en-3-one} + H_2O_2$$

The hydrogen peroxide created forms a red dyestuff by reacting with 4-aminophenazone and phenol under the catalytic action of peroxidase:

$$2H_2O_2 + \text{4-aminophenazone} + \text{phenol} \xrightarrow{\text{Peroxidase}} \text{4-(p-benzoquinone-monoimino)-phenazone} + 4H_2O$$

The color intensity is directly proportional to the concentration of cholesterol and can be determined photometrically.

Appendix F: Principle of the Triglyceride Analysis (Diagnostic chemicals Ltd., Oxford. CT)

Serum triglycerides are hydrolyzed to glycerol and free fatty acids by lipase:

$$\text{Triglycerides} + H_2O \xrightarrow{\text{Lipase}} \text{Glycerol} + \text{fatty acids}$$

In the presence of ATP and glycerol kinase (GK), the glycerol is phosphorylated to glycerol-1-phosphate:

$$\text{Glycerol} + \text{ATP} \xrightarrow{\text{GK, } Mg^{2+}} \text{Glycerol-1-phosphate} + \text{ADP}$$

Glycerol-1-phosphate is then oxidized by glycerol phosphate oxidase (GPO) to yield hydrogen peroxide (H_2O_2):

$$\text{Glycerol-1-phosphate} + O_2 \xrightarrow{\text{GPO}} H_2O_2 + \text{dihydroxyacetone phosphate}$$

The hydrogen peroxide causes oxidative coupling of p-chlorophenol and 4-aminoantipyrine, producing a red colored quinoneimine dye complex:

$$H_2O_2 + \text{p-cholorophenol} + \text{4-aminoantipyrine} \xrightarrow{\text{Peroxidase}} \text{quinoneimine dye} + H_2O_2$$

The increase in absorbance at 520 nm due to the formation of the quinoneimine dye is directly proportional to the concentration of triglycerides in the sample.

Appendix G: Porcine Cytokine Array / Chemokine Array 13-plex (Eve Technologies Corp., Calgary, AB, Canada) [https://www.evetechnologies.com/product/porcine-cytokine-array-chemokine-array-13-plex/]

Biomarker analytes: GM-CSF, IFNγ, IL-1α, IL-1ra, IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12, IL-18, TNFα.

Volume required: 50 µL single analysis, 75 µL duplicate analysis, and 100 µL triplicate analysis.

Multiplex assay overview: Discovery Assay measures 13 cytokine/chemokine biomarkers simultaneously in a single microwell. The assay is designed for protein analysis in serum, plasma, cell culture, and tissue homogenate.

Detection method: Multiplex Immunoassay analyzed with a BioPlex 200 System (Bio-Rad Laboratories, Hercules, CA).

Cytokine array assay kit source: Millipore MILLIPLEX (MilliporeSigma, Burlington, MA).