Effect of Dietary Fish Peptide and Enzyme Supplementation on Weanling Pigs

by

Taylor B. Wilson

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
May 1, 2021

Keywords: Weanling pigs, Fish peptides, Multienzyme complexes,
Growth performance

Approved by

Lee I. Chiba, Chair, Professor of Nutrition
Werner G. Bergen, Professor of Biochemistry and Nutrition
Nar K. Gurung, Associate Professor of Agricultural and Environmental Sciences,
Tuskegee University

ABSTRACT

A corn-soy diet is the gold standard for feeding pigs, however, such a diet may not be appropriate for weanling pigs. Weanling pigs are subjected to various stressors at weaning, and their digestive system is immature or the secretion of digestive enzymes is not sufficient to utilize corn-soybean meal (SBM) diets. For that reason, traditionally, weanling pigs have been provided with complex diets containing many special ingredients such as dried whey, plasma protein, fishmeal, soy protein concentrate, and others. Feeding such diets with highly palatable and digestible feed ingredients is very effective in promoting the growth performance of weanling pigs, however, using such diets can be rather costly. With the development of, e.g., fish peptides, which can be not only a great source of nutrients and but also considered as a bioactive or functional feed additive, and multienzyme complexes it might be possible to use corn-SBM diets more successfully for weanling pigs.

A total of 48 pigs (24 females and 24 castrated males) weaned at 3 to 4 wk of age (initial body weight, 7.77 ± 0.78 kg) were used to investigate the possibility of replacing a complex diet with a simple corn-soybean meal (SBM) diet by fish peptide and multienzyme complex supplementation. Pigs were randomly assigned to 3 dietary treatments with 4 replicate pens per treatment and 2 gilts and 2 castrated males per pen. A two-phase feeding program (2 wk each) was used, and 2 typical complexes, positive control (POS) diets were formulated to contain 12.15 and 11.07 g standard ileal digestible Lys/kg for Phase 1 and 2, respectively. In addition to corn and SBM, the complex diet for Phase 1 contained dried whey, fishmeal, plasma protein, poultry fat, and Zn oxide, whereas the complex diet for Phase 2 contained dried whey and

fishmeal. Two simple, corn-SBM negative control (NEG) diets were formulated to be isolysinic to the POS diets for Phase 1 and 2 and included 1.5% fish peptides. The NEG diets were supplemented with multienzyme complexes. During the fourth week of the study, a blood sample was collected from each pig, and samples were analyzed for serum metabolites. Pigs were allowed ad libitum access to feed and water throughout the 4-wk study period, and the pig weight and feed consumption data were collected weekly.

During the second week of the study (d 7 to 14), weight gain (WG) of pigs fed the POS diet was numerically lower ($P = 0.144$) than those fed the other 2 diets, which was reflected in the efficiency of feed utilization for WG. Pigs fed the NEG and ALL diets had greater gain to feed (G:F; $P = 0.034$), gain to Lys (G:Lys) intake ($P = 0.034$), and gain to DE (G:DE) intake ($P = 0.013$) than those fed the POS diet. Furthermore, G:F ($P = 0.037$), G:Lys intake ($P = 0.037$), and G:DE intake ($P = 0.010$) were greater in pigs fed the ALL diet than those fed the NEG diet. As in d 7 to 14, pigs fed the NEG and ALL diet had numerally greater weight gain than those fed the POS diets ($P = 0.147$) during the first 2 wk of the study, which was reflected in the efficiency of WG. During Phase 1 (d 0 to 14), the efficiency of feed ($P = 0.004$), Lys ($P = 0.004$), or DE ($P < 0.001$) utilization was greater in pigs fed the NEG and ALL diets than those fed the POS diet. In addition, pigs fed the ALL diet tended to have greater G:F ($P = 0.095$) and G:Lys intake ($P = 0.095$) and had greater G:DE intake ($P = 0.009$) than those fed the NEG diet. There seemed to be no clear effect of dietary treatments on overall growth performance (d 0 to 28) of weanling pigs or serum metabolite profile during the last week of the study.

During the first 2 wk of the study, fish peptides improved growth the performance of weanling pigs, and additional supplementation with multienzyme complexes improved growth performance further. The results indicated that it may be possible to replace a complex diet with a simple corn-SBM diet by supplementing the weanling pig diet with fish peptides and multienzyme complexes during Phase 1 (d 0 to 14).

ACKNOWLEDGMENTS

I thank my major professor, Dr. Lee I. Chiba, for his counsel, patience, and aid through my time in the graduate program. I would also like to thank my advisory committee members, Dr. Werner G. Bergen and Dr. Nar K. Gurung, for their assistance and serving on the committee. I acknowledge the faculty and staff of the Department of Animal Sciences and staff at the Swine Research and Education Center for their help and support. I also wish to acknowledge my mother for her continuous encouragement and support all through my graduate program.

TABLE OF CONTENTS

LIST OF TABLES

I. INTRODUCTION

The weaning period is not only the most critical time for a pig but also the most stressful time ~~event~~. During this time, a piglet is offered different diets, is separated from the sow, and brought into a new environment with young pigs from other sows. These various changes are considered as "weaning stressors." Some of those weaning stressors can lead to the phenomenon known as a “growth check.” A growth check is the inability of a weanling pig to gain weight for approximately a week or more after weaning. The diet change from liquid milk to feed that is solid, such as corn-soybean meal (SBM)-based diets can be a nutritional stress. Soon after weaning, their digestive system consisted of enzymes that are designed for the utilization of milk carbohydrates and lipids. Because of this, their digestive enzymes are not able to break down a simple corn-soy diet adequately. Thus, a simple corn-soy diet is not an adequate feed for weanling pigs.

Complex diets containing ingredients that are highly palatable and digestible ingredients that may allow for a gradual transition from a liquid diet, milk, to a solid feed. Those ingredients include milk products, spray-dried plasma protein, fishmeal, soy protein concentrate, oat groats, and others. With the addition of those ingredients, pigs can obtain necessary and sufficient energy and nutrients for normal growth. Research has proven over the years that complex diets are effective in enhancing growth performance compared with feeding "simple, corn-SBM diets." However, including those highly palatable and digestible ingredients can be very expensive. In addition, only a small amount of some of those ingredients can be required to prepare those complex diets and the procurement and storage and(or) maintenance of those ingredients can be rather challenging, economically and physically.

Another aspect of weanling pigs is their immunity. Pigs are weaned from the sow at a time when their passive immunity is declining and active immunity is not fully developed. Because of that, antibiotic feed additives have been used over the years. Research has shown that those additives can substantially improve growth rate and feed efficiency. With the concerns on using antibiotics because of the development of potential resistant bacteria, which can threaten public health, the use of antibiotics has been banned in some countries, and regulations have been imposed in some other countries. Research has shown that some alternative approaches to antibiotics, such as essential oils, benzoic acid, and zinc oxide, can be effective.

The overall research goal of the research project is to develop environmentally friendly optimum feeding strategies for successful and sustainable pig production. Our current research effort continues to be the exploration of the possibility of replacing complex weanling pig diets with simple corn-SBM diets. The current study was conducted to determine the possibility of replacing typical complex starter diets with simple corn-SBM diets by supplementation with feed additive or bioactive peptides, fish peptides, and multienzyme complex supplementation. Growth performance and serum metabolites were assessed to determine the efficacy of fish peptides and multienzyme complexes.

II. LITERATURE REVIEW

Introduction and Project Justification

The most consumed meat in the world in 2018 is pork. It contributes to the nutritional and economic wellbeing of people in numerous countries. In pig production, the most used feed is a corn-soybean meal (SBM) diet. It is the “gold standard” for feeding pigs because of its ability to satisfy both energy and amino acid requirements. However, a corn-SBM diet may not be a suitable diet for weanling pigs. A corn-SBM diet may be unsuitable because of one obvious reason. Weanling pigs’ digestive systems or their digestive enzymes are geared toward utilizing milk proteins, carbohydrates, and lipids. The necessary enzymes are increasing but insufficient to utilize corn-SBM diets. In addition to nutritional stress, weanling pigs are subjected to other weaning stressors such as environmental stress and social stress, which can further reduce the weanling pigs' ability to utilize corn-SBM diets. The inefficient utilization of energy and nutrients in feedstuffs may contribute to environmental problems such as "odor" problems and "eutrophication," which can result from the excretion of unutilized nutrients in the feces. Many odorous compounds originate from undigested dietary protein and other nitrogenous compounds. In addition, the runoff of N and P can pollute neighboring lakes, which can lead to algae blooms and depletion of O_2, resulting in the death of fish.

A complex diet, which contains highly palatable and digestible special ingredients, can be utilized by weanling pigs more efficiently, and it may curtail those adverse impacts of pig production on the environment to some extent. However, complex diets can also have some problems. Examples of some problems are the procurement and maintenance and(or) storage of a multitude of special ingredients. In addition, such diets can be rather expensive. If weanling pigs can somehow utilize corn-SBM diets more efficiently, such problems can be alleviated to a certain extent. With the continuous development of exogenous enzymes and enzyme technologies and so-called "functional feed additives," it may be possible for weanling pigs to effectively extract energy and nutrients from corn and SBM.

Traditionally, antibiotics have been included in the weanling pig diet because they have not yet developed their immunity fully. Including suitable antibiotics helps weanling pigs with the protection against harmful, pathogenic microorganisms, which can improve the growth performance of young pigs. However, the continuous use of antibiotics in weanling pig diets can lead to the development of antibiotic resistance, it can not only reduce the effectiveness of antibiotics in animal feed but also can have, theoretically, adverse effects on human health. For those reasons, the therapeutic use of antibiotics has been banned in many countries and many countries are restricting the use of antibiotics in animal feeds. In recent years, there have been intensive research efforts to find a viable alternative to antibiotics. Some functional bioactive feed additives, such as fish peptides" can contribute to such efforts.

Making some effort to replace complex diets with simple corn-SBM or semi simple corn-SBM for weanling pigs can contribute greatly to our ultimate research goal of our research program to contribute to the development of environmentally friendly, optimal feeding strategies for successful sustainable pig production. It is possible that by supplementing weanling pig diets with appropriate feed additives, such as functional bioactive peptides and multienzyme complex/complexes, we might be able to extract fully energy and nutrients from corn and SBM, which allow us to replace complex diets with more simplified weanling pig diets.

Diet Complexity

Simple Diets

As pointed out before, a corn-SBM diet, or a simple diet, has been the "standard" in feeding pigs. The weaning of pigs is the most important part of a pig’s life. In this period, weanling pigs are exposed to many very stressful events. These stressors can be environmentally, physiologically, and socially challenging (Campbell et al., 2013). Those challenges can lead to a "growth check," which can be characterized by the inability of a weanling pig to gain weight for approximately a week or so after weaning (Berkeveld et al., 2007). One specific stressor that can contribute to growth checks includes the weanling pig’s immature digestive system. Weanling pigs cannot break down corn and SBM efficiently because of insufficient enzymes necessary to utilize those feed ingredients, and feeding such diets may lead to a low feed intake and poor nutrient absorption, and eventually a poor growth performance. To compensate for their inability to utilize corn and SBM, it is necessary to include some highly digestible and palatable ingredients in corn-SBM-based diets.

Complex Diets

Because of weanling pigs' inability to utilize corn-SBM diets efficiently, complex diets are needed to ensure the smooth transition from the sow’s milk to solid diets (Mahan and Lepine 1991). A complex diet contains special ingredients such as dried whey, plasma protein, blood cells, fish meal, soy protein concentrate, and others, which are, again, highly palatable and digestible. Research has shown that newly weaned pigs generally require a complex diet to reach their optimum growth performance (Jones and Pond, 1964). However, the addition of various special ingredients and some additives can contribute to the increased cost of complex diets. For example, whey powder has decreased in availability and lactose has increased its price (Maxwell et al., 1998; Gould, 2014). This may contribute to the nursery phase of production being the greatest cost of the pig diets (Skinner et al., 2014), even though most of the feed for pig production would be consumed by grower-finisher pigs. The cost of weanling pig diets and others led to extensive research efforts to find alternative sources of energy and nutrients for weanling pigs and also the way to utilize corn and SBM more efficiently. As pointed out before, the development of enzyme technology may contribute greatly to such efforts.

Digestive Enzymes

Enzymes in General

Enzymes have multiple functional abilities such as catalyzing biochemical reactions (Cuesta, et al., 2015) and degrading proteins, lipids, and carbohydrates (Ianiro et al., 2016). Digestive enzymes are, obviously, essential for living organisms to survive, reproduce, and grow. Research shows that the nutritional value of food is partially dependent on the digestive enzymes in the animal's gut (Graf et al., 2015). The use of commercial enzymes as a feed additive has found widespread acceptance in animal feed. Those enzymes can provide a way to improve nutrient utilization and growth performance. In addition, they can reduce feed cost, and also the excretion of unutilized nutrients in feces. A steady increase in the use of enzymes is expected to increase in the coming years continuously. The 3 main commercially available enzymes are phytases, carbohydrases, and proteases. Phytases break down phytate, the principal storage form of P in many plant tissues. Carbohydrases aids in breaking down fibrous components such as xylans, β -glucans, and mannans. Proteases break down some complex proteins into peptides and amino acids. The most commonly used enzymes are phytases and carbohydrases.

Phytases

Phytate, also known as phytic acid, is found in grains and seeds, and it can account for 50 to 80 percent of the total amount of phosphate in the seed of plants (Selle et al., 2000; Kumar et al., 2012). Phytic acid has a high capacity to form insoluble complexes with divalent metal ions such as Ca, Cu, Fe, and Zn (Ekholm et al., 2002). Nonruminant animals, such as pigs, cannot break down phytate because of insufficient phytases in their system (Brumm et al., 2001), and inorganic P must be included in their diets. Unutilized P in the manure of those animals can contribute to some environmental problems such as eutrophication. Research has shown that the supplementation of the animal diet with exogenous phytase can increase the absorption of P, thereby, decreasing P found in the manure (Zeng et al., 2014).

Phytase is chemically known as myo-inositol hexakisphosphate phosphohydrolase, and it can reduce the chelation properties of phytate and hydrolyze phytate molecules (He et al., 2017). Phytases can be classified as 3- and 6-phytase based on their effect on the source and the site of the initial dephosphorylation. The 3-phytase is the microbial origin and 6-phytase is the plant origin, and they can both hydrolyze P at the carbon 3 atom and carbon 6 atom, respectively (Kornegay, 2001). Phytase stimulates the hydrolysis of phytate to a series of lower myo-inositol phosphates esters and inorganic phosphate and dephosphorylate in a step-wise fashion (Vats and Banerjee, 2004). The main sources of microbial phytases are bacteria1 and fungal origins (Haefner et al., 2005).

As the dietary phytase inclusion rate increased [0, 500, 1,500, and 4,500 phytase unit (FTU)/kg], P digestibility and weight gain in 21-d-old broiler chickens increased (Walk et al., 2018), but unlike in previous studies (Walk et al., 2013, 2014), the inclusion rates had no effect on feed conversion ratio. Simons et al. (1990) evaluated various dietary phytase concentrations (250, 375, 500, 750, 1,000, 1,500, and 2,000 FTU/kg) and reported that the addition of 1,500

FTU phytase/kg increased weight gain and improved feed conversion ratio in broiler chickens from 0 to 24 d of age. He et al. (2017) investigated the effect of microbial phytase supplementation (500, 750, and 1,000 FTU/kg) of low-P diets and concluded that the microbial phytase can improve growth performance, apparent ileal digestibility of some amino acids, apparent utilization of other nutrients in native ducks and reduce the excretion of P into the environment. Truong et al. (2014) evaluated the effect of phytase (1,000 FTU/kg) in the maize-, sorghum- and wheat-based diets, and concluded that phytase accelerated the disappearance of phytate regardless of the cereal grains used for broiler chicken diets.

Young et al. (1993) supplemented the corn-SBM weanling pig diet with 0, 500, and 1,000 FTU phytase/kg and concluded that the addition of the phytase increased feed intake and weight gain. Kornegay and Qian (1996) evaluated the effect of available P (0.7 and 1.6 g/kg) and phytase supplementation (0, 350, 700, 1,050, and 1,400 FTU/kg). They also included the diet containing the NRC recommended available P (3.2 g/kg) and supplemented the diet with 0 or 1,400 FTU phytase/kg. As supplemental phytase increased, average daily feed intake, average daily gain, and gain to feed ratio increased linearly in pigs fed the lower available P. Feed intake and weight gain increased further in weanling pigs fed the diet containing 3.2 g available P/kg and supplemented with 1,400 FTU phytase. Similarly, growing pigs fed the diet supplemented with 410 FTU microbial phytase/kg had greater weight gain and feed conversion ratio (Jongbloed et al., 2000). However, some researchers reported no response to the use of supplemental phytase. For instance, Omogbenigun et al. (2003) observed no differences in feed intake, weight gain, or gain to feed efficiency ratio in early-weaned pigs fed the diet containing 500 FTU phytase/kg and the control diet.

Carbohydrases

Carbohydrases are the most commonly used enzyme in the food industry. Carbohydrases are used by the beverage industry, used to produce prebiotics, and included in animal feed as a supplement. Some of the common carbohydrases include amylases, invertases, inulinases, β-galactosidases, β-glucosidases, and glucosyltransferases, and they are originated from fungi, bacteria, and yeast. Non-starch polysaccharides (NSP) include all the plant polysaccharides, obviously, other than starch. The NSP consisted of cellulose, hemicellulose, pentosans, β-glucans, and others (Bedford, 2000; Craig, et al. 2018). As summarized by Wang (2017), NSP comprises many compounds with very different structures, several exogenous enzymes have been developed to target this diverse group of carbohydrates. The NSP is largely associated with cell walls, which are made up of different structural components, and specific carbohydrases are required to break those down efficiently and release more energy. Xylanase and β-glucanase are most commonly used and account for 80% of the global carbohydrase market.

Xylan makes up one of the major components of hemicellulose such as xyloglucan, glucomannan, galactoglucomannan, and arabinogalactan (Shallom, et al., 2003). The heteropolysaccharides consist of β-1,4-linked xylose residues, which can be degraded by xylanase (Beg et al., 2001; Collins et al., 2004). Xylanase is any of a class of enzymes that degrade the linear polysaccharide xylan into xylose, and consists of, e.g., endo-1,4-β-d-xylanases, β-d-xylosidases, α-glucuronidase, acetylxylan esterase, α-l-arabinofuranosidases, p-coumaric esterase, and ferulic acid esterase (Bhardwaj et al., 2019). Glucanases are enzymes that break down glucan, a polysaccharide made up of several glucose subunits and components of the cell walls of the higher plant family, particularly abundant in the endosperm cell walls of cereals (Stone and Clarke, 1992). As summarized by Chaari and Chaabouni (2019), β-glucans are polysaccharides of d-glucose monomers linked via β-glycosidic bonds and can be found in a variety of natural sources, such as yeast, mushrooms, bacteria, algae, barley, and oats. The β-glucanases are one of the main enzymes used in the industry during recent decades. These enzymes hydrolyze β-glucans and are produced by a variety of bacteria, fungi, plants, and animals.

The supplementation of xylanase is an important part of poultry diets because of its capability to lessen the antinutritional impacts of NSP. The supplementation of wheat-based diets with xylanase improved weight gain and feed conversion ratio and increased the apparent ileal digestibility of crude protein, dry matter, gross energy, and most amino acids (Liu et al., 2017). Similarly, González-Ortiz et al. (2019) observed improved body weight gain and feed conversion ratio in broiler chickens fed the wheat-based diet supplemented with xylanase. The supplementation of the diets containing dried wheat distillers grains with soluble with xylanase improved body weight gain and apparent metabolizable energy (Whiting et al., 2019). On the other hand, supplementation of the corn-based diet with xylanase had no effect on feed intake (Gehring et al., 2012). Mathlouthi et al. (2002) evaluated the effect of supplementing rye- or corn-based diets with both xylanase and β-glucanase. Broiler chickens fed the rye-based diet reduced feed intake, weight gain, and feed efficiency compared with those fed the corn-based diet, but the addition of xylanase and β-glucanase to the rye-based diet alleviated growth performance depression associated with feeding the rye-based diet.

Similarly, supplementation of pig diets with xylanase or β-glucanase or both can be important because of the use of some cereal byproducts, which can increase the content of NSP, to offset the feed cost. Pedersen et al. (2015) investigated the effect of xylanase on the degradation of nondigestible carbohydrates and apparent ileal digestibility of dried wheat distiller's grains with solubles and found that xylanase supplementation decreased the concentration of soluble arabinoxylan in ileal digesta and decreased the concentration of soluble NSP in ileal digesta. Barrera et al. (2004) provided 5,500, 11,000, and 16,500 xylanase units/kg of the wheat-based diet, and their results indicated the improvement in weight gain and feed conversion ratio of growing pigs, especially with those supplemented with 11,000 xylanase units/kg. On the other hand, other researchers (e.., Olukosi et al., 2007b; Woyengo et al., 2008) reported that supplementing the grower-finisher pig diets with xylanase had no effect on growth performance. Owusu-Asiedu et al. (2010) concluded that adding a blend of exogenous xylanase and β-glucanase to wheat-barley-based diets can improve the performance in weanling pigs. Supplementation of a barley-based pig diet with β-glucanase increased the ileal apparent digestibility of starch and mixed-linked β-glucans (Graham et al., 1989). The digestibility of crude protein and energy in weanling pigs increased when their barley-based diets were supplemented with β-glucanase, but the enzyme supplementation had no effect on those fed the wheat-, corn-, or rye-based diet (Li et al., 1996).

Bioactive Peptides and Fish Peptides

Bioactive peptides can be defined as small amino acid sequences derived from food or feed proteins that possess diverse biological/physiological properties or activities (Udenigwe and Aluko, 2012). To produce bioactive peptides, proteolysis is necessary and it may occur through enzymatic digestion of the precursor protein, either in in vitro or in in vivo. In addition, food or feed processing and enzymes from microorganisms or plants can trigger proteolysis and release potential bioactive peptides (López-Barrios et al., 2014). Ryan et al. (2011) indicated that meat and fish bioactive peptides had antioxidative, antimicrobial, and antiproliferative activities. Perhaps, 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 the aforementioned properties plus antihypertensive, antithrombotic, and immunomodulatory properties (Kim and Wijesekara, 2010).

The effect or effectiveness of some potential bioactive peptides on humans or animals has been investigated in recent years. For instance, researchers have been interested in evaluating the potential of bioactive peptides in their ability to reduce the risk of chronic diseases or boosting natural immune protection to promote human health (e.g., Ma et al., 2006). Yang et al. (2009) reported the increase in some cytokines in the serum of mice that were exposed to marine oligopeptide preparation, and they concluded that the preparation has the capability of positively modulating the immune system. After assessing the effect of casein glycomacropeptide on growth performance, intestinal morphology, intestinal barrier permeability, and inflammatory responses in pigs challenged with E. coli K88, Rong et al. (2015) concluded that casein glycomacropeptide can reduce the inflammatory response caused by E. coli K88 challenge. Young et al. (2012) evaluated the anti-inflammatory activity of soy-derived peptides on pigs affected by colitis. Their results indicated that TNF-∀ concentrations were reduced in soy peptide-treated pigs, which were similar to the concentration observed in negative control pigs or pigs without colitis. An increase in TNF-α in serum can be an indirect indicator of inflammation. Although the positive effects of bioactive peptides have been well demonstrated (e.g., Zhang et al., 2019), it should be pointed out that the effects of bioactive peptides on animals have not been consistent (e.g., Zhao et al., 2008).

Again, fish peptides may have a greater potential as a bioactive feed additive in, e.g., pig diets. The fish industry is a pillar of their 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 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 limited. 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.

References

Ashter, S. A., 2018. Technology and Applications of Polymers Derived from Biomass. William Andrew Publishing, Norwich, New York, pp. 179-191.

Amad, A.A., Männer, K., Wendler, K.R., Neumann, K., Zentek, J., 2011. Effects of a phytogenic feed additive on growth performance and ileal nutrient digestibility in broiler chickens. Poult. Sci. 90, 2811–2816.

Awano, T., Takabe, K., Fujita, M., 2001. Xylan and lignin deposition on the secondary wall of *Fagus crenata* fibers. Prog. Biotechnol. 18, 137-142.

Azain, M.J., 1993. Impact of starter on nursery performance. Swine Report No. 86, 49-54. Univ. of Georgia, Athens, GA.

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

Bedford, M.R., 2000. Exogenous enzymes in monogastric nutrition — their current value and future benefits. Anim. Feed Sci. Technol. 86, 1-13.

Bedford, M.R., Schulze, H., 1998. Exogenous enzymes for pigs and poultry. Nutr. Res. Rev. 11, 91-114.

Beg, Q.K., Kapoor, M., Mahajan, L., Hoondal, G.S., 2001. Microbial xylanases and their industrial applications: a review. Appl. Microbiol. Biotechnol. 56, 326-338.

Berkeveld, M., P. Langendijk, Van Beers-Schreurs, H. M. G., Koets, A. P., Taverne, M. A. M., Verheijden J. H. M., 2007. Postweaning growth check in pigs is markedly reduced by intermittent suckling and extended lactation. J. Anim. Sci. 85, 258-266.

Bhardwaj, N., B. Kumar, P. Verma., 2019. A detailed overview of xylanases: an emerging biomolecule for current and future prospective. Bioresour. Bioprocess. 6, 40.

Brumm, M., 2001. Phytase in swine diets. Nebraska Swine Reports. http://digitalcommons.unl.edu/coopext_swine/93 (accessed 20 November 2020).

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

Campbell, J. M., Crenshaw, J. D., Polo, J., 2013. The biological stress of early weaned piglets. J. Anim. Sci. Biotechnol. 4, 2-5. doi:10.1186/2049-1891-4-19.

Chaari, F., Chaabouni S. E., 2019. Fungal β-1,3-1,4-glucanases: production, proprieties and biotechnological applications. J. Sci. Food Agric. 99, 2657-2664.

Charles, V.M. Jr., Scott D.C., 2000. Feeding the weaned pigs, in: Lewis, A.J., Southern, L.L. (Eds.), Swine Nutrition. 2nd ed., CRC Press, Boca Raton, pp. 691-698.

Choi, J., Sabikhi, L., Hassan, A., Anand, S., 2012. Bioactive peptides in dairy products. Int. J. Dairy Technol. 65, 1-12.

Collins, T., Gerday, C., Feller., G., 2004. Xylanases, xylanase families and extremophilic xylanases. FEMS Microbiol. Rev. 29, 3-23.

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

Craig, A. D., Bedford, M. R., Hastie, P., Khattak, F., Olukosi, O. A., 2018. The effect of carbohydrases or prebiotic oligosaccharides on growth performance, nutrient utilisation and development of small intestine and immune organs in broilers fed nutrient-adequate diets based on either wheat or barley. J. Sci. Food Agric. 99, 3246-3254.

Cromwell, G.L., Allee, G.L., Mahan, D.C., 2008. Assessment of lactose level in the mid- to late-nursery phase on performance of weanling pigs. J. Anim. Sci. 86, 127-133.

Duarte, J., Vinderola, G., Ritz, B., Perdigon, G., Matar, C., 2006. Immunomodulating capacity of commercial fish protein hydrolysate for diet supplementation. Immunobiol. 211, 341-350.

Duffy, S. K., Kelly, A. K., Rajauria, G., Clarke, L. C., Gath, V., Monahan, F. J., O’Doherty, J. V., 2018. The effect of 25-hydroxyvitamin D3 and phytase inclusion on pig performance, bone parameters and pork quality in finisher pigs. J. Anim. Physiol. Anim. Nutr. (Berl). 102, 1296-1305.

Ekhlm, P., Virkki, L., Ylinen, M., Johansson, L., 2003. The effect of phytic acid and some natural chelating agents on the solubility of mineral elements in oat bran. Food Chem. 80, 165-170.

Frølich, W., 1990. Chelating properties of dietary fiber and phytate. The role for mineral availability. Adv. Exp. Med. Biol. 270, 83-93.

Gehring, C. K., Bedford, M. R., Dozier, W. A., 2013. Extra-phosphoric effects of phytase with and without xylanase in corn-soybean meal-based diets fed to broilers. Poult. Sci. 92, 979-991.

Gevaert, B., L. Veryser, F. Verbeke, E. Wynendaele, B. De Spiegeleer., 2016. Fish hydrolysates: A regulatory perspective of bioactive peptides. Protein Peptide Lett. 23, 1-9.

González-Ortiz, G., dos Santos, T. T., Vienola, K., Vartiainen, S., Apajalahti,J., Bedford., M. R., 2019. Response of broiler chickens to xylanase and butyrate supplementation. Poult. Sci. 98, 3914-3925.

Graf, D., Di Cagno, R., Fåk, F., Flint, H. J., Nyman, M., Saarela, M., Watzl. B., 2015. Contribution of diet to the composition of the human gut microbiota. Microb. Ecol. Heal. Dis. 26, 1-11.

Graham, H., Fadel, J. G., Newman, C. W., Newman, R. K., 1989. Effect of pelleting and β-glucanase supplementation on the ileal and fecal digestibility of a barley-based diet in the pig. J. Anim. Sci. 67, 1293-1298.

Haefner, S., Knietsch, A., Scholten, E., Braun, J., Lohscheidt, M., Zelder, O., 2005. Biotechnological production and applications of phytases. Appl. Microbiol. Biotechnol. 68, 588-597.

Hajati, H., Rezaei, M., Sayyahzade, H., 2009. The Effects of Enzyme Supplementation on Performance, Carcass Characteristics and Some Blood Parameters of Broilers Fed on Corn-Soybean Meal-Wheat Diets. Int. J. Poult. Sci. 8, 1199-1205.

He, S., Medrano, R. F., Yu, Q., Cai, Y., Dai, Q., He, J., 2017. Effect of a microbial phytase on growth performance, plasma parameters and apparent ileal amino acid digestibility in Youxian Sheldrake fed a low-phosphorus corn-soybean diet. Asian-Australasian J. Anim. Sci. 30, 1442-1449.

Himmelberg, L. V., Peo, Jr., E. R., Lewis, A. J., Crenshaw, J. D., 1985. Weaning weight response of pigs to simple and complex diets. J. Anim. Sci. 61, 18-26.

Ianiro, G., Pecere, S., Giorgio, V., Gasbarrini, A., Cammarota, G., 2016. Digestive Enzyme Supplementation in Gastrointestinal Diseases. Curr. Drug Metab. 17, 187-193.

Jensen, M. S., Jensen, S. K., Jakobsen, K., 1997. Development of digestive enzymes in pigs with emphasis on lipolytic activity in the stomach and pancreas. J. Anim. Sci. 75, 437-455.

Jongbloed, A.W., Mroz, Z., van der Weij-Jongbloed, R., Kemme, P.A., 2000. The effects of microbial phytase, organic acids and their interaction in diets for growing pigs. Livest. Prod. Sci. 67, 113-122.

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

Kim, J.C., Hansen, C.F., Mullana, B.P., Pluske, J.R., 2012. Nutrition and pathology of weaner pigs: nutritional strategies to support barrier function in the gastrointestinal tract. Anim. Feed Sci. Technol. 173, 3-16.

Kim, S.K., Wijesekara, I., 2010. Development and biological activities of marine-derived bioactive peptides: a review. J. Funct. Foods 2, 1-9.

Kitts, D.D., Weiler, K., 2003. Bioactive proteins and peptides from food sources, applications of bioprocesses used in isolation and recovery. Curr. Pharma. Des. 9, 1309-1323.

Kornegay, E.T., 2001. Digestion of phosphorus and other nutrients: role of phytases and factors influencing their activity, in: Bedford, M. R., Partridge, G.G. (Eds.), Enzymes in Farm Animal Nutrition. CABI Publishing, Wallingford, UK, pp. 237-272.

Kumar, V., Sinha, A. K., Makkar, H. P. S., De Boeck, G., Becker, K,. 2012. Phytate and phytase in fish nutrition. 96, 335-364.

Langhout, P., 2000. New additives for broiler chickens. World Poult. 16, 22-27.

Li, S., Sauer, W. C., Mosenthin, R., Kerr., B., 1996. Effect of β-glucanase supplementation of cereal-based diets for starter pigs on the apparent digestibilities of dry matter, crude protein and energy. Anim. Feed Sci. Technol. 59, 223-231.

Lindemann, M.D., Cornelius, S.G., el Kandelgy, S.M., Moser, R.L., Pettigrew, J.E., 1986. Effect of age, weaning and diet on digestive enzyme levels in the piglet. J. Anim. Sci. 62, 1298-1307.

Liu, W. C., Kim, I. H., 2017. Metabolism and nutrition: Effects of dietary xylanase supplementation on performance and functional digestive parameters in broilers fed wheat-based diets. Poult. Sci. 96, 566-573.

López-Barrios, L., Gutiérrez-Uribe, J.A., Serna-Saldívar, S.O., 2014. Bioactive peptides and hydrolysates from pulses and their potential use as functional ingredients. J. Food Sci. 79, 273-283.

Mavromichalis, I., Hancock, J.D., Hines, R.H., Senne, B.W., Cao, H., 2001. Lactose, sucrose, and molasses in simple and complex diets for nursery pigs. Anim. Feed Sci. Technol. 93, 127-135.

Maenz, D.D., 2001. Enzymatic characteristics of phytases as they relate to their use in animal feeds, in: Bedford, M.R., Partridge, G.G. (Eds.), Enzymes in Farm Animal Nutrition. CABI Publishing, Wallingford, UK, pp. 61-84.

Mahan, D.C., Fastinger, N.D., Peters, J.C., 2004. Effects of diet complexity and dietary lactose levels during three starter phases on postweaning pig performance. J. Anim. Sci. 82, 2790-2797.

Mao, X., Piao, X., Lai, C., Li, D., Xing, J., Shi, B., 2005. Effects of beta-glucan obtained from the Chinese herb *Astragalus membranaceus* and lipopolysaccharide challenge on performance, immunological, adrenal, and somatotropic responses of weanling pigs. J. Anim. Sci. 83, 2775-2782.

Martínez Cuesta, S., Rahman, S. A., Furnham, N., Thornton, J. M., 2015. The Classification and Evolution of Enzyme Function. Biophys. J. 109, 1082-1086.

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

Maxwell, C. V, De Rodas, B. Z., Brock, K., 1998. Efficacy of lactose equivalent-80 (LE-80) as an alternative for Lactose in phase II nursery diets. Anim. Sci. Res. Rep. P-965. Oklahoma State Univ., Stillwater, OK. pp. 208-211.

Nørgaard, J.V., Blaabjerg, K., Poulsen, H.D., 2012. Salmon protein hydrolysate as a protein source in feed for young pigs. Anim. Feed Sci. Technol. 177, 124-129.

Ockenden, I., Dorsch, J.A., Reid, M.M., Lin, L., Grant, L.K., Raboy, V., Lott, J.N.A., 2004. Characterization of the storage of phosphorus, inositol phosphate and cations in grain tissues of four barley (Hordeum vulgare L.) low phytic acid genotypes. Plant Sci. 167, 1131-1142.

Olukosi, O.A., Adeola, O., 2008. Whole body nutrient accretion, growth performance and total tract nutrient retention responses of broilers to supplementation of xylanase and phytase individually or in combination in wheat-soybean meal-based diets. J. Poult. Sci. 45, 192-198.

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

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

Olukosi, O.A., Adeola, O., 2013. Enzymes and enzyme supplementation of swine diets, in: Chiba, L.I. (Eds.), Sustainable Swine Nutrition. Wiley-Blackwell, John Wiley & Sons, Inc., Hoboken, NJ. pp. 277-294.

Omogbenigun, F.O., Nyachoti, C.M., Slominski, B.A., 2003. The effect of supplementing microbial phytase and organic acids to a corn-soybean based diet fed to early-weaned pigs. J. Anim. Sci. 81, 1806-1813.

Owusu-Asiedu, A., Simmins, P. H., Brufau, J., Lizardo, R., Péron, A., 2010. Effect of xylanase and β-glucanase on growth performance and nutrient digestibility in piglets fed wheat–barley-based diets. Livest. Sci. 134, 76-78.

Rajabzadeh, M., Pourashouri, P., Shabanpour, B., Alishahi, A., 2018. Amino acid composition, antioxidant and functional properties of protein hydrolysates from the roe of rainbow trout (*Oncorhynchus mykiss*). Int. J. Food Sci. Technol. 53, 313-319.

Ravindran, V., Cabahug, S., Ravindra, G., Selle, P.H., Bryden, W.L., 2000. Response of broiler chickens to microbial phytase supplementation as influenced by dietary phytic acid and non-phytate phosphorous levels. II. Effects on apparent metabolisable energy, nutrient digestibility and nutrient retention. Br. Poult. Sci. 41, 193–200.

Ravindran, V., Selle, P.H., Ravindran, G., Morel, P.C.H., Kies, A.K., Bryden, W.L., 2001. Microbial phytase improves performance, apparent metabolizable energy, and ileal amino acid digestibility of broilers fed a lysine-deficient diet. Poult. Sci. 80, 338-344.

Ryan, J.T., Ross, R.P., Bolton, D., and Fitzgerald, G.F., Stanton, C., 2011. Bioactive peptides from muscle sources: meat and fish. Nutrients 3, 765-91.

Pedersen, M. B., Yu, S., Arent, S., Dalsgaard, S., Bach Knudsen, K. E., Lærke, H. N., 2015. Xylanase increased the ileal digestibility of nonstarch polysaccharides and concentration of low molecular weight nondigestible carbohydrates in pigs fed high levels of wheat distillers dried grains with solubles. J. Anim. Sci. 93, 2885-2893.

Poudel P., Levesque, C.L., Samuel, R., St-Pierre, B., 2020. Dietary inclusion of Peptiva, a peptide-base feed additive, can accelerate the maturation of the fecal bacterial microbiome in weaned pigs. BMC Vet. Res. 16, 60.

Selle, P. H., Ravindran, V., Caldwell, A., Bryden, W. L., 2000. Phytate and phytase: consequences for protein utilisation. Nutr. Res. Rev. 13, 255-278.

Shallom, D., Shoham, Y., 2003. Microbial hemicellulases. Curr. Opin. Microbiol. 6, 219-228.

Sila, A., Bougatef, A., 2016. Antioxidant peptides from marine by-products: isolation, identification and application in food systems: a review. J. Funct. Foods 21, 10-26.

Simons, P.C., Versteegh, H.A., Jongbloed, A.W., Kemme, P.A., Slump, P., Bos, K.D., Wolters, M.G., Beudeker, R.F., Verschoor, G.J., 1990. Improvement of phosphorus availability by microbial phytase in broilers and pigs. Br. J. Nutr. 64, 525-540.

Skinner, L. D., Levesque, C. L., Wey, D., Rudar, M., Zhu, J., Hooda, S., de Lange, C. F. M., 2014. Impact of nursery feeding program on subsequent growth performance, carcass quality, meat quality, and physical and chemical body composition of growing-finishing pigs. J. Anim. Sci. 92, 1044-1054.

Stoner, G. R., Allee, G.L, Nelssen, J.L., 1985. Effects of fish protein hydrolysate and dried whey in starter pig diets. Swine Day. Kansas Agric. Exp. Stn., Kansas State Univ., Manhattan, KS. pp. 67-73.

Sun, Z., Ma, Q., Li, Z., Ji, C., 2009. Effect of partial substitution of dietary spray-dried porcine plasma or fishmeal with soybean and shrimp protein hydrolysate on growth performance, nutrient digestibility and serum biochemical parameters of weanling piglets. Asian-Australas. J. Anim. Sci. 22, 1032-1037.

Thuy, N. ., Joseph, M., Ha, N. C., 2016. Effects of replacing marine fishmeal with graded levels of Tra Catfish by-product protein hydrolysate on the performance and meat quality of pigs. S. Afr. J. Anim. Sci. 46, 221-229.

Truong, H. H., Yu, S., Peron, A., Cadogan, D. J., Khoddami, A., Roberts, T. H., Liu, S. Y., Selle, P. H., 2014. Phytase supplementation of maize-, sorghum- and wheat-based broiler diets with identified starch pasting properties influences phytate (IP6) and sodium jejunal and ileal digestibility. Anim. Feed Sci. Technol. 198, 248-256. doi:10.1016/j.anifeedsci.2014.10.007.

Tucker, J. L., Naranjo, V.D., Bidner, T.D., Southern, L. L., 2011. Effect of salmon protein hydrolysate and spray-dried plasma protein on growth performance of weanling pigs. J. Anim. Sci. 89, 1466-1473.

Udenigwe, C.C., Aluko, R.E., 2012. Food protein-derived bioactive peptides: production, processing, and potential health benefits. J. Food Sci. 77, R11-R24.

Vats, P., Banerjee, U.C., 2004. Production studies and catalytic properties of phytases (myo-inositolhexakisphosphate phosphohydrolases): an overview. Enzyme Microb. Technol. 35, 3-14.

Walk, C. L., Bedford, M. R., Olukosi, O. A., 2018. Effect of phytase on growth performance, phytate degradation and gene expression of myo-inositol transporters in the small intestine, liver and kidney of 21 day old broilers. Poult. Sci. 97, 1155-1162.

Walk, C. L., Bedford, M. R., Santos, T. S., Paiva, D., Bradley, J. R., Wladecki, H., Honaker, C., McElroy, A. P., 2013. Extra-phosphoric effects of superdoses of a novel microbial phytase. Poult. Sci. 92:719-725.

Walk, C. L., Santos, T. T., Bedford, M. R., 2014. Influence of superdoses of a novel microbial phytase on growth performance, tibia ash, and gizzard phytate and inositol in young broilers. Poult. Sci. 93, 1172-1177.

Wang, Y. 2017. Effect of diet complexity, multi-enzyme complexes, essential oils, and benzoic acid on weanling pigs. M.S. Thesis. Auburn University, Auburn, AL.

Wenk, C., 2000. Recent advances in animal feed additives such as metabolic modifiers, antimicrobial agents, probiotics, enzymes and highly available minerals. Review. Asian-Austral. J. Anim. Sci. 13, 86-95.

Whang, K.Y., Easter, RA., 2000. Blood urea nitrogen as an index of feed efficiency and lean growth potential in growing-finishing swine. Asian-Australas. J. Anim. Sci. 13, 811-816.

Whiting, I. M., Rose, S. P., Mackenzie, A. M., Amerah, A. M., Pirgozliev, V. R., 2019. Effect of wheat distillers dried grains with solubles and exogenous xylanase on laying hen performance and egg quality. Poult. Sci. 98, 3756–3762.

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

Young, L.G., Leunissen, M., Atkinson, J.L., 1993. Addition of microbial phytase to diets of young pigs. J. Anim. Sci. 71, 2147-2150.

Zamora-Sillero, J., Gharsallaoui, A., Prentice, C., 2018. Peptides from fish by-product protein hydrolysates and its functional properties: An overview. Marine Biotechnol. 20, 118-130.

Zeng, Z. K., Wang, D., Piao, X. S., Li, P. F., Zhang, H. Y., Shi, C. X., Yu., S. K., 2014. Effects of adding super dose phytase to the phosphorus-deficient diets of young pigs on growth performance, bone quality, minerals and amino acids digestibilities. Asian-Australas. J. Anim. Sci. 27, 237-246.

Zhang, G.G., Yang, Z.B., Wang, Y., Yang, W.R., Zhou, H.J., 2014. Effects of dietary supplementation of multi-enzyme on growth performance, nutrient digestibility, small intestinal digestive enzyme activities, and large intestinal selected microbiota in weanling pigs. J. Anim. Sci. 92, 2063-2069.

Zhang, Y., Chen, S., Zong, X., Wang, C., Shi, C., Wang, F., Wang, Y, Lu, Z., 2020. Peptides derived from fermented soybean meal suppresses intestinal inflammation and enhances epithelial barrier function in piglets. Food Agric. Immunol. 31, 120-135.

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

Effect of dietary fish peptide and enzyme supplementation on weanling pigs

T.B. Wilson[a], L.I. Chiba[a,]*, J. Guo[a], E.A. Spangler[b], T.S. Shieh[c]

[a] Department of Animal Sciences, College of Agriculture, Auburn University, Auburn University, AL 36849, United States

[b] Department of Pathology, College of Veterinary Medicine, Auburn University, Auburn University, AL 36849, United States

[c] Vitech Bio-Chem Corp., Orange, CA 92867, United States

* Corresponding author.

Email address: chibale@auburn.edu (L.I. Chiba).

A B S T R A C T

A total of 48 pigs (24 females and 24 castrated males) weaned at 3 to 4 wk of age (initial body weight, 7.77 ± 0.78 kg) were used to investigate the possibility of replacing a complex diet with a simple corn-soybean meal (SBM) diet by fish peptide and multienzyme complex supplementation. Pigs were randomly assigned to 3 dietary treatments with 4 replicate pens per treatment and 2 gilts and 2 castrated males per pen. A two-phase feeding program (2 wk each) was used, and 2 typical complexes, positive control (POS) diets were formulated to contain 12.15 and 11.07 g standard ileal digestible Lys/kg for Phase 1 and 2, respectively. In addition to corn and SBM, the complex diet for Phase 1 contained dried whey, fishmeal, plasma protein, poultry fat, and Zn oxide, whereas the complex diet for Phase 2 contained dried whey and fishmeal. Two simple, corn-SBM negative control (NEG) diets were formulated to be isolysinic to the POS diets for Phase 1 and 2 and included 1.5% fish peptides. The NEG diets were supplemented with multienzyme complexes. During the fourth week of the study, a blood sample was collected from each pig, and samples were analyzed for serum metabolites. Pigs were allowed ad libitum access to feed and water throughout the 4-wk study period, and the pig weight and feed consumption data were collected weekly. During the second week of the study (d 7 to 14), weight gain (WG) of pigs fed the POS diet was numerically lower ($P = 0.144$) than those fed the other 2 diets, which was reflected in the efficiency of feed utilization for WG. Pigs fed the NEG and ALL diets had greater gain to feed (G:F; $P = 0.034$), gain to Lys (G:Lys) intake ($P = 0.034$), and gain to DE (G:DE) intake ($P = 0.013$) than those fed the POS diet. Furthermore, G:F ($P = 0.037$), G:Lys intake ($P = 0.037$), and G:DE intake ($P = 0.010$) were greater in pigs fed the ALL diet than those fed the NEG diet. As in d 7 to 14, pigs fed the NEG and ALL diet had numerally greater weight gain than those fed the POS diets ($P = 0.147$) during the first 2 wk of

the study, which was reflected in the efficiency of WG. During Phase 1 (d 0 to 14), the efficiency of feed (*P* = 0.004), Lys (*P* = 0.004), or DE (*P* < 0.001) utilization was greater in pigs fed the NEG and ALL diets than those fed the POS diet. In addition, pigs fed the ALL diet tended to have greater G:F (*P* = 0.095) and G:Lys intake (*P* = 0.095) and had greater G:DE intake (*P* = 0.009) than those fed the NEG diet. There seemed to be no clear effect of dietary treatments on overall growth performance (d 0 to 28) of weanling pigs or serum metabolite profile during the last week of the study. During the first 2 wk of the study, fish peptides improved the growth performance of weanling pigs, and additional supplementation with multienzyme complexes improved growth performance further. The results indicated that it may be possible to replace a complex diet with a simple corn-SBM diet by supplementing the weanling pig diet with fish peptides and multienzyme complexes during Phase 1 (d 0 to 14).
Keywords: Weanling pigs, Fish peptides, Multienzyme complexes, Growth performance

1. Introduction

Young pigs are subjected to various stressors at weaning (Le Dividich and Sève, 2000; van Beers-Schreurs et al., 1998), and providing a highly palatable and digestible diet is, obviously, important in preventing or alleviating the so-called "growth check" soon after weaning and also 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 (Jensen et al., 1997; Lindemann et al., 1986). For instance, their enzyme profile is designed to utilize "milk" protein, carbohydrate, and fat, not corn and SBM (e.g., Lindemann et al., 1986; Le Dividich and Sève, 2000). For those reasons, weanling pigs have been fed so-called "complex diets" that contain many "special ingredients," such as dried whey, soy protein concentrate, plasma protein, fish meal, blood meal, oat groats, and others, which are highly palatable and digestible, to improve growth performance (Himmelberg et al., 1985; Whang et al., 2000; Wolter et al., 2003; 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. There might be some viable alternative supplements, such as fish peptides or hydrolysate that can be not only an excellent source of nutrients but also a "bioactive" or "functional" feed additive/supplement (Gevaert et al., 2016; Zamora-Sillero, 2018). Such an additive/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(or) availability of various enzymes, it might be possible for us to use a corn-soy-based diet more efficiently or successfully for weanling pigs (e.g., Olukosi et al., 2007; Zhang et al., 2014). Enzymes such as carbohydrases, proteases, phytase, and lipase can be used to extract more energy and nutrients from a simple corn-soy-based diet. With 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 study, the possibility to replace a complex diet with a simple corn-SBM diet for weanling pigs by supplementation with fish peptides and multienzyme complexes was investigated by assessing the effect of treatment on growth performance and serum metabolites.

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). A total of 48 pigs (24 females and 24 castrated males) weaned at 3 to 4 wk of age (initial body weight, 7.77 ± 0.78 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 3 dietary treatments with 4 replicate pens per treatment and 2 gilts and 2 castrated males per pen. Pigs were allowed ad libitum access to feed and water throughout the 4-wk study period, and the pig weight 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 the beginning of the study. A two-phase feeding program was used, and each phase consisted of 2 wk. Two typical complex, positive control (POS) diets were formulated to contain 12.15 and 11.07 g 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). In addition to corn and SBM, the complex diet for Phase 1 contained dried whey (Bakers Authority, Maspeth, NY), fishmeal (Omega Protein, Reedville, VA), plasma protein (AP920, APC Inc., Ankey, IA), poultry fat, and Zn oxide (Nutra Blend, LLC, Neosho, MO), whereas the complex diet for Phase 2 contained dried whey and fishmeal. Two simple, corn-SBM negative control (NEG) diets were formulated to be isolysinic to the POS diets for Phase 1 and 2 and included 1.5% fish peptides (Peptiva; Vitech Bio-Chem Corp., Orange, CA; Guo, 2020). The NEG diets were supplemented with multienzyme complexes (Ronozyme Multigrain GT and Ronozyme Hiphos 2500 GT; DSM Nutritional Products, Inc., Ames, IA; ALL). 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 and analysis of blood samples

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. 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 the analysis for metabolites. After thawing, samples were analyzed for serum metabolites including urea N, total protein, albumin, globulin, (albumin to globulin ratio), glucose, triglycerides, and cholesterol concentrations using an automated analyzer at Auburn University Clinical Pathology Laboratory (Auburn, AL).

2.5. Statistical analysis

Data were analyzed using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC). The pen was considered as the experimental unit. Covariates considered for the analysis were initial body weight for the growth performance data and third-week body weight for the serum metabolite data. Preplanned contrasts were: consisted of POS diet vs. NEG diet + ALL diet, NEG diet vs. ALL diet. The results are considered statistically significant if $P \leq 0.05$ and trends if $P \leq 0.10$.

3. Results

From d 0 to 7, pigs fed the POS diet tended to consume more digestible energy (DE) than those fed the NEG and All diets (P = 0.067), which was reflected in a tendency for those pigs to have slightly less gain to DE (G:DE) intake than pigs fed those diets (P = 0.104; Table 3). There was no effect of dietary treatments on other response criteria. During the second week of the study (d 7 to 14), weight gain (WG) of pigs fed the POS diet was numerically lower (P = 0.144) than those fed the other 2 diets, which was reflected in the efficiency of WG (Table 3). Pigs fed the NEG and ALL diets had greater gain to feed (G:F; P = 0.034), gain to Lys (G:Lys) intake (P = 0.034), and G:DE intake (P = 0.013) than those fed the POS diet. Furthermore, G:F (P = 0.037), G:Lys intake (P = 0.037), and G:DE intake (P = 0.010) were greater in pigs fed the ALL diet than those fed the NEG diet.

From d 14 to 21, there was no clear effect of dietary treatments on the intake of feed, Lys, or DE, or the efficiency of utilization of feed, Lys, or DE intake. Similarly, although there seemed to be numerical differences in some response criteria, dietary treatments had no clear effect on the intake of feed, energy, and Lys or the rate and efficiency of weight gain in pigs from d 14 to 21 (Table 4). Similarly, during the last week of the study (d 21 to 28), the POS, NEG, or ALL diets had no clear effect on growth performance of pigs (Table 4).

From d 0 to 14 d, pigs fed the NEG and ALL diet had numerally greater weight gain than those fed the POS diets (P = 0.147), and it was reflected in the efficiency of feed utilization for WG (Table 5). During the first 2 wk of the study (d 0 to 14), the efficiency of feed (P = 0.004), Lys (P = 0.004), or DE ($P < 0.001$) utilization was greater in pigs fed the NEG and ALL diets than those fed the POS diet. Moreover, pigs fed the ALL diet tended to have greater G:F (P =

0.095) and G:Lys intake (*P* = 0.095) and had greater G:DE intake (*P* = 0.009) than those fed the NEG diet. There was no effect of dietary treatments on the intake of feed, Lys, or DE intake. During the entire study (d 0 to 28), pigs fed the POST diet tended to have slightly lower G:DE intake than those fed the NEG and ALL diets (P = 0.06), but dietary treatments had no effect on other response criteria.

The effect of dietary fish peptide and multienzyme supplementation on metabolites in the serum of weanling pigs during the fourth week of the study is presented in Tables 6. Pigs fed the NEG and ALL diets seemed to be greater total protein than those fed the POS diet (*P* = 0.002). Globulin concentration was greater in pigs fed the NEG and ALL diets than those fed the POS diets (*P* = 0.007). Albumin to globulin ratio (*P* = 0.063) tended to be greater in those fed the POS diet than those fed the NEG and ALL diets. There was no clear effect of treatments on serum albumin, blood urea nitrogen, glucose, cholesterol, or triglyceride concentrations.

4. Discussion

A corn-soy diet is the standard for feeding pigs, however, such a diet may not be appropriate for weanling pigs. Weanling pigs are subjected to various stressors at weaning (Campbell et al., 2013), and their digestive system is immature or the secretion of digestive enzymes is not sufficient (Lindemann et al., 1986; Jensen et al., 1997; Le Dividich and Sève, 2000) to utilize corn-SBM diets. For that reason, traditionally, weanling pigs have been provided with complex diets containing many special ingredients such as dried whey, plasma protein, fish meal, soy protein concentrate, and others. Feeding such diets with highly palatable and digestible feed ingredients is very effective in promoting the growth performance of weanling pigs (Himmelberg et al., 1985; Mahan et al., 2004; Cromwell et al., 2008; Tran et al., 2014), but using such diets can be rather costly. With the development of, e.g., 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), and multienzyme complexes (e.g., Olukosi et al., 2007; Zhang et al., 2014), it might be possible to use corn-SBM diets more successfully for weanling pigs.

In the present study, from d 7 to 14, pigs fed the NEG and ALL diets grew numerically faster than those fed the POS diet or the complex diet. Such a numerical trend was reflected in the efficiency of feed, Lys, and DE utilization. The G:F, G:Lys intake, and G:DE intake from d 7 to 14 were greater in weanling pigs fed the NEG and ALL diets than those fed the POS diet, indicating that fish peptides may have had some positive effects on the rate and efficiency of weight gain. In addition, G:F, G:Lys intake, and G:DE intake were greater in pigs fed the ALL diets than those fed the NEG diet. The results may indicate that the multienzyme complexes containing phytase and carbohydrases may have had some beneficial effects on the utilization of feed, Lys, and energy.

The responses observed during d 7 to 14 were, obviously, reflected in the rate of efficiency of weight gain during the first 2 wk of the study. Pigs fed the NEG and ALL diets seemed to grow faster than those fed the POS diet. The efficiency of feed, Lys, or DE utilization was greater in pigs fed the NEG and ALL diets than those fed the POS diet. Furthermore, Pigs fed the ALL diet tended to utilize feed, Lys, or energy more efficiently than those fed the POS diet. The results indicated again, fish peptides had some beneficial effects on the growth performance of weanling pigs and multienzyme complexes had an additional positive effect on weanling pig performance. Those differences were, however, not reflected in the overall growth performance of weanling pigs.

Many previous studies have shown that feeding piglets a simple diet can result in reduced growth performance of weanling pigs (e.g., Wang et al., 2018), and feeding a complex diet improved weanling pig performance (e.g., Whang et al., 2000; Wolter et al., 2003; Cervantes-Pham and Stein, 2010). Such a growth depression in pigs offered a simple corn-SBM diet was not apparent in the current study. However, the NEG diet contained fish peptides, and the ALL diet contained both fish peptides and multienzyme complexes.

Although the literature on the use of fish peptides hydrolysates as a source of protein or amino acids for weanling pigs has been rather scarce, the use of such products is not a new idea (e.g., Stoner et al., 1985). In recent years, researchers reported that growth performance of young pigs can be effectively improved by dietary supplementation of fish peptides or hydrolysates (Thuy et al., 2016) or those products can effectively replace some commonly used protein supplements 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 for animals, as well as for humans, depending on the degree of refinement, and also as a functional dietary ingredient. Various biological activities have been demonstrated in animals and also humans (Gevaert et al., 2016), including antioxidative, antimicrobial, antihypertensive, and other activities (Rajabzadeh et al., 2018; Zamora-Sillero, 2018). Perhaps, those biological activities collectively had some beneficial effects on the growth performance of weanling pigs in the present study.

In the present study, again, supplementation of the simple diets containing fish peptides with multienzyme complexes had some beneficial effect on the utilization of feed, Lys, and DE, which is contrary to several reports on the lack of response to multienzyme complex supplementation (Kiarie et al., 2007; Omogbenigun et al., 2003). The lack of could be attributed to the age of pigs (Harper et al., 1997). In addition, as far as carbohydrases are concerned, the effectiveness may have something to do with the variation on the fiber content among various feed ingredients (Emiola, 2009; Omogbenigun et al., 2004). For instance, Jones et al. (2010) found that carbohydrases seemed to be less effective when included in diets based on corn and SBM. They reported that galactomannanase, β-glucanase, or xylanase supplementation did not improve the animal performance when those enzymes were incorporate into corn–SBM based diets containing 30% corn distillers dried grains with solubles. Perhaps, the substrate composition and concentration are likely to play a role in the efficacy of carbohydrase enzymes (Bedford, 2000), and corn is highly digestible and low in fiber, carbohydrases may not consistently improve growth performance of pigs (Partridge, 2000). In the present study, however, multienzyme complexes consisting of phytases and carbohydrases in the weanling pig diet seemed to be effective in improving the efficiency of feed, Lys, and energy utilization.

Serum metabolite data may provide some information on the animal's metabolic activities. Lowrey et al. (1962) indicated that the total protein concentration can be used as an indicator of efficiency of utilization of dietary protein. In the current study, pigs fed the NEG and ALL diet had greater serum total protein during the last week of the study. Mule et al. (2016) reported that positive correlations were observed between serum albumin and growth performance in young pigs, but there was no effect of dietary treatments on serum albumin. Serum globulin, which is generally determined by the difference between total protein and albumin, was greater in pigs fed the NEG and ALL diet than those fed the POS diet, which was caused by its greater concentration in pigs fed the ALL diet. Considering the pooled SEM, the difference between the NEG & ALL diet should be statistically different, but it was not. The increase in the globulin fraction usually results from an increase in immunoglobulins. Similarly, albumin to globulin ratio should have been statistically lower in pigs fed the ALL diet than those fed the NEG diet based on the pooled SEM, but it was not different. Serum urea N is another important indicator of protein and amino acid adequacy and efficiency of amino acid utilization (e.g., Eggum, 1970; Coma et al., 1995), but dietary treatments had no effect on serum urea N. In general, dietary treatments seemed to have no clear effect on the serum metabolite profile.

5. Conclusion

During the first 2 wk of the study, pigs fed the NEG and ALL diet had numerally greater weight gain than those fed the POS diets, which was reflected in the efficiency of feed utilization for WG. The efficiency of feed, Lys, and DE utilization was greater in pigs fed the NEG and ALL diets than those fed the POS diet. In addition, pigs fed the ALL diet tended to have greater G:F and G:Lys intake and had greater G:DE intake than those fed the NEG diet. The results may indicate that it may be possible to replace a complex diet with a simple corn-SBM diet by supplementing the weanling pig diet with fish peptides and enzyme complexes during Phase 1 (d 0 to 14).

Declaration of Competing 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 to construed as influencing the current article.

Acknowledgments

This research project was funded in part by Alabama Agricultural Experiment Station

(Auburn University, Auburn, AL) and Leveking Bio-Engineering Co., Ltd. (Shenzhen, China)/Vitech Bio-Chem Corporation (Orange, CA). Appreciation is extended to Omega Protein (Reedville, VA), APC, Inc. (Ankeny, IA), and Nutra Blend LLC (Neosho, MO, US) for donating fishmeal, plasma protein, and vitamin-trace mineral premix 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.

References

AOAC, 2000. Official Method of Analysis, 17th ed. Assoc. Off. Anal. Chem., Washington, DC.

Bedford, M.R., 2000. Exogenous enzymes in monogastric nutrition — their current value and future benefits. Anim. Feed Sci. Technol. 86, 1–13.

Campbell, J. M., Crenshaw, J. D., Polo, J., 2013. The biological stress of early weaned piglets. J. Anim. Sci. Biotechnol. 4, 2-5.

Cervantes-Pahm, S.K., Stein, H.H., 2010. Ileal digestibility of amino acids in conventional, fermented, and enzyme-treated soybean meal and in soy protein isolate, fish meal, and casein fed to weanling pigs. J. Anim. Sci. 88, 2674-2683.

Coma, J., Zimmerman, D.R., Carrion, D., 1995. Relationship of rate of lean tissue growth and other factors to concentration of urea in plasma of pigs. J. Anim. Sci. 73, 3649-3656.

Cromwell, G.L., Allee, G.L., Mahan, D.C., 2008. Assessment of lactose level in the mid- to late-nursery phase on performance of weanling pigs. J. Anim. Sci. 86, 127-133.

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

Emiola, I.A., Opapeju, F.O., Slominski, B.A., Nyachoti, C.M., 2009. Growth performance and nutrient digestibility in pigs fed wheat distillers dried grains with solubles-based diets supplemented with a multicarbohydrase enzyme. J. Anim. Sci. 87, 2315-2322.

Gevaert, B., L. Veryser, F. Verbeke, E. Wynendaele, and B. De Spiegeleer. 2016. Fish hydrolysates: A regulatory perspective of bioactive peptides. Protein Peptide Lett. 23:1-9.

Guo, J. 2020. Effect of diet complexity and dietary fish peptide supplementation on weanling pigs. MS Thesis. Auburn University, AL.

Harper, A.F., Kornegay, E.T., Schell, T.C., 1997. Phytase supplementation of low-phosphorus growing-finishing pig diets improves performance, phosphorus digestibility, and bone mineralization and reduces phosphorus excretion. J. Anim. Sci. 75, 3174-3186.

Himmelberg, L.V., Peo, Jr., E.R., Lewis, A.J., Crenshaw, J.D., 1985. Weaning weight response of pigs to simple and complex diets. J. Anim. Sci. 61, 18-26.

Jensen, M. S., Jensen, S. K., Jakobsen, K., 1997. Development of digestive enzymes in pigs with emphasis on lipolytic activity in the stomach and pancreas. J. Anim. Sci. 75, 437-455.

Jones, C.K., Bergstrom, J.R., Tokach, M.D., DeRouchey, J.M., Goodband, R.D., Nelssen, J.L., Dritz, S.S., 2010. Efficacy of commercial enzymes in diets containing various concentrations and sources of dried distillers grains with solubles for nursery pigs. J. Anim. Sci. 88, 2084–2091.

Le Dividich, J., and Sève, B., 2000. Effects of underfeeding during the weaning period on growth, metabolism, and hormonal adjustments in the piglet. Domest. Anim. Endocrinol. 19, 63-74.

Lindemann, M.D., Cornelius, S.G., el Kandelgy, S.M., Moser, R.L., Pettigrew, J.E., 1986. Effect of age, weaning and diet on digestive enzyme levels in the piglet. J. Anim. Sci. 62, 1298-1307.

Lowrey, R.S., Pond, W.G., Barnes, R.H., Krook, L., Loosli, J.K., 1962. Influence of caloric level and protein quality on the manifestations of protein deficiency in the young pig. J. Nutr. 78, 245-253.

Mahan, D.C., Fastinger, N.D., Peters, J.C., 2004. Effects of diet complexity and dietary lactose levels during three starter phases on postweaning pig performance. J. Anim. Sci. 82, 2790-2797.

Mule, H.R., Chiba, L.I., Fabian, J., Kuhlers, D.L., Jungst, S.B., Frobish, L.T., Nadarajah, K., Bergen, W.G., Welles, E.G., 2006. Effect of early dietary amino acid restrictions on serum metabolites in pigs selected for lean growth efficiency. Can. J. Anim. Sci. 86, 489-500.

Nørgaard, J.V., Blaabjerg, K., Poulsen, H.D., 2012. Salmon protein hydrolysate as a protein source in feed for young pigs. Anim. Feed Sci. Technol. 177, 124-129.

NRC, 2012. Nutrient Requirement of Swine. 11th rev. ed., Natl. Acad. Press, Washington, DC.

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

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

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

Partridge, G.G., 2000. The role and efficacy of carbohydrase enzymes in pig nutrition, in: Bedford, M. R., Partridge, G.G. (Eds.), Enzymes in Farm Animal Nutrition. CABI, Wallingford, UK, pp. 161-198.

Rajabzadeh, M., Pourashouri, P., Shabanpour, B., Alishahi, A., 2018. Amino acid composition, antioxidant and functional properties of protein hydrolysates from the roe of rainbow trout (Oncorhynchus mykiss). Int. J. Food Sci. Technol. 53, 313-319.

Stoner, G.R., Allee, G.L., Nelssen, J.L., 1985. Effects of fish protein hydrolysate and dried whey in starter pig diets. Swine Day. Kansas Agric. Exp. Stn., Kansas State Univ., Manhattan, KS, US. p. 67-73.

Sun, Z., Ma, Q., Li, Z., Ji, C., 2009. Effect of partial substitution of dietary spray-dried porcine plasma or fishmeal with soybean and shrimp protein hydrolysate on growth performance, nutrient digestibility and serum biochemical parameters of weanling piglets. Asian-Australas. J. Anim. Sci. 22, 1032-1037.

Thuy, N.T., Joseph, M., Ha, N.C., 2016. Effects of replacing marine fishmeal with graded levels of Tra Catfish by-product protein hydrolysate on the performance and meat quality of pigs. S. Afr. J. Anim. Sci. 46, 221-229.

Tran, H., Bundy, J.W., Li, Y.S., Carney-Hinkle, E.E, Miller, P.S., Burkey, T.E., 2014. Effects of spray-dried porcine plasma on growth performance, immune response, total antioxidant capacity, and gut morphology of nursery pigs. J. Anim. Sci. 92, 4494-4504.

Tucker, J. L., V. D. Naranjo, T. D. Bidner, and L. L. Southern. 2011. Effect of salmon protein hydrolysate and spray-dried plasma protein on growth performance of weanling pigs. J. Anim. Sci. 89:1466-1473.

van Beers-Schreurs, H.M., Nabuurs, M.J., Vellenga, L., Kalsbeek-van der Valk, H.J., Wensing, T., Breukink, H.J., 1998. Weaning and the weanling diet influence the villous height and crypt depth in the small intestine of pigs and alter the concentrations of short-chain fatty acids in the large intestine and blood. J. Nutr. 128, 947-953.

Wang, Y. 2018. L.I. Chiba, C. Huang, I.M. Torres, L. Wang, E.G. Welles. 2018. Effect of diet complexity, multi-enzyme complexes, essential oils, and benzoic acid on weanling pigs. Livest. Sci. 209:32-38.

Whang, K. Y., McKeith, F. K., Kim, S. W., Easter, R. A., 2000. Effect of starter feeding program on growth performance and gains of body components from weaning to market weight in swine. J. Anim. Sci. 78, 2885-2895.

Wolter, B.F., Ellis, M., Corrigan, B.P., DeDecker, J.M., Curtis, S.E., Parr, E.N., Webel, D.M., 2003. Impact of early postweaning growth rate as affected by diet complexity and space allocation on subsequent growth performance of pigs in a wean-to-finish production system. J. Anim. Sci. 81, 353-359

Zamora-Sillero, J., Gharsallaoui, A., Prentice, C., 2018. Peptides from fish by-product protein hydrolysates and its functional properties: an overview. Marine Biotechnol. 20, 118-130.

Zhang, G.G., Yang, Z.B., Wang, Y., Yang, W.R., Zhou, H.J., 2014. Effects of dietary supplementation of multi-enzyme on growth performance, nutrient digestibility, small intestinal digestive enzyme activities, and large intestinal selected microbiota in weanling pigs. J. Anim. Sci. 92, 2063-2069.

Table 1
Composition of Phase 1 weanling pig diets (as-fed basis; d 0 to 14) [1,2,3,4].

Item	POS	NEG	ALL
Ingredient (g/kg)			
Corn	465.6	542.3	536.0
Soybean meal (48% CP)	269.7	410.2	410.7
Dried whey	150	-	-
Fishmeal	40.0	-	-
Plasma protein	20.0	-	-
Fish peptides	-	15.0	15.0
Poultry fat	30.0	-	-
Dicalcium phosphate	7.50	13.4	13.5
Limestone	6.90	8.90	8.80
Salt	3.50	3.50	3.50
ZnO	4.20	4.20	4.20
Vitamin-mineral premix[5]	2.50	2.50	2.50
Multienzyme complexes	-	-	0.90
Calculated composition			
DE (Mcal/kg)	3.69	3.41	3.38
CP (g/kg)	226.2	247.7	247.4
Ca (g/kg)	8.0	8.0	8.0
P (g/kg)	7.0	7.0	7.0
Ca:P	1.14	1.14	1.14
SID Lys (g/kg)	12.15	12.15	12.15
SID Lys:DE (g/Mcal)	3.29	3.56	3.59
Analyzed composition (g/kg)			
CP	197.4	209.5	228.1

[1] POS = positive complex diet, NEG = negative simple corn-soybean meal diet with 1.5% fish peptides (Peptiva; Vitech Bio-Chem Corp., Orange, CA), and ALL = diet supplemented with 1.5% fish peptides and multienzyme complexes [Ronozyme Hiphos (GT) 2500 and Ronozyme Multigrain (GT)]; DSM Nutritional Products, Inc., Ames, IA].

[2] Fed starter diets from 7.77 ± 0.78 to 22.20 ± 1.55 kg.

[3] Dried whey = Bakers Authority, Maspeth, NY; fishmeal = Omega Protein, Reedville, VA; plasma protein (AP920) = APC Inc, Ankey, IA; ZnO = Nutra Blend, Neosho, MO.

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

[5] Provided the following (unit/kg diet; Nutra Blend): 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 weanling pig diets (as-fed basis; d 14 to 28) [1,2,3,4].

Item	POS	NEG	ALL
Ingredient (g/kg)			
Corn	567.5	587.2	580.8
Soybean meal (48% CP)	334.4	366.1	366.5
Dried whey	50.0	-	-
Fishmeal	20.0	-	-
Plasma protein	-	-	-
Fish peptides	-	15.0	15.0
Poultry fat	-	-	-
Dicalcium phosphate	11.8	14.4	14.5
Limestone	7.50	8.50	8.50
Salt	3.50	3.50	3.50
ZnO	2.80	2.80	2.80
Vitamin-mineral premix[5]	2.50	2.50	2.50
Multienzyme complexes	-	-	0.90
Calculated composition			
DE (Mcal/kg)	3.42	3.40	3.38
CP (g/kg)	224.8	230.3	230.0
Ca (g/kg)	8.0	8.0	8.0
P (g/kg)	7.0	7.0	7.0
Ca:P	1.14	1.14	1.14
SID Lys (g/kg)	11.07	11.07	11.07
SID Lys:DE (g/Mcal)	3.24	3.26	3.28
Analyzed composition (g/kg)			
CP	219.9	219.1	217.0

[1] POS = positive complex diet, NEG = negative simple corn-soybean meal diet with 1.5% fish peptides (Peptiva; Vitech Bio-Chem Corp., Orange, CA), and ALL = diet supplemented with 1.5% fish peptides and multienzyme complexes [Ronozyme Hiphos (GT) 2500 and Ronozyme Multigrain (GT)]; DSM Nutritional Products, Inc., Ames, IA].

[2] Fed starter diets from 7.77 ± 0.78 to 22.20 ± 1.55 kg.

[3] Dried whey = Bakers Authority, Maspeth, NY; fishmeal = Omega Protein, Reedville, VA; plasma protein (AP920) = APC Inc, Ankey, IA; ZnO = Nutra Blend, Neosho, MO.

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

[5] Provided the following (unit/kg diet; Nutra Blend): 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 and enzyme supplementation on growth performance of weanling pigs (first and second week) [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	540	6.56	1.99	182	339	27.9	92
NEG	523	6.35	1.78	159	303	25.0	89
ALL	487	5.91	1.65	170	354	29.1	104
SEM	15	0.18	0.06	9	18	1.5	6
P-value							
POS vs. NEG + ALL	0.214	0.214	0.067	0.946	0.251	0.251	0.104
NEG vs. ALL	0.667	0.667	0.145	0.229	0.272	0.272	0.714
d 7 to 14							
POS	863	10.49	3.18	202	232	19.1	63
NEG	820	9.96	2.80	245	301	24.8	88
ALL	813	9.88	2.76	267	328	27.0	97
SEM	24	0.29	0.10	13	14	1.2	5
P-value							
POS vs. NEG + ALL	0.645	0.645	0.295	0.144	0.034	0.034	0.013
NEG vs. ALL	0.548	0.548	0.148	0.202	0.037	0.037	0.010

[1] POS = positive complex diet, NEG = negative simple corn-soybean meal diet with 1.5% fish peptides, and ALL= diet supplemented with 1.5% fish peptides and multienzyme complexes.

[2] Fed starter diets from 7.77 ± 0.78 to 22.20 ± 1.55 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 growth performance of weanling pigs (third and fourth week) [1,2,3]

Item	ADFI (g/d)	LysI (g/d)	DEI (Mcal/d)	WG (g/d)	G:F (g/kg)	G:LysI (g/d)	G:DEI (g/Mcal)
d 14 to 21							
POS	1,207	13.36	4.13	256	210	19.0	61
NEG	1,175	13.01	4.00	209	178	16.1	52
ALL	1,173	12.99	3.97	180	155	14.0	46
SEM	20	0.22	0.07	18	15	1.3	4
P-value							
POS vs. NEG + ALL	0.688	0.688	0.524	0.246	0.288	0.288	0.307
NEG vs. ALL	0.526	0.526	0.451	0.355	0.443	0.443	0.459
d 21 to 28							
POS	1,302	14.42	4.46	321	246	22.2	72
NEG	1,278	14.14	4.35	274	213	19.2	63
ALL	1,281	14.18	4.33	339	264	23.9	78
SEM	29	0.32	0.10	20	13	1.2	4
P-value							
POS vs. NEG + ALL	0.901	0.902	0.783	0.406	0.285	0.285	0.258
NEG vs. ALL	0.780	0.780	0.719	0.410	0.380	0.380	0.400

[1] POS = positive complex diet, NEG = negative simple corn-soybean meal diet with 1.5% fish peptides, and ALL= diet supplemented with 1.5% fish peptides and multienzyme complexes.

[2] Fed Phase 1 and 2 diets from 7.77 ± 0.78 to 22.20 ± 1.55 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 (first 2 wk and overall) [1,2,3]

Item	ADFI (g/d)	LysI (g/d)	DEI (Mcal/d)	WG (g/d)	G:F (g/kg)	G:LysI (g/d)	G:DEI (g/Mcal)
d 0 to 14							
POS	702	8.52	2.59	192	275	22.6	74
NEG	671	8.16	2.29	202	301	24.8	88
ALL	650	7.90	2.20	218	337	27.7	99
SEM	17	0.21	0.07	7	10	0.8	4
P-value							
POS vs. NEG + ALL	0.406	0.406	0.142	0.147	0.004	0.004	< 0.001
NEG vs. ALL	0.550	0.550	0.113	0.528	0.095	0.095	0.009
d 0 to 28							
POS	978	11.21	3.44	240	245	21.4	70
NEG	949	10.87	3.23	222	233	20.4	69
ALL	938	10.74	3.18	240	254	22.2	75
SEM	17	0.20	0.07	7	5	0.4	2
P-value							
POS vs. NEG + ALL	0.580	0.569	0.311	0.651	0.157	0.151	0.060
NEG vs. ALL	0.575	0.572	0.256	0.368	0.304	0.307	0.702

[1] POS = positive complex diet, NEG = negative simple corn-soybean meal diet with 1.5% fish peptides, and ALL= diet supplemented with 1.5% fish peptides and multienzyme complexes.

[2] Fed Phase 1 and 2 diets from 7.77 ± 0.78 to 22.20 ± 1.55 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 and enzyme supplementation 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	4.31	3.65	0.68	6.04	14.46	119	66.6	49.1
NEG	4.48	3.90	0.58	7.68	14.60	112	66.1	43.8
ALL	4.80	3.73	1.08	3.84	15.41	114	60.9	49.4
SEM	0.07	0.06	0.08	0.77	0.32	2	1.7	2.0
P-value								
POS vs. NEG + ALL	0.002	0.705	0.007	0.063	0.228	0.709	0.168	0.514
NEG vs. ALL	0.166	0.114	0.511	0.347	0.865	0.241	0.919	0.320

[1] POS = positive complex diet, NEG = negative simple corn-soybean meal diet with 1.5% fish peptides, and ALL= diet supplemented with 1.5% fish peptides and multienzyme complexes.

[2] Fed Phase 1 and 2 diets from 7.77 ± 0.78 to 22.20 ± 1.55 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.

IV. SUMMARY AND CONCLUSION

Young pigs are subjected to various stressors at weaning, and providing a highly palatable and digestible diet is, obviously, important in preventing or alleviating the so-called "growth check" soon after weaning and also optimizing growth performance thereafter. Although a corn-soybean meal (SBM) diet is the 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, not corn and SBM. For those reasons, weanling pigs have been fed so-called "complex diets" that contain many "special ingredients," such as dried whey, soy protein concentrate, plasma protein, fish meal, blood meal, oat groats, and others, which are highly palatable and digestible, to improve growth performance.

Providing such diets with highly palatable and digestible ingredients to weanling pigs can be, however, a costly proposition. There might be some viable alternative supplements, such as fish peptides or hydrolysate that can be not only an excellent source of nutrients but also a "bioactive" or "functional" feed additive/supplement. Such an additive/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(or) availability of various enzymes, it might be possible for us to use a corn-soy-based diet more efficiently or successfully for weanling pigs. Enzymes such as carbohydrases, proteases, phytase, and lipase can be used to extract more energy and nutrients from a simple corn-soy-based diet. With 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 study, the possibility to replace a complex diet with a simple corn-SBM diet for weanling pigs by supplementation with fish peptides and multienzyme complexes was investigated by assessing the effect of treatment on growth performance and serum metabolites.

A total of 48 pigs (24 females and 24 castrated males) weaned at 3 to 4 wk of age (initial body weight, 7.77 ± 0.78 kg) were used to investigate the possibility of replacing a complex diet with a simple corn-soybean meal (SBM) diet by fish peptide and multienzyme complex supplementation. Pigs were randomly assigned to 3 dietary treatments with 4 replicate pens per treatment and 2 gilts and 2 castrated males per pen. A two-phase feeding program (2 wk each) was used, and 2 typical complexes, positive control (POS) diets were formulated to contain 12.15 and 11.07 g standard ileal digestible Lys/kg for Phase 1 and 2, respectively. In addition to corn and SBM, the complex diet for Phase 1 contained dried whey, fishmeal, plasma protein, poultry fat, and Zn oxide, whereas the complex diet for Phase 2 contained dried whey and fishmeal. Two simple, corn-SBM negative control (NEG) diets were formulated to be isolysinic to the POS diets for Phase 1 and 2 and included 1.5% fish peptides. The NEG diets were supplemented with multienzyme complexes. During the fourth week of the study, a blood sample was collected from each pig, and samples were analyzed for serum metabolites. Pigs were allowed ad libitum access to feed and water throughout the 4-wk study period, and the pig weight and feed consumption data were collected weekly.

During the second week of the study (d 7 to 14), weight gain (WG) of pigs fed the POS diet was numerically lower than those fed the other 2 diets, which was reflected in the efficiency of feed utilization for WG. Pigs fed the NEG and ALL diets had greater gain to feed (G:F), gain to Lys (G:Lys) intake, and gain to DE (G:DE) intake than those fed the POS diet. Furthermore, G:F, G:Lys intake, and G:DE intake were greater in pigs fed the ALL diet than those fed the NEG diet. As in d 7 to 14, pigs fed the NEG and ALL diet had numerally greater weight gain than those fed the POS diets during the first 2 wk of the study, which was reflected in the efficiency of WG. During Phase 1 (d 0 to 14), the efficiency of feed, Lys, or DE utilization was greater in pigs fed the NEG and ALL diets than those fed the POS diet. In addition, pigs fed the ALL diet tended to have greater G:F and G:Lys intake and had greater G:DE intake than those fed the NEG diet. There seemed to be no clear effect of dietary treatments on overall growth performance (d 0 to 28) of weanling pigs or serum metabolite profile during the last week of the study.

During the first 2 wk of the study, fish peptides improved the growth performance of weanling pigs, and additional supplementation with multienzyme complexes improved growth performance further. The results indicated that it may be possible to replace a complex diet with a simple corn-SBM diet by supplementing the weanling pig diet with fish peptides and multienzyme complexes during Phase 1 (d 0 to 14).

V. CUMULATIVE BIBLIOGRAPHY

AOAC, 2000. Official Method of Analysis, 17th ed. Assoc. Off. Anal. Chem., Washington, DC.

Ashter, S. A., 2018. Technology and Applications of Polymers Derived from Biomass. William Andrew Publishing, Norwich, New York, pp. 179-191.

Amad, A.A., Männer, K., Wendler, K.R., Neumann, K., Zentek, J., 2011. Effects of a phytogenic feed additive on growth performance and ileal nutrient digestibility in broiler chickens. Poult. Sci. 90, 2811–2816.

Awano, T., Takabe, K., Fujita, M., 2001. Xylan and lignin deposition on the secondary wall of *Fagus crenata* fibers. Prog. Biotechnol. 18, 137-142.

Azain, M.J., 1993. Impact of starter on nursery performance. Swine Report No. 86, 49-54. Univ. of Georgia, Athens, GA.

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

Bedford, M.R., 2000. Exogenous enzymes in monogastric nutrition — their current value and future benefits. Anim. Feed Sci. Technol. 86, 1-13.

Bedford, M.R., Schulze, H., 1998. Exogenous enzymes for pigs and poultry. Nutr. Res. Rev. 11, 91-114.

Beg, Q.K., Kapoor, M., Mahajan, L., Hoondal, G.S., 2001. Microbial xylanases and their industrial applications: a review. Appl. Microbiol. Biotechnol. 56, 326-338.

Berkeveld, M., P. Langendijk, Van Beers-Schreurs, H. M. G., Koets, A. P., Taverne, M. A. M., Verheijden J. H. M., 2007. Postweaning growth check in pigs is markedly reduced by intermittent suckling and extended lactation. J. Anim. Sci. 85, 258-266.

Bhardwaj, N., B. Kumar, P. Verma., 2019. A detailed overview of xylanases: an emerging biomolecule for current and future prospective. Bioresour. Bioprocess. 6, 40.

Brumm, M., 2001. Phytase in swine diets. Nebraska Swine Reports. http://digitalcommons.unl.edu/coopext_swine/93 (accessed 20 November 2020).

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

Campbell, J. M., Crenshaw, J. D., Polo, J., 2013. The biological stress of early weaned piglets. J. Anim. Sci. Biotechnol. 4, 2-5.

Cervantes-Pahm, S.K., Stein, H.H., 2010. Ileal digestibility of amino acids in conventional, fermented, and enzyme-treated soybean meal and in soy protein isolate, fish meal, and casein fed to weanling pigs. J. Anim. Sci. 88, 2674-2683.

Chaari, F., Chaabouni S. E., 2019. Fungal β-1,3-1,4-glucanases: production, proprieties and biotechnological applications. J. Sci. Food Agric. 99, 2657-2664.

Charles, V.M. Jr., Scott D.C., 2000. Feeding the weaned pigs, in: Lewis, A.J., Southern, L.L. (Eds.), Swine Nutrition. 2nd ed., CRC Press, Boca Raton, pp. 691-698.

Choi, J., Sabikhi, L., Hassan, A., Anand, S., 2012. Bioactive peptides in dairy products. Int. J. Dairy Technol. 65, 1-12.

Collins, T., Gerday, C., Feller., G., 2004. Xylanases, xylanase families, and extremophilic xylanases. FEMS Microbiol. Rev. 29, 3-23.

Coma, J., Zimmerman, D.R., Carrion, D., 1995. Relationship of rate of lean tissue growth and other factors to concentration of urea in plasma of pigs. J. Anim. Sci. 73, 3649-3656.

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

Craig, A. D., Bedford, M. R., Hastie, P., Khattak, F., Olukosi, O. A., 2018. The effect of carbohydrases or prebiotic oligosaccharides on growth performance, nutrient utilisation and development of small intestine and immune organs in broilers fed nutrient-adequate diets based on either wheat or barley. J. Sci. Food Agric. 99, 3246-3254.

Cromwell, G.L., Allee, G.L., Mahan, D.C., 2008. Assessment of lactose level in the mid- to late-nursery phase on performance of weanling pigs. J. Anim. Sci. 86, 127-133.

Duarte, J., Vinderola, G., Ritz, B., Perdigon, G., Matar, C., 2006. Immunomodulating capacity of commercial fish protein hydrolysate for diet supplementation. Immunobiol. 211, 341-350.

Duffy, S. K., Kelly, A. K., Rajauria, G., Clarke, L. C., Gath, V., Monahan, F. J., O'Doherty, J. V., 2018. The effect of 25-hydroxyvitamin D3 and phytase inclusion on pig performance, bone parameters and pork quality in finisher pigs. J. Anim. Physiol. Anim. Nutr. (Berl).

Eggum, B.O., 1970. Blood urea measurement as a technique for assessing protein quality. Br. J. Nutr. 24, 983-988.102, 1296-1305.

Ekhlm, P., Virkki, L., Ylinen, M., Johansson, L., 2003. The effect of phytic acid and some natural chelating agents on the solubility of mineral elements in oat bran. Food Chem. 80, 165-170.

Emiola, I.A., Opapeju, F.O., Slominski, B.A., Nyachoti, C.M., 2009. Growth performance and nutrient digestibility in pigs fed wheat distillers dried grains with solubles-based diets supplemented with a multicarbohydrase enzyme. J. Anim. Sci. 87, 2315-2322.

Frølich, W., 1990. Chelating properties of dietary fiber and phytate. The role for mineral availability. Adv. Exp. Med. Biol. 270, 83-93.

Gehring, C. K., Bedford, M. R., Dozier, W. A., 2013. Extra-phosphoric effects of phytase with and without xylanase in corn-soybean meal-based diets fed to broilers. Poult. Sci. 92, 979-991.

Gevaert, B., L. Veryser, F. Verbeke, E. Wynendaele, B. De Spiegeleer., 2016. Fish hydrolysates: A regulatory perspective of bioactive peptides. Protein Peptide Lett. 23, 1-9.

González-Ortiz, G., dos Santos, T. T., Vienola, K., Vartiainen, S., Apajalahti,J., Bedford., M. R., 2019. Response of broiler chickens to xylanase and butyrate supplementation. Poult. Sci. 98, 3914-3925.

Graf, D., Di Cagno, R., Fåk, F., Flint, H. J., Nyman, M., Saarela, M., Watzl. B., 2015. Contribution of diet to the composition of the human gut microbiota. Microb. Ecol. Health Dis. 26, 1-11.

Graham, H., Fadel, J. G., Newman, C. W., Newman, R. K., 1989. Effect of pelleting and β-glucanase supplementation on the ileal and fecal digestibility of a barley-based diet in the pig. J. Anim. Sci. 67, 1293-1298.

Haefner, S., Knietsch, A., Scholten, E., Braun, J., Lohscheidt, M., Zelder, O., 2005. Biotechnological production and applications of phytases. Appl. Microbiol. Biotechnol. 68, 588-597.

Hajati, H., Rezaei, M., Sayyahzade, H., 2009. The Effects of Enzyme Supplementation on Performance, Carcass Characteristics and Some Blood Parameters of Broilers Fed on Corn-Soybean Meal-Wheat Diets. Int. J. Poult. Sci. 8, 1199-1205.

Harper, A.F., Kornegay, E.T., Schell, T.C., 1997. Phytase supplementation of low-phosphorus growing-finishing pig diets improves performance, phosphorus digestibility, and bone mineralization and reduces phosphorus excretion. J. Anim. Sci. 75, 3174-3186.

He, S., Medrano, R. F., Yu, Q., Cai, Y., Dai, Q., He, J., 2017. Effect of a microbial phytase on growth performance, plasma parameters and apparent ileal amino acid digestibility in Youxian Sheldrake fed a low-phosphorus corn-soybean diet. Asian-Australasian J. Anim. Sci. 30, 1442-1449.

Himmelberg, L. V., Peo, Jr., E. R., Lewis, A. J., Crenshaw, J. D., 1985. Weaning weight response of pigs to simple and complex diets. J. Anim. Sci. 61, 18-26.

Ianiro, G., Pecere, S., Giorgio, V., Gasbarrini, A., Cammarota, G., 2016. Digestive Enzyme Supplementation in Gastrointestinal Diseases. Curr. Drug Metab. 17, 187-193.

Jensen, M. S., Jensen, S. K., Jakobsen, K., 1997. Development of digestive enzymes in pigs with emphasis on lipolytic activity in the stomach and pancreas. J. Anim. Sci. 75, 437-455.

Jongbloed, A.W., Mroz, Z., van der Weij-Jongbloed, R., Kemme, P.A., 2000. The effects of microbial phytase, organic acids and their interaction in diets for growing pigs. Livest.

Jones, C.K., Bergstrom, J.R., Tokach, M.D., DeRouchey, J.M., Goodband, R.D., Nelssen, J.L., Dritz, S.S., 2010. Efficacy of commercial enzymes in diets containing various concentrations and sources of dried distillers grains with solubles for nursery pigs. J. Anim. Sci. 88, 2084-2091.

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

Kim, J.C., Hansen, C.F., Mullana, B.P., Pluske, J.R., 2012. Nutrition and pathology of weaner pigs: nutritional strategies to support barrier function in the gastrointestinal tract. Anim. Feed Sci. Technol. 173, 3-16.

Kim, S.K., Wijesekara, I., 2010. Development and biological activities of marine-derived bioactive peptides: a review. J. Funct. Foods 2, 1-9.

Kitts, D.D., Weiler, K., 2003. Bioactive proteins and peptides from food sources, applications of bioprocesses used in isolation and recovery. Curr. Pharma. Des. 9, 1309-1323.

Kornegay, E.T., 2001. Digestion of phosphorus and other nutrients: role of phytases and factors influencing their activity, in: Bedford, M. R., Partridge, G.G. (Eds.), Enzymes in Farm Animal Nutrition. CABI Publishing, Wallingford, UK, pp. 237-272.

Kumar, V., Sinha, A. K., Makkar, H. P. S., De Boeck, G., Becker, K,. 2012. Phytate and phytase in fish nutrition. 96, 335-364.

Langhout, P., 2000. New additives for broiler chickens. World Poult. 16, 22-27.

Le Dividich, J. & Sève, B., 2000. Effects of underfeeding during the weaning period on growth, metabolism, and hormonal adjustments in the piglet. Domest. Anim. Endocrinol. 19, 63-74.

Li, S., Sauer, W. C., Mosenthin, R., Kerr., B., 1996. Effect of β-glucanase supplementation of cereal-based diets for starter pigs on the apparent digestibilities of dry matter, crude protein and energy. Anim. Feed Sci. Technol. 59, 223-231.

Lindemann, M.D., Cornelius, S.G., el Kandelgy, S.M., Moser, R.L., Pettigrew, J.E., 1986. Effect of age, weaning and diet on digestive enzyme levels in the piglet. J. Anim. Sci. 62, 1298-1307.

Liu, W. C., Kim, I. H., 2017. Metabolism and nutrition: Effects of dietary xylanase supplementation on performance and functional digestive parameters in broilers fed wheat-based diets. Poult. Sci. 96, 566-573.

López-Barrios, L., Gutiérrez-Uribe, J.A., Serna-Saldívar, S.O., 2014. Bioactive peptides and hydrolysates from pulses and their potential use as functional ingredients. J. Food Sci. 79, 273-283.

Lowrey, R.S., Pond, W.G., Barnes, R.H., Krook, L., Loosli, J.K., 1962. Influence of caloric level and protein quality on the manifestations of protein deficiency in the young pig. J. Nutr. 78, 245-253.

Mavromichalis, I., Hancock, J.D., Hines, R.H., Senne, B.W., Cao, H., 2001. Lactose, sucrose, and molasses in simple and complex diets for nursery pigs. Anim. Feed Sci. Technol. 93, 127-135.

Maenz, D.D., 2001. Enzymatic characteristics of phytases as they relate to their use in animal feeds, in: Bedford, M.R., Partridge, G.G. (Eds.), Enzymes in Farm Animal Nutrition. CABI Publishing, Wallingford, UK, pp. 61-84.

Mahan, D.C., Fastinger, N.D., Peters, J.C., 2004. Effects of diet complexity and dietary lactose levels during three starter phases on postweaning pig performance. J. Anim. Sci. 82, 2790-2797.

Mao, X., Piao, X., Lai, C., Li, D., Xing, J., Shi, B., 2005. Effects of beta-glucan obtained from the Chinese herb *Astragalus membranaceus* and lipopolysaccharide challenge on performance, immunological, adrenal, and somatotropic responses of weanling pigs. J. Anim. Sci. 83, 2775-2782.

Martínez Cuesta, S., Rahman, S. A., Furnham, N., Thornton, J. M., 2015. The Classification and Evolution of Enzyme Function. Biophys. J. 109, 1082-1086.

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

Maxwell, C. V, De Rodas, B. Z., Brock, K., 1998. Efficacy of lactose equivalent-80 (LE-80) as an alternative for Lactose in phase II nursery diets. Anim. Sci. Res. Rep. P-965. Oklahoma State Univ., Stillwater, OK. pp. 208-211.

Mule, H.R., Chiba, L.I., Fabian, J., Kuhlers, D.L., Jungst, S.B., Frobish, L.T., Nadarajah, K., Bergen, W.G., Welles, E.G., 2006. Effect of early dietary amino acid restrictions on serum metabolites in pigs selected for lean growth efficiency. Can. J. Anim. Sci. 86, 489-500.

Nørgaard, J.V., Blaabjerg, K., Poulsen, H.D., 2012. Salmon protein hydrolysate as a protein source in feed for young pigs. Anim. Feed Sci. Technol. 177, 124-129.

NRC, 2012. Nutrient Requirement of Swine. 11th rev. ed., Natl. Acad. Press, Washington, DC.

Ockenden, I., Dorsch, J.A., Reid, M.M., Lin, L., Grant, L.K., Raboy, V., Lott, J.N.A., 2004. Characterization of the storage of phosphorus, inositol phosphate and cations in grain tissues of four barley (Hordeum vulgare L.) low phytic acid genotypes. Plant Sci. 167, 1131-1142.

Olukosi, O.A., Adeola, O., 2008. Whole body nutrient accretion, growth performance and total tract nutrient retention responses of broilers to supplementation of xylanase and phytase individually or in combination in wheat-soybean meal-based diets. J. Poult. Sci. 45, 192-198.

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

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

Olukosi, O.A., Adeola, O., 2013. Enzymes and enzyme supplementation of swine diets, in: Chiba, L.I. (Eds.), Sustainable Swine Nutrition. Wiley-Blackwell, John Wiley & Sons, Inc., Hoboken, NJ. pp. 277-294.

Omogbenigun, F.O., Nyachoti, C.M., Slominski, B.A., 2003. The effect of supplementing microbial phytase and organic acids to a corn-soybean based diet fed to early-weaned pigs. J. Anim. Sci. 81, 1806-1813.

Owusu-Asiedu, A., Simmins, P. H., Brufau, J., Lizardo, R., Péron, A., 2010. Effect of xylanase and β-glucanase on growth performance and nutrient digestibility in piglets fed wheat–barley-based diets. Livest. Sci. 134, 76-78.

Rajabzadeh, M., Pourashouri, P., Shabanpour, B., Alishahi, A., 2018. Amino acid composition, antioxidant and functional properties of protein hydrolysates from the roe of rainbow trout (*Oncorhynchus mykiss*). Int. J. Food Sci. Technol. 53, 313-319.

Ravindran, V., Cabahug, S., Ravindra, G., Selle, P.H., Bryden, W.L., 2000. Response of broiler chickens to microbial phytase supplementation as influenced by dietary phytic acid and non-phytate phosphorous levels. II. Effects on apparent metabolisable energy, nutrient digestibility and nutrient retention. Br. Poult. Sci. 41, 193–200.

Ravindran, V., Selle, P.H., Ravindran, G., Morel, P.C.H., Kies, A.K., Bryden, W.L., 2001. Microbial phytase improves performance, apparent metabolizable energy, and ileal amino acid digestibility of broilers fed a lysine-deficient diet. Poult. Sci. 80, 338-344.

Ryan, J.T., Ross, R.P., Bolton, D., and Fitzgerald, G.F., Stanton, C., 2011. Bioactive peptides from muscle sources: meat and fish. Nutrients 3, 765-91.

Partridge, G.G., 2000. The role and efficacy of carbohydrase enzymes in pig nutrition, in: Bedford, M. R., Partridge, G.G. (Eds.), Enzymes in Farm Animal Nutrition. CABI, Wallingford, UK, pp. 161-198.

Pedersen, M. B., Yu, S., Arent, S., Dalsgaard, S., Bach Knudsen, K. E., Lærke, H. N., 2015. Xylanase increased the ileal digestibility of nonstarch polysaccharides and concentration of low molecular weight nondigestible carbohydrates in pigs fed high levels of wheat distillers dried grains with solubles. J. Anim. Sci. 93, 2885-2893.

Poudel P., Levesque, C.L., Samuel, R., St-Pierre, B., 2020. Dietary inclusion of Peptiva, a peptide-base feed additive, can accelerate the maturation of the fecal bacterial microbiome in weaned pigs. BMC Vet. Res. 16, 60.

Rajabzadeh, M., Pourashouri, P., Shabanpour, B., Alishahi, A., 2018. Amino acid composition, antioxidant and functional properties of protein hydrolysates from the roe of rainbow trout (Oncorhynchus mykiss). Int. J. Food Sci. Technol. 53, 313-319.

Selle, P. H., Ravindran, V., Caldwell, A., Bryden, W. L., 2000. Phytate and phytase: consequences for protein utilisation. Nutr. Res. Rev. 13, 255-278.

Shallom, D., Shoham, Y., 2003. Microbial hemicellulases. Curr. Opin. Microbiol. 6, 219-228.

Sila, A., Bougatef, A., 2016. Antioxidant peptides from marine by-products: isolation, identification and application in food systems: a review. J. Funct. Foods 21, 10-26.

Simons, P.C., Versteegh, H.A., Jongbloed, A.W., Kemme, P.A., Slump, P., Bos, K.D., Wolters, M.G., Beudeker, R.F., Verschoor, G.J., 1990. Improvement of phosphorus availability by microbial phytase in broilers and pigs. Br. J. Nutr. 64, 525-540.

Skinner, L. D., Levesque, C. L., Wey, D., Rudar, M., Zhu, J., Hooda, S., de Lange, C. F. M., 2014. Impact of nursery feeding program on subsequent growth performance, carcass quality, meat quality, and physical and chemical body composition of growing-finishing pigs. J. Anim. Sci. 92, 1044-1054.

Stoner, G. R., Allee, G.L, Nelssen, J.L., 1985. Effects of fish protein hydrolysate and dried whey in starter pig diets. Swine Day. Kansas Agric. Exp. Stn., Kansas State Univ., Manhattan, KS. pp. 67-73.

Sun, Z., Ma, Q., Li, Z., Ji, C., 2009. Effect of partial substitution of dietary spray-dried porcine plasma or fishmeal with soybean and shrimp protein hydrolysate on growth performance, nutrient digestibility and serum biochemical parameters of weanling piglets. Asian-Australas. J. Anim. Sci. 22, 1032-1037.

Thuy, N., Joseph, M., Ha, N. C., 2016. Effects of replacing marine fishmeal with graded levels of Tra Catfish by-product protein hydrolysate on the performance and meat quality of pigs. S. Afr. J. Anim. Sci. 46, 221-229.

Tran, H., Bundy, J.W., Li, Y.S., Carney-Hinkle, E.E, Miller, P.S., Burkey, T.E., 2014. Effects of spray-dried porcine plasma on growth performance, immune response, total antioxidant capacity, and gut morphology of nursery pigs. J. Anim. Sci. 92, 4494-4504.

Truong, H. H., Yu, S., Peron, A., Cadogan, D. J., Khoddami, A., Roberts, T. H., Liu, S. Y., Selle, P. H., 2014. Phytase supplementation of maize-, sorghum- and wheat-based broiler diets with identified starch pasting properties influences phytate (IP6) and sodium jejunal and ileal digestibility. Anim. Feed Sci. Technol. 198, 248-256.

Tucker, J. L., Naranjo, V.D., Bidner, T.D., Southern, L. L., 2011. Effect of salmon protein hydrolysate and spray-dried plasma protein on growth performance of weanling pigs. J. Anim. Sci. 89, 1466-1473.

Udenigwe, C.C., Aluko, R.E., 2012. Food protein-derived bioactive peptides: production, processing, and potential health benefits. J. Food Sci. 77, R11-R24.

van Beers-Schreurs, H.M., Nabuurs, M.J., Vellenga, L., Kalsbeek-van der Valk, H.J., Wensing, T., Breukink, H.J., 1998. Weaning and the weanling diet influence the villous height and crypt depth in the small intestine of pigs and alter the concentrations of short-chain fatty acids in the large intestine and blood. J. Nutr. 128, 947-953.

Vats, P., Banerjee, U.C., 2004. Production studies and catalytic properties of phytases (myo-inositolhexakisphosphate phosphohydrolases): an overview. Enzyme Microb. Technol. 35, 3-14.

Walk, C. L., Bedford, M. R., Olukosi, O. A., 2018. Effect of phytase on growth performance, phytate degradation and gene expression of myo-inositol transporters in the small intestine, liver and kidney of 21 day old broilers. Poult. Sci. 97, 1155-1162.

Walk, C. L., Bedford, M. R., Santos, T. S., Paiva, D., Bradley, J. R., Wladecki, H., Honaker, C., McElroy, A. P., 2013. Extra-phosphoric effects of superdoses of a novel microbial phytase. Poult. Sci. 92:719-725.

Walk, C. L., Santos, T. T., Bedford, M. R., 2014. Influence of superdoses of a novel microbial phytase on growth performance, tibia ash, and gizzard phytate and inositol in young broilers. Poult. Sci. 93, 1172-1177.

Wang, Y. 2017. Effect of diet complexity, multi-enzyme complexes, essential oils, and benzoic acid on weanling pigs. M.S. Thesis. Auburn University, Auburn, AL.

Wang, Y. 2018. L.I. Chiba, C. Huang, I.M. Torres, L. Wang, E.G. Welles. 2018. Effect of diet complexity, multi-enzyme complexes, essential oils, and benzoic acid on weanling pigs. Livest. Sci. 209:32-38.

Wenk, C., 2000. Recent advances in animal feed additives such as metabolic modifiers, antimicrobial agents, probiotics, enzymes and highly available minerals. Review. Asian-Austral. J. Anim. Sci. 13, 86-95.

Whang, K.Y., Easter, RA., 2000. Blood urea nitrogen as an index of feed efficiency and lean growth potential in growing-finishing swine. Asian-Australas. J. Anim. Sci. 13, 811-816.

Whang, K. Y., McKeith, F. K., Kim, S. W., Easter, R. A., 2000. Effect of starter feeding program on growth performance and gains of body components from weaning to market weight in swine. J. Anim. Sci. 78, 2885-2895.

Whiting, I. M., Rose, S. P., Mackenzie, A. M., Amerah, A. M., Pirgozliev, V. R., 2019. Effect of wheat distillers dried grains with solubles and exogenous xylanase on laying hen performance and egg quality. Poult. Sci. 98, 3756–3762.

Wolter, B.F., Ellis, M., Corrigan, B.P., DeDecker, J.M., Curtis, S.E., Parr, E.N., Webel, D.M., 2003. Impact of early postweaning growth rate as affected by diet complexity and space allocation on subsequent growth performance of pigs in a wean-to-finish production system. J. Anim. Sci. 81, 353-359.

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

Young, L.G., Leunissen, M., Atkinson, J.L., 1993. Addition of microbial phytase to diets of young pigs. J. Anim. Sci. 71, 2147-2150.

Zamora-Sillero, J., Gharsallaoui, A., Prentice, C., 2018. Peptides from fish by-product protein hydrolysates and its functional properties: An overview. Marine Biotechnol. 20, 118-130.

Zeng, Z. K., Wang, D., Piao, X. S., Li, P. F., Zhang, H. Y., Shi, C. X., Yu., S. K., 2014. Effects of adding super dose phytase to the phosphorus-deficient diets of young pigs on growth performance, bone quality, minerals and amino acids digestibilities. Asian-Australas. J. Anim. Sci. 27, 237-246.

Zhang, G.G., Yang, Z.B., Wang, Y., Yang, W.R., Zhou, H.J., 2014. Effects of dietary supplementation of multi-enzyme on growth performance, nutrient digestibility, small intestinal digestive enzyme activities, and large intestinal selected microbiota in weanling pigs. J. Anim. Sci. 92, 2063-2069.

Zhang, Y., Chen, S., Zong, X., Wang, C., Shi, C., Wang, F., Wang, Y, Lu, Z., 2020. Peptides derived from fermented soybean meal suppresses intestinal inflammation and enhances epithelial barrier function in piglets. Food Agric. Immunol. 31, 120-135.

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:

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

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 N Analysis (Roche Diagnostics, Indianapolis, 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, Indianapolis, 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:

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

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.