SUPPLEMENTATION OF VALINE, ISOLEUCINE, AND TRYPTOPHAN IN CORN-CORN FERMENTED PROTEIN BASED DIETS FOR WEANLING PIGS

BY

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THESIS

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ABSTRACT

Corn is the main source of starch used in the production of ethanol, and because there has been an extensive increase in biodiesel production, the yield of corn co-products has increased as well. As a result, new corn co-products with a greater nutritional value for animal nutrition has been developed. One of these new ingredients is the fermented corn protein (**FCP**), however, as other corn co-products, Leu concentration in FCP is almost as twice as in other protein ingredients. Exccess of Leu can reduce the growth performance of pigs by increasing the catabolism of Val and Ile, and reduce the serotonin synthesis in the brain by preventing Trp transport into the brain. Therefore, the objective of this study was to test the hypothesis that FCP may be included in diets for weanling pigs if these diet are fortified with crystalline sources of Val, Trp, and (or) Ile. Three-hundred and twenty weanling pigs [body weight (**BW**): 6.11 ± 0.66 kg] were randomly allotted to a randomized complete block design with 10 diets and 2 blocks with weaning group as the blocking factor. There were 2 gilts and 2 barrows per pen and eight replicate pens per treatment. A two-phase feeding program was used with d 1 to 14 as phase 1 and d 15 to 28 as phase 2. For each phase, a corn-soybean meal (**SBM**) diet was formulated and 2 basal diets based on corn and 10% FCP or corn and 20% FCP were used as well. Seven additional diets were formulated by adding Val, Ile, Trp, Val and Ile, Val and Trp, Ile and Trp, or Val, Ile, and Trp to the basal diet with 20% FCP. Average daily feed intake (**ADFI**), average daily gain (**ADG**), and average gain:feed ratio (**G:F**) were calculated for each phase and for the overall experiment. Fecal scores were recorded every other day in a five points scale by one single observer. Blood samples were collected on d 14 and d 28 from one pig per pen; plasma samples were analyzed for blood urea N, total protein, albumin, peptide YY, and immunoglobulin G. Ileal tissue and fecal samples were collected from one pig per pen on d 14 from the treatments fed the corn-SBM diet and from pigs fed the two basal diets containing 10 or 20% FCP. Fecal samples were analyzed for volatile fatty acids (**VFA**) and ammonium concentration, and microbial protein. Results indicated that inclusion of 10 or 20% FCP in diets reduced (P <0.05) final BW on d 28, ADG, and ADFI in phase 2 and for the entire experimental period. However, pigs fed the diet supplemented with Val, Ile, and Trp had a greater ($P < 0.05$) final BW and ADG in phase 2 and for the overall experiment than pigs fed the other FCP-containing diets. Fecal scores in phase 2 were reduced ($P < 0.05$) if FCP was used instead of SBM. On d 28, pigs fed the diet with 20% FCP and only Val, Val and Trp, or Val, Trp, and Ile had reduced ($P < 0.01$) blood urea N compared with pigs fed the corn-SBM diet or the other FCP-based diets. The values obtained for peptide YY ranged between 1.72 to 1.93 ng/mL and between 2.72 to 3.16 ng/mL for d 14 and d 28, respectively. The values obtained for immunoglobulin G ranged between 3.05 to 5.35 mg/mL and 3.91 to 6.59 mg/mL for d 14 and d 28, respectively. There were no effects of dietary treatments on peptide YY, immunoglobulin G, ileum morphology, VFA in feces, ammonium concentration, or microbial protein. In conclusion, FCP may be included up to 20% diets for weanling pigs with added Val, Ile, and Trp without having any negative effect on growth performance, gut health, or hindgut fermentation rate.

Key words: branched-chain amino acids, growth performance, high protein corn co-product, leucine, tryptophan, weanling pigs

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CHAPTER 1: INTRODUCTION

Ethanol is the most important biofuel worldwide with United States and Brazil as the major ethanol producers, accounting for 84% of the total production in the world (Duque et al., 2021). Ethanol is produced by microbial fermentation of carbohydrate compounds of plants or algae such as cereal grains, food and beverage waste, and cellulosic biomass (Parsons, 2021; Tse et al., 2021). In the U.S., corn is the main crop used to produce more than 90% of the annual ethanol production due to the high starch content of corn (Li et al., 2022). The rapid expansion of the ethanol industry in the last 40 years is attributed to the renewable fuel standard program (Newes et al., 2021).

The U.S. ethanol industry is the largest producer of corn co-products, with an annual production of approximately 38 million metric tons (U.S. Grains Council, 2018). Corn distillers dried grains with solubles (**DDGS**) is the primary co-product from the ethanol industry, but ethanol plants are becoming bioreneries to enhance ethanol yield and to create other corn co-products with potentially higher value for livestock feeding (U.S. Grains Council, 2018). Indeed, the use of emerging technologies allow companies to produce a co-product with around 50% of crude protein. This product has a greater digestibility of amino acids than DDGS and is known as fermented corn protein (**FCP**; Acosta et al., 2021).

Substantial reductions in feed costs have been achieved when using corn co-products in diets for swine (Shurson, 2018). However, if corn co-products are included in the diets, several factors need to be considered to maintain the growth performance of pigs. The high fiber content of DDGS and other corn co-products may increase the Thr requirement because of increased endogenous losses of digestive enzymes, enterocytes, and mucin (Dilger et al., 2004; Mathai et al., 2016), and endogenous protein has a greater concentration of Thr than any other amino acid

(Stein et al., 1999). Therefore, pigs fed high fiber diets will have a greater SID Thr:Lys than pigs fed a low fiber diet (Mathai et al., 2016).

Corn co-products also contain high amounts of Leu when compared with other protein sources, and excess Leu can increase the catabolism of the other two branched-chain amino acids (i.e., Val and Ile). As a result, there will be a reduced availability of Val and Ile for protein synthesis (Harris et al., 2004). In addition, excess Leu impairs uptake of Trp in the brain and thereby reduce the amount of Trp available for serotonin synthesis, which is a neurotransmitter that regulates feed intake (Wessels et al., 2016). Therefore, feeding diets containing high amounts of corn co-products may result in reduced growth performance if these challenges are not taken into account when formulating the diets. As a consequence, research is needed to identify feeding practices that can overcome the limitations of using corn co-products in diets for swine.

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CHAPTER 2: FERMENTED CORN PROTEIN IN DIETS FOR WEANLING PIGS: REVIEW OF LITERATURE

Introduction

Corn and soybean meal (**SBM**) are the two most important ingredients in diets for pigs in many countries. Diets based on corn and SBM are widely used and corn and SBM are currently the predominant ingredient combination in diets for pigs in the Americas, Asia, and some European countries (Garavito, 2022).

Corn and soybeans contain essential nutrients such as carbohydrates, proteins, and fats. However, ingredients may be commercially fractionated into their components to produce human food and animal feed (Zijlstra et al., 2004). In the last decades, corn and soybeans have also been used to produce biofuels (Zijlstra and Beltranena, 2007).

Biodiesel or ethanol derived from corn crops are considered sustainable, eco-friendly, and bioeconomic, and has the potential to decrease reliance on non-renewable motor fuels, leading to various environmental advantages, such as diminished emissions during the combustion process. (Zijlstra and Beltranena, 2013; Sekhon et al., 2015; Garraín et al., 2016; Cherwoo et al., 2023). However, because it is only the starch in the corn kernel and the oil in the soybeans that are used for biofuels, some parts of the ingredients are left for other uses, including incorporation in diets for pigs. Incorporating co-products in pig diets may be challenging because commercial pig breeds are sensitive to fluctuations in diet quality, protein levels, and energy density (Burton et al., 2013).

Due to the considerable expansion in ethanol production during the last four decades, there is a significantly heightened demand for fresh revenue sources for co-products derived from ethanol (Parsons, 2021). The primary co-product from ethanol production is the corn distillers dried grains with solubles (**DDGS**), and it has been used traditionally in the cattle industry due to its high fiber content and variable quality (Cromwell et al., 1993). However, the U.S. fuel ethanol production has been increasing during the last decades because of the fuel blending requirements of the Renewable Fuel Standard program (U.S. Energy Information Administration, 2022). The overall capacity of ethanol production in the U.S. increased from 13.6 billion gallons per year in 2011 to 17.5 billion gallons per year in 2021 (U.S. Energy Information Administration, 2022), and 95% of the total ethanol produced was corn-derived ethanol (Kurambhatti et al., 2018), increasing the production of DDGS by 318% from around 9 million MT in 2005/06 to 38.5 million MT in 2017/18 (USDA, 2019). This resulted in a greater availability of DDGS, leading to a growing interest in including DDGS in diets for poultry and pigs (Parsons, 2021). While incorporating DDGS into diets for swine may reduce feed cost due to its lower price than other ingredients (Donohue and Cunningham, 2009; Buenavista et al., 2021), there is hesitancy among nutritionists to include high levels of DDGS due to its elevated fiber content and variable quality because nutritional value of DDGS is highly affected by the fermentation efficiency, process design, and operation practice of ethanol production among different suppliers (Buenavista et al., 2021).

Corn and corn co-products

Corn, also known as maize, is a cereal grain used for food and feed, and is one of the most important crops in the United States. In 2022, global production of corn was 1,156 million metric tons with the United States, China, and Brazil being the top corn producing countries with 30, 24, and 12% of total production, respectively (USDA, 2023). Corn is the predominant feed grain in the United States, constituting more than 95% of total feed grain production and utilization (USDA, 2023). In addition to food and feed, corn is also used to produce ethanol and other manufactured goods (Statista, 2023). Corn also accounts for around 80 percent of the total volume of traded feed grain in the past decade (USDA, 2023). Corn is the major energy source in diets for pigs (Lammers et al., 2007), and due to its high content of starch and oil, and low fiber concentration, corn has a greater energy value than most other cereal grains (Menegat et al., 2019).

The apparent total tract digestibility of dry matter in corn is approximately 90% whereas the apparent total tract digestibility of starch is between 85 and 98% (McGhee and Stein, 2020). Starch digestibility may increase by reducing the particle size or by extruction (Rojas and Stein, 2015; Rodriguez et al., 2020). The crude protein (**CP**) is between 7 and 9% and Lys concentration is 0.25%, which is lower than in the majority of other cereal grains. However, the SID of most indispensable amino acids (**AA**) in corn is between 75 and 85% (Cervantes-Pahm et al., 2014; McGhee and Stein, 2018), being slightly greater than in barley and sorghum and comparable to wheat (Pedersen et al., 2007a; 2007b). The total dietary fiber is below 10% and primarily composed by arabinoxylans and cellulose (Jaworski et al., 2015).

Corn contains 0.26% P, but standardized total tract digestibility of P is only 25 to 42.5% because at least 2/3 of the P is bound to phytate (Almeida and Stein, 2010; 2012; Stein et al., 2016). However, the addition of microbial phytase to corn-based diets results in an elevated phosphorus digestibility ranging between 45% and 60% (Almeida and Stein, 2010; 2012; Stein et al., 2016).

Diets for livestock accounts for approximately 40% of the overall domestic corn consumption in the U.S. (USDA, 2023). The remaining 60% is used for food, seed, and industrial uses including production of ethanol. For most of these uses, corn is further processed to obtain the final product. In addition to the primary products produced, many co-products are also generated. During processing, corn is usually subjected to either dry or wet milling, or dry grinding depending on the desired final product. Wet milling involves the introduction of corn into a liquid medium, and therefore, is a more complicated process than dry milling. Nevertheless, wet milling is used to separate the corn kernel into smaller parts, enabling the creation of a number of end products including high fructose corn syrup, glucose, dextrose, starch, corn oil, beverage alcohol, industrial alcohol, and fuel ethanol (USDA, 2023). However, a number of co-products from the wet milling industry are also produced including maize gluten meal, high fat maize germ, maize germ meal, maize bran, maize wet distiller grains, DDGS, and maize gluten feed (USDA, 2023). Dry milling does not include any liquification of corn and is a less intensive process than wet milling (U.S. Grain Council, 2018). Dry millers process corn into flakes for cereals, corn flour, corn grits, corn meal, and brewers grits for beer production. The major co-product from dry milling is hominy feed (USDA, 2023). Dry grinding of corn is currently the main source of ethanol production in the United States (Parsons, 2021). Co-products from dry grind plants include CO_2, crude corn oil, and DDGS or distillers grains without solubles (**DDG**; NCR, 2012; U.S. Grains Council, 2018).

Fermented corn protein

Whereas the traditional dry grinding method in the ethanol industry results in the production of DDGS, which contains approximately 27% CP, several variations in the process has been introduced, which results in production of different co-products. Isolation of the protein fraction and the low-fiber fraction fermentation of DDGS results in generation of a co-product known as high protein dried distillers grains (**HPDDGS**; Cristobal et al., 2020; Acosta et al., 2021; Cemin et al., 2021), which contains 38 to 48% CP (Widmer et al., 2007; Kim et al., 2009; Rho et al., 2017; Espinosa and Stein, 2018; Yang et al., 2019; Cristobal et al., 2020; Cemin et al., 2021; Rao et al., 2021). Production of HPDDGS differs from traditional DDGS in that the corn grain is dehulled and degermed prior to the fermentation and distilling process to increase the concentration of starch by reducing unfermentable components such as fiber and fat and thereby enhance the efficiency of ethanol production (NRC, 2012; Clizer, 2021).

However, new technologies facilitate the extraction of fiber before or after fermentation and removal of variable amounts of oil which concentrates the protein and yeast in the co-product. This development has resulted in a novel co-product containing approximately 50% CP, which is called corn fermented protein (**CFP**) or high protein fermented corn protein. Fermented corn protein has a different nutritional value than conventional DDGS with reduced oil (~27% CP), HP-DDGS (38-48% CP), or corn protein concentrate (> 67% CP; U.S. Grains Council, 2023). One unique characteristic of FCP is the yeast content is approximately 20 to 29% (U.S. Grains Council, 2023). This proportion is greater than the estimated 7 to 10% of spent yeast in conventional DDGS (Shurson, 2018; U.S. Grains Council, 2023). The yeast contribution towards DDGS total protein is approximately 20% (Hand and Liu, 2010). Having a high amount of residual yeast in FCP has no benefit other than increasing protein concentration, because the spent yeast does not have the properties of direct fed microbials or probiotics in diets for pigs (U.S. Grains Council, 2023). Likewise, the yeast cell wall components (i.e., mannan oligosaccharides, β-glucans, and nucleotides) do not represent any added value due to the inconsistency of results regarding their benefits on growth performance or health of pigs (Shurson, 2018; U.S. Grains Council, 2023).

There are three providers of the technologies to produce FCP: ICM, Inc. (Colwich, KS), Fluid Quip Technologies (Cedar Rapids, IA), and Marquis Energy (Hennepin, IL), and there are two FCP categories (U.S. Grains Council, 2023). The FCP mechanically separated category where no additive is used, and the FCP without mechanical separation, where a flocculant or polymer is used (U.S. Grains Council, 2023). Most of the FCP in the market are mechanically separated during the production process.

Nutritional value

Because there are different technologies to concentrate the protein and yeast in FCP, there are differences in the reported values for CP (> 50%) and AA (Cristobal et al., 2020; Acosta et al., 2021; Clizer, 2021; Yang et al., 2021; Garavito, 2022; Stas et al., 2022; U.S. Grains Council, 2023). However, all of these values are greater than values reported for CP and AA in HPDDG or HPDDGS (Widmer et al., 2007, Kim et al., 2009; Rho et al., 2017; Espinosa and Stein, 2018; Yang et al., 2019; Cemin et al., 202l; Rao et al., 2021). The recovery of yeast and corn gluten protein from the stillage after fermentation results in a greater CP in FCP than in HPDDGS (Acosta et al., 2021).

In contrast to CP, there is greater variability among sources for ether extract (crude fat) and acid hydrolyzed ether extract (**AEE**), neutral detergent fiber (**NDF**), total dietary fiber (**TDF**), and ash (U.S. Grains Council, 2023). The reported concentration of gross energy in FCP (Cristobal et al., 2020; Acosta et al., 2021; Yang et al., 2021) is greater than in conventional DDGS (Yang et al., 2021), but comparable to HPDDG or HPDDGS (Widmer et al., 2007; Kim et a., 2009; Espinosa and Stein, 2018; Rao et al., 2021). Total dietary fiber and NDF are lower in FCP than in conventional DDGS because there is a mechanical separation of fiber from the protein during production (Acosta et al., 2021; Yang et al., 2021). Ether extract varies from

3.48% to 13.7% (Yang et al., 2021; Garavito, 2022) and AEE is between 5.60% and 9.49% (Cristobal et al., 2020; Acosta et al., 2021). However, values between 2.50% and 3.00% have also been reported (Clizer, 2021).

The concentration of Ca in FCP is low as in other corn co-products, ranging from 0.01 to 0.10% and the concentration of P ranges from 0.68 to 1.04% (Cristobal et al., 2020; Acosta et al., 2021; Yang et al., 2021).

The FCP contains more Lys than HPDDGS (~2.17 vs. 1.22%; NRC, 2012; Cristobal et al., 2020; Acosta et al., 2021). The greater Lys-to-CP ratio in FCP is likely a result of the presence of yeast because yeast contains 46.5% CP and 2.5% Lys (Shurson, 2018). Likewise, the concentration of Met, Trp, Val, and Ile in FCP is greater than in HPDDGS (Cristobal et al., 2020; Acosta et al., 2021; Yang et al., 2021). The concentration of Leu in FCP and HPDDGS is not different, but FCP contains twice as much Leu as conventional DDGS (NRC, 2012; U.S. Grains Council, 2023). As a result, Leu in FCP is two to four times greater than Lys (Yang et al., 2021).

The digestible energy ranges from 3,837 to 4,560 kcal/kg, and the metabolizable energy ranges from 3,643 to 4,306 kcal/kg (Cristobal et al., 2020; Acosta et al., 2021; Yang et al., 2021). The variability is likely a consequence of the variability in AEE. These values are greater than the DE and ME in some sources of HPDDGS (Espinosa and Stein et al., 2018; Yang et al., 2021), but DE and ME for HPDDG or HPDDGS that are closer to FCP also have been reported (Widmer et al., 2009; Rho et al., 2017).

The apparent ileal digestibility and standardized ileal digestibility of AA in FCP varies from 61 to 90% (Cristobal et al., 2020; Acosta et al., 2021, Yang et al., 2021). These values are in agreement with values for HPDDGS (Adeola and Ragland, 2016; Rho et al., 2017; Espinosa and Stein, 2018).

Amino acids

Amino acids are the building block of proteins and serve as a precursor for other nitrogenous compounds (Blanco and Blanco, 2022). These biomolecules contain an amine (–NH_2) and a carboxylic acid (–COOH), that are attached to a carbon atom, known as the α carbon (Blanco and Blanco, 2022). Each AA has a specific functional side-chain, called the R group (Akram et al., 2011; Moreira et al., 2015). Although there are more than 500 AA in nature, only 20 AA can be used for protein synthesis (Wu, 2009). Pigs cannot synthesize 9 of the AA required for protein synthesis, or not in adequate quantity, thus, they are classified as indispensable AA, and must be provided in the diet (Table 2.1; Rezaei et al., 2013). There are also some AA that have limitations regarding the rate to which they can be synthesized, these AA are termed conditionally indispensable AA (Reeds, 2000). As an example, young pigs have limited capacity to synthetize Pro from Glu in the small intestine, and therefore, Pro is indispensable for young pigs, whereas older pigs easily can synthesize sufficient Pro to maximize protein synthesis (Kang et al., 2014). All other AA needed in protein synthesis are classified as dispensable AA, and can be synthesized by the animals provided the necessary precursors are available. These AA, therefore, do not need to be included in the diets (Lopez and Mohiuddin, 2023).

Branched-chain amino acids

The branched-chain AA (**BCAA**) Val, Ile, and Leu, are nutritionally indispensable AA for pigs. The BCAA are used not only for protein synthesis, but they are important for the immune system and energy homeostasis as well (Habibi et al., 2022), because BCAA are important for synthesis of ketone bodies and glucose (Bifari and Nisoli, 2017). The BCAA have an exclusive feature in that they are not primarily de-aminated in the liver, but in skeletal muscle

(Platell et al., 2000), and they belong to the same family of AA because the side chain is branched, small, and hydrophobic (Neinast et al., 2019). As a consequence, all three BCAA share the first two steps of their catabolism (Bifari and Nisoli, 2017).

Valine. Valine is an indispensable AA for protein synthesis and optimum growth. Valine may be the next limiting AA after Lys, Met, Trp, and Thr in a conventional corn and SBM based diet for pigs (Figueroa et al., 2003). However, Val is the second limiting AA in a corn-SBM diet for lactating sows because Val is the most efficiently used AA by the mammary gland for milk synthesis (Holen et al., 2022).

A deficiency of Val will decrease average daily feed intake (**ADFI**), and as a consequence, growth performance of pigs will be reduced (Soumeh et al., 2015). The reduced ADFI by pigs fed a Val deficient diet may be a mechanism to avoid anorexia due to the unbalanced diet (Gloaguen et al., 2012). However, more research is needed to elucidate the mechanism of how Val influences ADFI. The negative effects caused by Val deficiency may be reduced if Ile and Leu are deficient as well, suggesting that the impaired growth performance is more related to a BCAA imbalance rather than to Val deficiency (Gaines et al., 2011). The lack of a negative effect on growth performance of excess Val indicates that Val is not adversely impacting the metabolism of the other BCAA (Soumeh et al., 2015).

Isoleucine. The BCAA are involved in glucose metabolism and uptake. In a previous oral glucose tolerance test, administration of Ile had a greater impact on reducing plasma glucose levels than Leu. Likewise, Ile had a greater stimulation on insulin-independent glucose uptake than Leu in C2C12 myotubes using phosphatidylinositol 3-kinase and protein kinase C, but not Mammalian target of rapamycin pathways (Doi et al., 2003). Theses observations were confirmed in rats, where Ile stimulated glucose uptake in the skeletal muscle and reduced plasma glucose without any effect on plasma insulin (Doi et al., 2005).

Isoleucine up-regulated the protein expression of GLUT1 in red muscle, GLUT4 in red, white, and intermediate muscle, and SGLT-1 and GLUT2 in the small intestine, enhancing glucose uptake of piglets fed a Ile supplemented diet (Zhang et al., 2016). However, the mechanism of how Ile translocate those transporters remain unclear (Zhang et al., 2017).

Leucine. Leucine stimulates directly mammalian target of rapamycin, which is involved in protein synthesis not only in the skeletal muscle, but in other tissues such as adipose tissue (Li et al., 2011), and the protein synthesis by Leu is dependent on availability of other AA (Wilson et al., 2010). The mammalian target of rapamycin signaling is important for detection of nutrient availability and regulates energy balance (Zhang et al., 2017). An excess of Leu will cause an overstimulation of mammalian target of rapamycin leading to a decreased feed intake of rats as elevated levels of leptin does as well (Cota et al., 2006). Leucine enhances glucose uptake in the muscle by increasing translocation of GLUT1 and GLUT4 by up-regulating insulin levels (Zhang et al., 2017). Also Leu enhance glucose recycling through the glucose–alanine cycle to ensure glucose homeostasis (Li et al., 2011).

Leucine regulates the immune system mainly via the mammalian target of rapamycin signaling pathway, modulating innate and adaptive immune responses; promoting differentiation, activation, and function in T cells, B cells and antigen-presenting cells (Zhang et al., 2017). Likewise, Leu decrease expression of pro-inflammatory cytokines such a TNF-α and IL-1β, and increase TGF-β and IL-10 mRNA expression, which are anti-inflammatory cytokines (Zhou et al., 2020).

Metabolism of BCAA. Metabolism of the BCAA starts with deamination in skeletal muscle because the majority of the branched chain aminotransferase (**BCAT**) enzyme is located in muscle and not in liver. The BCAT enzyme is shared by all three BCAA and its reaction results in synthesis of three separate branched chain keto acids (**BCKA**) that are transported to the liver for further metabolism. The second step of BCAA metabolism involves oxidative decarboxylation of the three BCKA, which is catalyzed by the branched-chain α-keto acid dehydrogenase complex (**BCKDH**; Mann et al., 2021), resulting in three coa-acids, that are metabolized following distinct pathways (Holecek, 2018). Leucine is crucial to control of the second step in BCAA metabolism because the BCKA that is generated by deamination of Leu, alpha-ketoisocaproate (**KIC**), triggers activation of the BCKDH complex in the liver (Harper et al. 1984). When BCKDH is activated, metabolism of not only KIC, but also the other BCKA, will be initiated (Holecek, 2018). Therefore, feeding diets containing excessive Leu results in increased metabolism of all three BCAA, which may induce a deficiency of Val and Ile (Kwon et al., 2019). In contrast, there is no evidence that Leu reduces digestibility of Ile and Val.

Tryptophan. Tryptophan is an indispensable acid that needs to be supplied in the diet (Le Floc'h and Seve, 2007). Swine diets primarily composed of corn and soybean meal, usually have Trp as the second or third limiting AA (Burgoon et al., 1992). Tryptophan is involved in multiple biological roles, including protein synthesis, synthesis of the neuromediator serotonin, and activation of the immune response through the kynurenine pathway (Sainio et al., 1996; Le Floc'h and Seve, 2007).

Tryptophan is the only precursor of serotonin, and its conversion takes place in the hypothalamus in the brain (Richard et al., 2009). However, Trp and Leu share the same transporter into the brain and if there is excess Leu in the diet, Trp entry into the brain is reduced because Leu will occupy the transporter needed for Trp transport into the brain (Kwon et al., 2022). Thus, diets high in Leu affect the availability of Trp for synthesis of serotonin (Wessels et al., 2016), and there is reduced synthesis of serotonin in the brain if Trp entry into the brain is limited by excess Leu (Kwon et al., 2022). Serotonin plays a key role in controlling feed consumption, and as consequence, excess dietary Leu results in reduced feed intake (Kwon et al., 2022).

Challenges using fermented corn protein in diets for pigs

The concentration of CP and AA in FCP makes this co-product an attractive ingredient to be included in diets for swine. However, the concentration of Leu in FCP is around twice the concentration in other protein sources, such as SBM (NRC, 2012; Acosta et al., 2021). Therefore, it was concluded that using FCP in diets for pigs will result in excess dietary Leu in the diets (Kwon et al., 2024).

Fermented corn protein may be included in diets for weanling pigs by up to 14% as a partial replacement of SBM or other protein sources (Garavito, 2022). However, after phase 2, growth performance of pigs may be negatively affected if more than 15% of FCP is used (Garavito, 2022). These observations are in agreement with Acosta et al. (2021), who reported that growth performance or fecal score did not differ among treatments if FCP was included by up to 10% in diets for weanling pigs as partial replacement for enzyme-treated SBM or plasma protein. However, inclusion of 10% FCP diets for weanling pigs decreased average daily gain (**ADG**) and gain to feed ratio (**G:F**) compared with enzyme-treated SBM (Stas et al., 2022). These results indicate that FCP may not be used as a complete replacement for other protein ingredients. Increasing the Ile: Leu and Val:Leu ratios in diets containing FCP improved G:F ratio, but had no effect on final body weight, ADG, or ADFI (Stas et al., 2022). These observations indicate that FCP may be used in diets for weanling pigs at a low inclusion rate, however, supplementation with added AA may be required if FCP is used at greater inclusion rates. Results of a meta-analysis indicated that increasing the concentration of Val, Ile, or Trp may overcome the negative effects of an imbalanced BCAA diet due to excess dietary Leu (Cemin et al. 2019). Likewise, adding extra Val and Trp to diets containing FCP may fully or partially prevent negative effects on growth performance (Kwon et al. 2024). However, adding Ile does not appear to be as helpful as adding Val and Trp (Kwon et al. 2024). In contrast, late-finishing pigs fed a diet with added Ile overcame the negative effects of excess Leu (Kerkaert et al., 2021), thus adding Ile may be helpful depending on the levels of Leu and the level of supplementation of Ile in the diet.

Conclusion

Corn is the most used crop to obtain biofuels, and the demand for ethanol has increased in the last three decades, causing an increase in the availability of corn co-products derived from the dry grinding ethanol industry. To increase the production efficiency of ethanol, new technologies have been developed to increase the concentration of protein fraction after distillation, resulting in a novel co-product known as FCP. This new ingredient contains around 50% CP, and Lys is similar or greater than in HPDDGS. However, FCP contains twice as much Leu as other protein sources, such as SBM. Therefore, including FCP in diets for swine may result in a BCAA imbalance, causing an increased catabolism of Val and Ile, that will affect growth performance by reducing ADFI and ADG. However, due to the negative impact of excess Leu on the metabolism of Trp, Val, and Ile, it is possible that the use of FCP requires synthetic AA supplementation to ameliorate the negative effects of excess Leu in the diet. It is possible that with an appropriate supplementation of Val, Ile, and Trp, the increased catabolism of Val and Ile, and the impaired availability of Trp for serotonin synthesis, may be counteracted and as a consequence, FCP may be used as a protein source in diets for pigs without negative effects on growth performance.

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Tables and Figures

Table 2.1. Nutritional composition of corn and corn co-products[1,2]

Item	Corn	Wet milling co-products					Dry milling co-products	Dry grinding
		Corn germ meal	Corn gluten meal	Corn gluten feed	High fat corn germ	Corn bran	Hominy feed	Distillers dried grains with solubles
Gross energy, kcal/kg	3, 924	4,230	5,102	4,324	5, 929	4,368	4,407	4,769
Dry matter, %	86.74	88.24	91.03	85.87	93.59	89.94	87.62	87.55
Crude protein, %	6.68	23.70	62.88	23.00	17.00	9.85	8.71	25.43
AEE, %	3.40	3.12	4.29	4.15	30.60	3.10	9.47	10.36
Starch, %	67.29	18.63	6.68	9.77	11.90	16.20	59.52	4.56
NDF, %	8.53	37.37	10.45	30.88	40.90	61.00	21.79	35.20
ADF, %	2.00	14.31	5.23	7.68	18.20	16.70	5.51	10.02
SDF[4] %	7.86	33.41						
IDF, %	0.41	2.48						
TDF, %	8.27	35.89	5.80	35.40	-	24.2	11.8	29.8
Calcium, %	0.01	0.14	0.01	0.11	0.03	0.07	0.01	0.22

Table 2.1. (cont.)

Phosphorus, %	0.19	2.04	0.60	0.90	0.70	0.14	0.71	0.82
Indispensable AA %								
Arg	0.33	1.15	2.26	0.95	1.26	0.40	0.47	1.13
His	0.19	0.76	1.31	0.61	0.49	0.34	0.24	0.67
Ile	0.23	0.79	2.60	0.79	0.63	0.34	0.30	0.92
Leu	0.76	1.58	10.09	1.86	1.30	1.13	0.91	2.75
Lys	0.22	1.13	1.18	1.02	0.73	0.30	0.33	0.75
Met	0.14	0.53	1.61	0.32	0.31	0.16	0.15	0.48
Phe	0.31	0.87	4.03	0.87	0.77	0.46	0.41	1.24
Thr	0.24	0.84	2.03	1.21	0.63	0.37	0.30	0.97
Trp	0.04	0.32	0.44	0.16	0.12	0.07	0.06	0.19
Val	0.32	1.16	2.89	1.12	1.04	0.48	0.42	1.33
Total			28.44	8.91	7.28	4.05	3.59	10.43
Dispensable AA, %								
Ala	0.47	1.34	5.30	1.48	1.04	0.64	0.60	1.62

Table 2.1. (cont.)

Asp	0.44	1.59	3.85	1.44	1.25	0.55	0.60	1.60
Cys	0.15	0.34	1.14	0.43	0.21	0.23	0.17	0.50
Glu	1.13	2.45	12.06	2.70	2.10	1.64	1.35	3.04
Gly	0.27	1.57	1.84	1.03	0.96	0.40	0.37	0.99
Pro	0.31	1.4	5.68	1.61	0.76	0.97	0.61	1.75
Ser	0.30	0.93	2.54	0.73	0.67	0.37	0.35	1.07
Tyr	0.21	0.68	3.27	0.64	0.49	0.33	0.27	0.91
Total			35.68	10.06	7.48	5.13	4.32	11.48

[1]AA, amino acids; AEE, acid hydrolyzed ether extract; NDF, neutral detergent fiber; ADF, acid detergent fiber; SDF, soluble dietary fiber; IDF, insoluble dietary fiber; TDF, total dietary fiber.

[2]Adapted from Rojas et al., 2013; Liu et al., 2014; Stein et al., 2016.

Table 2.2. Concentration of digestible and metabolizable energy and apparent total tract digestibility (ATTD) of energy and standardized total tract digestibility (STTD) of P in corn and corn co-products[1,2]

Item	Corn	Wet milling co-products					Dry milling co-products	Dry grinding co-products
		Corn germ meal	Corn gluten meal	Corn gluten feed	High fat corn germ	Corn bran	Hominy feed	Distillers dried grains with solubles
ATTD of GE, %	89.4	73.9	92.5	70.6	72.67	79.20	78.7	72.9
DE, kcal/kg	3,498	3,073	4,896	3,051	3,397	2,881	3,399	3,556
ME, kcal/kg	3,375	2,817	4,006	2,721	3,123	2,768	3,271	3,235
STTD of P without phytase, %	42.5	53.2	75.2	84.6	-	-	37.7	76.5
STTD of P with phytase, %	64.1	68.3	87.4	87.1	-	-	60.1	82.8

[1]ATTD, apparent total tract digestibility; GE, gross energy; DE, digestible energy; ME, metabolizable energy; STTD, standardized total tract digestibility.

[2]Adapted from Rojas et al., 2013; Liu et al., 2014.

Table 2.3. Standardized ileal digestibility (%) of crude protein (CP) and amino acids (AA) in corn and corn co-products[1]

Item	Corn	Wet milling co-products					Dry milling co-products	Dry grinding co-products
		Corn germ meal	Corn gluten meal	Corn gluten feed	High fat corn germ	Corn bran	Hominy feed	Distillers dried grains with solubles
CP, %	83.4	58.5	85.5	70.9	56.5	35.4	74.8	70.5
Indispensable AA %								
Arg	91.6	89.0	93.7	90.4	81.3	70.4	96.3	81.3
His	90.7	77.0	82.8	75.9	74.6	73.4	79.8	75.5
Ile	88.0	77.0	86.4	76.6	66.7	60.6	70.8	74.7
Leu	92.3	80.0	91.3	82.2	70.9	77.5	83.8	84.4
Lys	72.7	65.0	78.7	68.8	58.4	0.1	58.8	46.0
Met	91.7	81.0	90.6	78.8	67.7	73.5	77.0	81.6
Phe	90.2	81.0	89.4	81.2	70.6	68.5	79.2	81.1
Thr	87.0	69.0	83.5	75.2	64.3	48.0	65.8	69.5

Table 2.3. (cont.)

Trp	88.0	83.0	91.8	87.5	77.5	71.2	82.4	82.6
Val	87.6	76.0	84.9	74.9	66.5	55.2	71.7	73.9
Mean	88.2		88.6	78.7	69.8	58.9	78.0	76.7
Dispensable AA %								
Ala	88.6	75.0	87.8	77.9	63.3	47.5	79.3	77.8
Asp	87.4	65.0	83.2	66.4	59.4	45.2	68.8	68.1
Cys	87.6	61.0	81.0	65.2	57.5	63.8	76.1	73.4
Glu	91.3	78.0	87.6	77.1	72.7	73.6	82.9	81.8
Gly	83.2	60.0	66.5	76.2	64.9	-23.6	97.1	64.8
Pro	87.8	62.0	97.2	130.9	17.6	-19.5	190.2	86.4
Ser	89.5	73.0	89.7	77.1	69.6	59.0	82.4	76.9
Tyr	90.2	79.0	89.6	81.0	69.3	66.9	77.8	81.1
Mean	87.6		83.6	83.5	59.3	38.5	95.6	77.8

[1]Adapted from Almeida et al., 2011; Liu et al., 2014.

Table 2.4. Nutritional composition of high protein corn co-products[1,2]

Item	Fermented corn protein	High protein - distillers dried grains with solubles	High protein yeast-based distillers dried grains with solubles
Gross energy, kcal/kg	4,937		
Dry matter, %	93.00	91.20	91.52
Crude protein, %	50.10	45.30	50.00
AEE, %	5.60	3.50	3.66
Starch, %	-	10.15	5.19
Crude fiber, %	-	-	4.05
ADF, %		20.6	-
NDF, %		33.6	4.41
SDF[4] %	3.4	-	-
IDF, %	24.4	-	-
TDF, %	27.8	-	-
Calcium, %	-	0.02	-
Phosphorus, %	-	0.36	-
Indispensable AA%			

Table 2.4. (cont.)

Arg	2.31	1.62	2.80
His	1.33	1.07	1.42
Ile	2.19	1.83	2.32
Leu	5.68	6.18	5.24
Lys	1.98	1.22	2.22
Met	1.01	0.93	1.10
Phe	2.49	2.42	2.72
Thr	2.00	1.59	2.04
Trp	0.42	0.24	0.54
Val	2.83	2.12	0.99
Dispensable AA, %			
Ala	3.47	3.32	3.32
Asp	3.55	2.75	3.70
Cys	0.87	0.82	0.94
Glu	7.39	7.52	6.98
Gly	2.01	1.39	2.17

Table 2.4. (cont.)

Pro	3.50	3.65	3.29
Ser	2.17	1.96	2.08
Tyr	1.98	1.92	2.22

[1]AA, amino acids; EE, acid hydrolyzed ether extract; NDF, neutral detergent fiber; ADF, acid detergent fiber; SDF, soluble dietary fiber; IDF, insoluble dietary fiber; TDF, total dietary fiber.

[2]Adapted from Cristobal et al., 2020; Acosta et al., 2021; Garavito, 2022.

Table 2.5. Indispensable, condititionally indispensable, and dispensable amino acids for pigs[1]

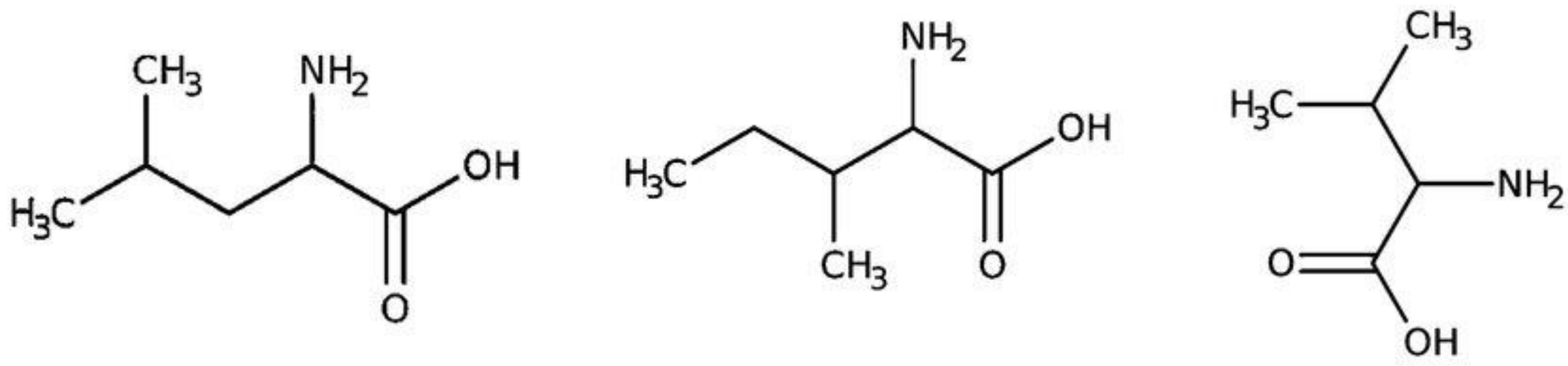
Indispensable	Conditionally Indispensable	Dispensable
His	Arg	Ala
Ile	Glu	Asp
Leu	Pro	Asn
Lys	Tyr	Gln
Met	Cys	Gly
Phe		Ser
Thr		
Trp		
Val		

[1] From NRC. 2012.

Primary products		
Wet milling	Dry milling	Dry grinding
High fructose corn syrup Starch Corn oil	Corn grits	Ethanol

Co-products		
Wet milling	Dry milling	Dry grinding
Corn gluten meal High fat corn germ Corn bran Corn WDG	Hominy feed	Corn distillers dried grains with solubles

Figure 2.1. Corn processing end products and co-products.



Leucine **Isoleucine** **Valine**

Figure 2.2. Chemical structures of branched-chain amino acids (Kim et al., 2022).

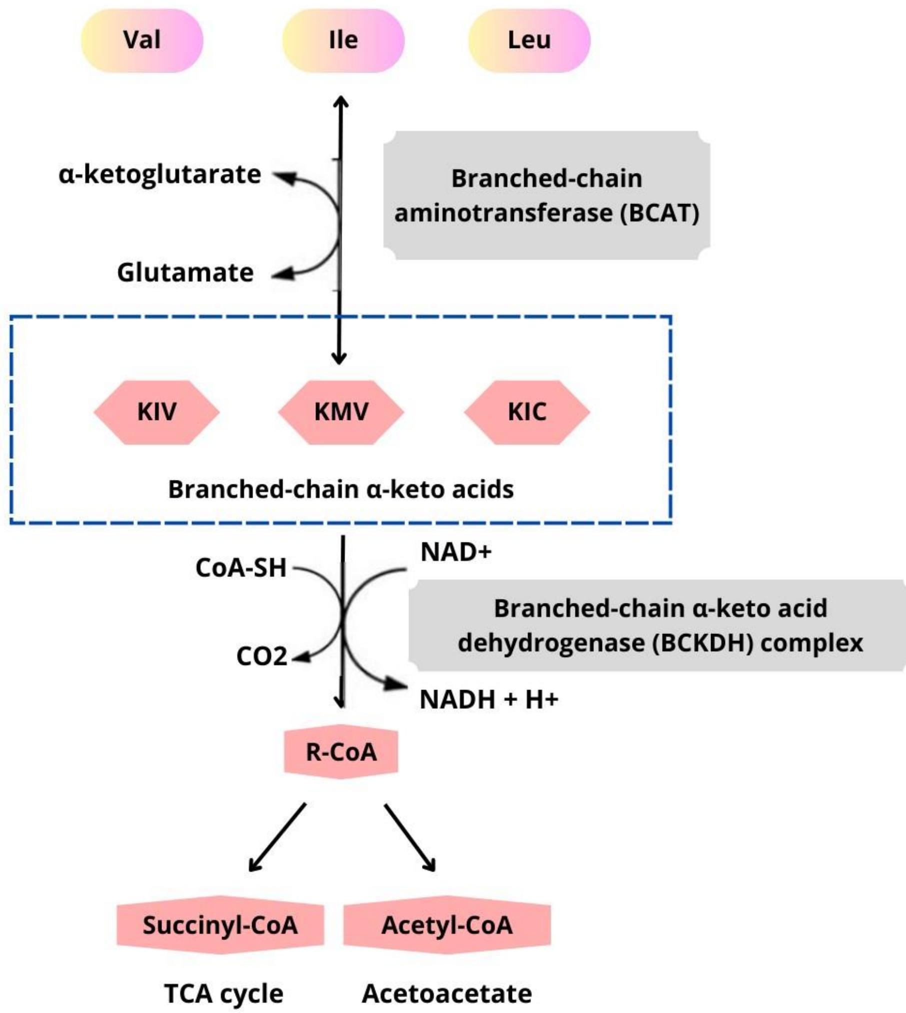


Figure 2.3. Catabolism of branched-chain amino acids (adapted from Brosnan and Brosnan, 2006).

CHAPTER 3: DIETARY SUPPLEMENTATION OF VALINE, ISOLEUCINE, AND TRYPTOPHAN MAY OVERCOME THE NEGATIVE EFFECTS OF EXCESS LEUCINE IN DIETS CONTAINING FERMENTED CORN PROTEIN

ABSTRACT

Diets with high inclusion of high-protein distillers dried grains with solubles (**HP-DDGS**) or corn fermented protein (**CFP**) may have an excess of dietary Leu, and therefore, have a detrimental impact on growth performance of pigs. However, it was hypothesized that the negative effect of using FCP in diets for weanling pigs may be overcome if diets are fortified with crystalline sources of Val, Trp, and (or) Ile. A total of 320 weanling pigs [body weight (**BW**): 6.11 ± 0.66 kg] were randomly allotted to one of 10 dietary treatments in a completely randomized design. There were four pigs per pen (i.e., 2 gilts and 2 barrows) and eight replicate pens per treatment. A two-phase feeding program was used with d 1 to 14 as phase 1 and d 15 to 28 as phase 2. Within each phase, a corn-soybean meal (**SBM**) diet was formulated and 2 basal diets based on corn and 10% FCP or corn and 20% FCP were used as well. Seven additional diets were formulated by adding Val, Ile, or Trp individually, Val and Ile, Val and Trp, Ile and Trp, or Val, Ile, and Trp to the basal diet with 20% FCP. Average daily feed intake (**ADFI**), average daily gain (**ADG**), and average gain:feed ratio (**G:F**) were calculated for each phase and for the overall experiment. Fecal scores were recorded every other day using a score from 1 to 5 (1 = normal feces; 5 = watery diarrhea). Blood samples were collected on d 14 and d 28; plasma samples were analyzed for blood urea N, total protein, albumin, peptide YY, and immunoglobulin G. On d 14, ileal tissue and fecal samples were collected from one pig per pen from the treatments fed the corn-SBM diet and from pigs fed the two basal diets containing 10 or

20% FCP to determine volatile fatty acids (**VFA**) and ammonium concentration, and microbial protein. Results indicated that inclusion of 10 or 20% FCP in diets reduced (P <0.05) final BW on d 28, ADG, and ADFI in phase 2 and for the entire experimental period. However, pigs fed the diet supplemented with Val, Ile, and Trp had a greater ($P < 0.05$) final BW and ADG in phase 2 and for the overall experiment than pigs fed the other FCP-containing diets. Fecal scores in phase 2 were reduced ($P < 0.05$) if FCP was used instead of SBM. On d 28, pigs fed the diet with 20% FCP and only Val, Val and Trp, or Val, Trp, and Ile had reduced ($P < 0.01$) blood urea N compared with pigs fed the corn-SBM diet or the other FCP-based diets. There were no effects of dietary treatments on peptide YY, immunoglobulin G, ileum morphology, VFA in feces, ammonium concentration, or microbial protein. In conclusion, FCP may be included in diets for weanling pigs without affecting growth performance, gut health, or hindgut fermentation rate, if these diets are fortified with supplemental Val, Trp, and Ile.

Key words: branched-chain amino acids, growth performance, fermented corn protein, leucine, tryptophan, weanling pigs

INTRODUCTION

High-protein distillers dried grains with solubles (**HP-DDGS**) and other corn co-products such as fermented corn protein (**FCP**) have been developed in recent years, and most of these ingredients are produced by fractionating corn co-products from the ethanol industry (U.S. Grains Council, 2023). The FCP contains approximately 50% CP and has a greater amino acid (**AA**) digestibility than conventional corn distillers grains with solubles, and therefore may be used in diets for pigs as a source of AA. Indeed, it is possible to formulate diets for pigs based on corn and HP-DDGS or other corn co-products, but such diets will contain more than twice as much Leu as recommended. Results of recent research indicated that there is a negative relationship between dietary Leu and brain synthesis of serotonin, which results in reduced feed intake of pigs fed diets containing excess Leu (Wessels et al., 2016; Kwon et al., 2019). Pigs fed diets with elevated levels of Leu also have a reduced protein synthesis because of increased Val and Ile metabolism caused by the excess dietary Leu (Harris et al., 2004). As a consequence, pigs often have reduced growth performance if fed diets with high concentrations of FCP. However, it may be possible to counteract the negative effects of the high Leu concentrations in corn co-products by adding crystalline sources of Val, Ile, and/or Trp to the diets and it may, therefore, be possible that FCP can be used in diets for weanling pigs without influencing growth performance or intestinal health of weanling pigs. However, at this time, limited research has been conducted to confirm this hypothesis. Therefore, the hypothesis that FCP may be used as the primary source of AA in diets for weanling pigs if diets are fortified with crystalline AA was tested.

MATERIALS AND METHODS

The protocol for the experiment was submitted to the Institutional Animal Care and Use Committee at the University of Illinois and was approved before the initiation of the experiment. Pigs that were the offspring of Line 800 boars mated to Camborough females were used (Pig Improvement Company, Hendersonville, TN, USA). The FCP used in the experiment was sourced from Green Plains Energy (Omaha, NE; Table 3.1), and soybean meal (**SBM**) was sourced from Stutzman's Feed & Supply (Arthur, IL). Ground yellow corn was obtained from the University of Illinois Feed Mill (Champaign, IL).

Diets, animals, and experimental design

A two-phase feeding program was used with day 1 to 14 as phase 1 and day 15 to 28 as phase 2. A total of 320 weanling pigs with an initial body weight (**BW**) of 6.11 ± 0.66 kg were used in 2 blocks of 160 pigs. Within each block, pigs were randomly assigned to 10 dietary treatments in a randomized complete block design. There were two barrows and two gilts in each pen and eight replicate pens per treatment. A control diet based on corn and SBM was formulated and two basal diets were formulated based on corn and 10% FCP or corn and 20% FCP (Tables 3.2 and 3.3). Seven additional diets were formulated by supplementing the diet with 20% FCP with crystalline Ile, Trp, and Val as follows: 1) FCP + 0.10% Ile; 2) FCP + 0.05% Trp; 3) FCP + 0.10% Val; 4) FCP + 0.10% Ile + 0.10% Val; 5) FCP + 0.10% Ile + 0.05% Trp; 6) FCP + 0.10% Val + 0.05% Trp; 7) FCP + 0.10% Ile + 0.10% Val + 0.05% Trp. All diets in phases 1 and 2 were formulated to meet the current estimates for nutrient requirements by nursery pigs (NRC, 2012).

Pigs were housed in pens with fully slatted plastic floors. Each pen was equipped with a feeder and a nipple drinker, and pigs had free access to feed and water throughout the experiment.

Sample and data collection

Individual pig weights were recorded at the beginning of the experiment, on day 14, and at the end of the 28-day experiment. Daily feed allotments were recorded and the weight of feed left in the feeders were recorded on day 14 and on the last day of the experiment to calculate feed consumption. Fecal scores were assessed visually per pen every other day using a score from 1 to 5 (1 = normal feces; 2 = moist feces; 3 = mild diarrhea; 4 = severe diarrhea; and 5 = watery diarrhea).

On day 14 and on the last day of the experiment, blood samples were collected from the jugular vein of one pig in each pen that had a body weight that was closest to the pen average. Two blood samples were collected from pigs in heparinized vacutainers and vacutainers containing EDTA. Blood samples were centrifuged at 4,000 × g at 4 °C for 13 min, and plasma was collected and stored at -20 ºC until analysis. Heparinized plasma samples were analyzed for blood urea nitrogen, total protein, and albumin using a Beckman Coulter Clinical Chemistry AU analyzer at the University of Illinois Veterinary Diagnostic Laboratory. Plasma samples treated with EDTA were also analyzed for peptide YY (**PYY**) and immunoglobulin G (**IgG**) using pig specific ELISA kits according to the recommendations from the manufacturer (Phoenix Pharmaceuticals Inc., Burlingame, CA, Bethyl Laboratories Inc., Montgomery, TX, respectively). All analyses were performed in duplicates.

On d 14, one pig per pen (the pig with a body weight closest to the pen average) in 3 of the 10 dietary treatments (i.e., corn-SBM basal diet and the two basal diets containing 10 and 20% FCP) was euthanized via captive bolt penetration. Ileal tissue samples between 2 and 3 cm long were collected approximately 80 cm from the ileal-cecal junction. Samples were cut and pinned with the serosa side down on a piece of cardboard. Samples were then fixed in 10% neutral buffered formalin until processing for immunohistochemistry staining and morphological evaluation. After fixation, all tissue samples were delivered to Veterinary Diagnostic Pathology, University of Illinois (Urbana, IL, USA). All tissue samples were sectioned and transferred to slides. Villus height was measured from the villus tip to the base, and the crypt depth was measured from the crypt-villus junction to the base of the crypt. Lamina propria thickness were also measured at the midpoint of the villus. Villus height:crypt depth (**VH:CD**) was also calculated.

Fecal samples from these pigs were also collected and analyzed for microbial protein, fecal NH_3, and volatile fatty acids (**VFA**). For microbial protein, 5 to 10 g of feces were collected and stored at -20 °C until analyzed. For VFA and NH_3 analysis, 5 g of feces were placed in 15 mL tubes and samples were stabilized in 2*N* HCl and stored at -20 °C until analyzed. Fecal ammonia concentrations were determined according to the method by Chaney and Marbach (1962) and VFA were determined using previously established procedures (Sunvold et al., 1995). Microbial protein was determined following the procedure described by Espinosa et al. (2019).

Chemical analysis

All diet and ingredient samples were analyzed in duplicate for concentrations of gross energy using a bomb calorimeter (Model 6400, Parr Instruments, Moline, IL, USA), and N was analyzed by combustion (method 990.03; AOAC Int., 2019) using a LECO FP628 analyzer (LECO Corp., Saint Joseph, MI, USA) with the subsequent calculation of crude protein as N × 6.25 (Tables 3.3, 3.4 and 3.5). Dry matter was also analyzed in diet and ingredient samples by oven drying at 135°C for 2 h (method 930.15, AOAC Int., 2019) and these samples were also analyzed for dry ash (method 942.05; AOAC Int., 2019). All diet and ingredient samples were analyzed for acid hydrolyzed ether extract using the acid hydrolysis filter bag technique (Ankom HCl Hydrolysis System; Ankom Technology, Macedon, NY, USA) followed by crude fat extraction using petroleum ether (AnkomXT15 Extractor; Ankom Technology, Macedon, NY, USA). All diet and ingredient samples were analyzed for AA [method 982.30 E (a, b, c); AOAC Int., 2019].

Statistical analysis

Data for growth performance were summarized to calculate average daily gain (**ADG**), average daily feed intake (**ADFI**), and gain to feed ratio (**G:F**) for each pen of pigs and for each treatment group at the conclusion of the experiment. Normality of data was verified and outliers were identified using the UNIVARIATE procedure of SAS. Outliers were removed if the value deviated from the 1st or 3rd quartiles by more than 3 times the interquartile range. Data were analyzed using the PROC MIXED of SAS with the experimental unit being the pen. The model included diet as fixed effect and block and replicate within block as random effects. Treatment means were calculated using the LSMEANS statement. Statistical significance and tendency was considered as $P < 0.05$ and $0.05 \leq P < 0.10$, respectively.

RESULTS

All animals remained healthy throughout the experiment and readily consumed their assigned diets. Inclusion of 10 or 20% FCP in diets reduced ($P < 0.05$) final BW on d 28, ADG and ADFI in phase 2 and for the entire experimental period (Table 3.6). Pigs fed the diet containing 20% FCP supplemented with all three AA had a greater ($P < 0.05$) final BW and ADG in phase 2 and for the overall experiment than pigs fed diets supplemented with only one or two AA. However, pigs fed diets supplemented with Val, Val and Ile, or Val and Trp had greater ($P < 0.05$) final BW and greater ADG in phase 2 and for the overall experiment than pigs fed diets containing only Ile, only Trp, or Ile and Trp. Pigs fed the control diet or the diet with 20% FCP and Val, Val and Ile, Val and Trp, or Val, Ile and Trp had greater ($P < 0.05$) ADFI in phase 2 and for the overall experiment than pigs fed diets with 10 or 20% FCP and no AA or diets with 20% FCP and Ile and Trp. Pigs fed the control diet had greater ($P < 0.05$) G:F for the entire experiment than pigs fed a diet with FCP regardless of AA supplementation.

From d 15 to d 28 and for the overall experiment, fecal scores were reduced ($P < 0.05$) if FCP was included in the diet (Table 3.7). No differences among experimental diets were observed for blood urea N, total protein, albumin, peptide YY, or IgG on d 14 (Table 3.8). However, on d 28, pigs fed the diet with 20% FCP and only Val, Val and Trp, or Val, Trp, and Ile had reduced ($P < 0.01$) blood urea N compared with pigs fed the control diet (Table 3.9). No differences in villus height, crypth depth, or VH:CD of pigs were observed among pigs fed the control diet or the 2 basal diets containing FCP (Table 3.10). Inclusion of FCP in experimental diets did not affect the concentration of microbial protein in feces of pigs (Table 3.11). Likewise, concentrations of VFA and ammonium in feces were not affected by inclusion of FCP in the diets.

DISCUSSION

Soybean meal is the most commonly used protein source in diets for swine (Parrini et al., 2023). However, SBM inclusion is usually limited to less than 20% during the post-weaning period due to hypersensitivity reactions that can cause damage to the intestinal microvilli and reduce the absorption capacity (Friesen et al., 1993; Engle, 1994). In addition, SBM has a high cost and fluctuating price (Lestingi, 2024). Therefore, SBM can only furnish some of the AA needed by weanling pigs. Historically, animal proteins have also been used in weanling pig diets, but due to high costs and reduced availability of these ingredients, there is a need to identify other sources of AA that can be used in diets for weanling pigs. New technologies in the corn ethanol industry allow for removing some of the fiber in corn before fermentation, which results in a co-product with a greater AA concentration (Widmer et al., 2007; Acosta et al., 2021). One of these new products is FCP, which contains around 50% CP, and FCP may, therefore, be included in diets for weanling pigs (Acosta et al., 2021). However, reduced growth performance by pigs fed FCP or other proteins has been reported (Yang et al., 2019; Acosta et al., 2021). The reason for the reduced growth performance may be the high concentration of Leu in corn protein because excess Leu in diets for growing pigs influences the metabolism of Val and Ile by increasing Val and Ile catabolism, resulting in a deficiency of Val and Ile for protein synthesis (Harris et al., 2004). This is likely because all three BCAA share the first two steps in their catabolism. In the first step, there is a reversible transamination by branched-chain amino acid aminotransferase (**BCAT**) which produces α-keto isovalerate (**KIV**), α-ketoβ-methylvalerate (**KMV**), and α-ketoisocaproate (**KIC**), from deamination of Val, Ile, and Leu, respectively (Harris et al., 2005). The second step is an irreversible decarboxylation by the branched-chain α-keto acid dehydrogenase complex (**BCKD**; Brosnan and Brosnan, 2006; Wiltafsky et al., 2010). However, if there is an oversupply of Leu, there will be a greater concentration of KIC, which is the most powerful stimulator of BCAT and BCKD. Therefore, high Leu levels increase the catabolism of Val and Ile as well (Harper et al., 1984) causing a BCAA imbalance (Kwon et al., 2019) and therefore, reduced growth performance of pigs is usually observed if there is excess Leu in the diet (Cemin et al., 2019a and b; Kwon et al., 2019). As a consequence, greater concentrations of Val and Ile in high-Leu diets may be required to obtain mantain growth performance (Yang et al., 2019).

High levels of Leu in diets is also detrimental to Trp metabolism. Tryptophan is a precursor for serotonin, which is a neurotransmitter that influences feed intake regulation (Zhang et al., 2007), and excess dietary Leu may reduce synthesis of serotonin in the brain (Wessels et al., 2016) by preventing Trp from being transported to the brain (Kwon et al., 2019). Reduced serotonin synthesis can result in reduced feed intake and pigs with reduced feed intake due to excess Leu also have reduced growth performance.

The observation that the final BW, ADG, and ADFI decreased as the FCP without AA supplementation increased in the basal diets is in agreement with previous data, indicating that 20 and 30% HP-DDGS resulted in a reduced growth performance of pigs (Yang et al., 2019). Likewise, weanling pigs fed up to 10% FCP at the expense of enzyme-treated SBM had reduced BW, ADG, and G:F (Stas et al., 2022). In contrast, FCP was also included in diets for weanling pigs by up to 10% without negative effects on growth performance (Acosta et al., 2021), and up to 14% FCP was used in the first two phases after weaning without adversely affecting final BW, ADG, and ADFI (Garavito-Duarte et al., 2024). Thus, responses to inclusion of FCP in diets for weanling pigs have been inconsistent, which may be a result of different degrees of imbalances among the BCAA depending on diet composition. Indeed, modeling the impact of excess dietary Leu resulted in a model to predict the increased requirement for Val, Ile, and Trp in high Leu diets (Cemin et al., 2019b).

In the current experiment, the objective was to determine effects of extra Val, Ile, and Trp, independently or in combination, on growth performance of pigs fed diets with excess dietary Leu. The observation that addition of Val, Ile, and Trp resulted in final BW and ADG of pigs that was not different from that of pigs fed the control diet support the hypothesis that requirements of all three AA is increased in high Leu diets. Likewise, the observation that supplementation with Ile or Trp alone or in combination without Val did not increase the performance, whereas Val alone or in combination with Trp or Ile resulted in ADG that was greater than if Val was not used indicates that Val was the first limiting AA in the diets. However, because Val alone was not enough to restore growth performance, it is concluded that requirements for Ile and Trp were also increased in the high Leu diets. Increasing ADG of pigs fed high Leu diets by increasing dietary Val has been demonstrated previously (Gloaguen et al., 2011; Millet et al., 2015). The observation that effects of adding only Trp was limited is also in agreement with a previous data (Kerkaert et al., 2021). This may be because only 3% of metabolized Trp is used for serotonin synthesis (Richard et al., 2009), and in rats fed excess Leu, it was necessary to supplement Trp in combination with Val and Ile to overcome a reduction in growth performance (Rogers et al., 1967).

The observation that there is no added advantage of adding Ile and Val compared with only Val is in agreement with data demonstrating that Ile is less able to counteract the negative effects of excess Leu than Val (Cemin et al., 2019b). This is also in agreement with earlier research that determined that Ile supplementation only partially overcame the negative effects of excess Leu (Harper et al., 1954). This may be because the uptake competition for Leu is higher for Ile than Val (Kerkaert et al., 2021), which may be because the Km of the neutral AA transporter is highest for Val, followed by Leu and Ile (Hargreaves and Pardridge, 1988). Therefore, there will be a faster plasma pool clearance for Ile than for Val, suggesting that Ile may be more susceptible to excess Leu than Val (Kerkaert et al., 2021).

The observation that pigs fed the control diet or diets containing FCP and Val, Ile, and Trp had growth performance that was not different indicates that the negative effects of high-Leu diets can be overcome when FCP-diets are fortified with crystalline Val, Ile, and Trp. These observations are also in agreement with data demonstrating that the optimal SID Ile to Lys ratio increased as Leu:Lys ratio increased in diets for 8 to 25 kg pigs (Htoo et al., 2017). Adding both Trp and Val to a diet high in Leu and fed to growing pigs may also prevent the detrimental effects of excess Leu on growth performance (Kwon et al., 2024).

The recovery of ADG of pigs fed supplemented diets with Val and Ile may be a result of increased ADFI (Kerkaert et al., 2021). However, because addition of only Val and Ile in the current experiment did not fully restore ADFI, whereas the addition of the combination of Ile, Val, and Trp did, it is likely that the requirement of all three AA was increased by excess Leu. It is also likely that it was the reduced ADFI that resulted in reduced ADG and final BW. Nevertheless, because ADFI and ADG were reduced at the same time, G:F was not impacted by dietary treatments.

Results of previous research determined that the detrimental effects of an oversupply of Leu may be partially ameliorated by increasing Trp (Kwon et al., 2022). However, it was also concluded that Trp concentration in the brain may not be the only reason for reduced ADFI of pigs fed high Leu diets. The mammalian target of rapamycin (mTOR) detects signals related to nutrients, energy, and hormones, influencing the regulation of metabolism and energy balance (Hu et al., 2016). The activity of mTOR in the hypothalamus highly regulates feed intake (Mori et al., 2009). Branched-chain amino acids among other AA are stimulators of mTOR even though it mechanism is not fully elucidated (Hu et al., 2016). However, an excess of Leu can overstimulate mTOR, suppressing feed intake, whereas excess Val does not have negative effects on feed intake (Cota et al., 2006). In addition, Val inhibits the transport of Leu through the blood-brain barrier to a larger extend than Ile or Trp (Hjelle et al., 1978). However, increasing both Val and Ile in high Leu diets partially recovered G:F ratio, but did not correct the reduced ADFI when compared with a control diet without inclusion of FCP (Stas et al., 2022), which further indicated that a part of the reduced ADFI is caused by reduced availability of Trp to synthesize serotonin.

The observation that pigs fed diets with 20% FCP had improved fecal score compared with control diet is in agreement with data demonstrating softer feces from pigs fed a corn-SBM diet than pigs fed a diet with FCP (Garavito-Duarte et al., 2024). The reason for these observations may be the presence of yeast in FCP because yeast may reduce diahrrea in pigs (Boontiam et al., 2022).

Blood urea N is positively correlated with urinary N excretion, and is, therefore, an indicator of AA utilization efficiency (Kohn et al., 2005). The observation that pigs fed the FCP diets with supplemented Val, Val and Trp, or Val, Trp, and Ile had reduced blood urea N compared with pigs fed the control diet indicates that protein utilization in these pigs was more efficient. This is likely a result of these diets having a more balanced AA composition with less excess AA. Specifically, reducing SBM and including FCP in the diets reduced excesses of Arg and also reduced concentrations of Asp and Glu, which likely contributed to the reduced blood urea N. Likewise, the reduced feed intake may also contributed to the reduced blood urea N because pigs had a lower feed intake.

Albumin binds and transports nutrients in the blood (Bern et al., 2015). Thus, the observation that there were only few differences in albumin concentration among treatments indicates that the reduced ADFI observed for pigs fed FCP diets did not impact the ability of albumin to transport nutrients. The lack of a difference in plasma IgG among treatments indicate that the inclusion of FCP did not influence the immune response or the systemic health of pigs.

Changes in the morphological structure in weanling pigs, such as villus atrophy and crypt hyperplasia, have been reported if pigs are fed diets that are inadequate in nutrient supply (Zheng et al., 2021). These changes may result in a decrease in surface area, and a reduction in nutrient absorption. Thus, the lack of differences in morphology of pigs fed experimental diets indicates that inclusion of FCP in diets does not have a negative impact on nutrient absorption when compared with pigs fed a diet based on SBM.

Distillers dried grains with solubles may increase growth of bacteria in the hindgut of pigs (Bindelle et al., 2008), and increased microbial growth may result in increased synthesis of VFA because of increased fermentation (Espinosa et al., 2019). To the best of our knowledge, there is no data reported demonstrating the effects of FCP on intestinal microbial protein, VFA, and ammonium concentration in feces from pigs. Therefore, lack of a difference among treatments in microbial protein, VFA, and ammonium concentration indicates that the inclusion of FCP does not impact microbial growth and fermentation, which is likely due to the lower concentration of TDF in FCP compared with distillers dried grains with solubles.

CONCLUSIONS

Inclusion of FCP in diets for weanling pigs did not affect immune system, ileum morphology, microbial protein, VFA, or fecal ammonium. Likewise, pigs fed FCP with added Val, Val and Trp, or Val, Trp, and Ile had a better protein utilization than pigs a fed without those AA. However, using FCP in diets for weanling pigs had a negative impact on growth performance, but this effect could be mostly overcome with supplementation of Val, Trp, and Ile. Therefore, up to 20% FCP may be included in diets for weanling pigs without affecting growth performance or blood characteristics, provided that provisions of Val and Ile are increased by 0.10% and Trp is increased by 0.05%.

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Tables

Table 3.1. Analyzed composition of main ingredients, as-fed basis[1]

Item	Soybean meal	Fermented corn protein	Corn
Gross energy, kcal/kg	4,194	4,950	3,847
Crude protein, %	46.98	50.26	6.72
Dry matter, %	89.35	91.72	86.41
Ash, %	12.73	9.34	1.95
Acid hydrolyzed ether extract, %	2.86	5.82	3.53
Insoluble dietary fiber, %	16.50	24.20	10.20
Soluble dietary fiber, %	2.90	2.50	Not detected
Total dietary fiber, %	19.40	26.70	10.20
Indispensable AA, %			
Arg	3.26	2.30	0.29
His	1.35	1.36	0.18
Ile	2.24	2.34	0.24
Leu	3.55	6.51	0.69

Table 3.1. (cont.)

Lys	2.66	1.80	0.22
Met	0.62	1.11	0.12
Phe	2.36	2.92	0.30
Thr	1.82	1.96	0.22
Trp	0.62	0.49	0.05
Val	2.25	2.84	0.33
Dispensable AA, %			
Ala	2.00	3.81	0.44
Asp	5.25	3.62	0.42
Cys	0.66	0.93	0.14
Glu	8.33	8.52	1.11
Gly	1.95	2.11	0.27
Pro	2.25	3.99	0.52
Ser	2.13	2.22	0.27
Tyr	1.70	2.19	0.15

[1]Fermented corn protein sourced from Green Plains Energy, Omaha, NE.

Table 3.2. Ingredient composition of phase 1 experimental diets, as-fed basis[1,2,3,4,5]

Ingredient, %	Control diet	FCP[1], 10%	FCP, 20%	FCP + Ile	FCP + Trp	FCP + Val	FCP + Ile + Val	FCP + Ile + Trp	FCP + Val + Trp	FCP + Ile + Val + Trp
Ground corn	44.70	47.25	48.89	48.79	48.84	48.79	48.69	48.74	48.74	48.64
Soybean meal	20.00	10.00	-	-	-	-	-	-	-	-
Fermented corn protein	-	10.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00
Soybean oil	2.80	2.80	2.80	2.80	2.80	2.80	2.80	2.80	2.80	2.80
Whey powder	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00
ESBM[1]	9.00	6.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Limestone	1.04	1.18	1.31	1.31	1.31	1.31	1.31	1.31	1.31	1.31
Dicalcium phosphate	0.70	0.65	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60
L-Lysine	0.47	0.74	0.96	0.96	0.96	0.96	0.96	0.96	0.96	0.96
DL-Methionine	0.23	0.18	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17
L-Threonine	0.13	0.17	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
L-Tryptophan	-	0.04	0.07	0.07	0.12	0.07	0.07	0.12	0.12	0.12

Table 3.2. (cont.)

L-Valine	0.03	0.04	0.03	0.03	0.03	0.13	0.13	0.03	0.13	0.13
L-Isoleucine	-	-	-	0.10	-	-	0.10	0.10	-	0.10
L-Histidine	-	0.05	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07
Salt	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Vitamin-mineral premix[2]	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Calculated values										
ME, kcal/kg	3,456	3,443	3,439	3,436	3,438	3,436	3,433	3,434	3,434	3,431
Crude protein, %	19.26	18.42	18.10	18.09	18.10	18.09	18.09	18.09	18.09	18.08
Total Ca, %	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80
P[2], %	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Amino acids[4], %										
Arg	1.13	0.92	0.74	0.74	0.74	0.74	0.74	0.74	0.74	0.74
His	0.46	0.46	0.46	0.46	0.46	0.46	0.46	0.46	0.46	0.46
Ile	0.79	0.73	0.69	0.79	0.69	0.69	0.79	0.79	0.69	0.79

Table 3.2. (cont.)

Leu	1.45	1.63	1.84	1.84	1.84	1.84	1.84	1.84	1.84	1.84
Lys	1.40	1.40	1.40	1.40	1.40	1.40	1.40	1.40	1.40	1.40
Met	0.48	0.45	0.47	0.47	0.47	0.47	0.47	0.47	0.47	0.47
Met + Cys	0.74	0.74	0.74	0.74	0.74	0.74	0.74	0.74	0.74	0.74
Phe	0.82	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80
Thr	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80
Trp	0.23	0.23	0.23	0.23	0.28	0.23	0.23	0.28	0.28	0.28
Val	0.86	0.86	0.86	0.86	0.86	0.96	0.96	0.86	0.96	0.96

[1]ESBM, enzyme-treated soybean meal: Hamlet Protein, Findley, OH.; FCP, fermented corn protein (Green Plains Energy, Omaha, NE).

[2]The vitamin-mineral premix provided the following quantities of vitamins and micro-minerals per kilogram of complete diet: vitamin A as retinyl acetate, 11,150 IU; vitamin D3 as cholecalciferol, 2,210 IU; vitamin E as DL-alpha tocopheryl acetate, 66 IU; vitamin K as menadione nicotinamide bisulfate, 1.42 mg; thiamin as thiamine mononitrate, 1.10 mg; riboflavin, 6.59 mg; pyridoxine as pyridoxine hydrochloride, 1.00 mg; vitamin B12, 0.03 mg; D-pantothenic acid as D-calcium pantothenate, 23.6 mg; niacin, 44.1 mg; folic acid, 1.59 mg; biotin, 0.44 mg; Cu, 20 mg as copper chloride; Fe, 125 mg as iron sulfate; I, 1.26 mg as ethylenediamine dihydriodide; Mn, 60.2 mg as manganese hydroxychloride; Se, 0.30 mg as sodium selenite and selenium yeast; and Zn, 125.1 mg as

Table 3.2. (cont.)

zinc hydroxychloride.

[3]Standardized total tract digestible P.

[4]Amino acids are indicated as standardized ileal digestible amino acids.

[5]Diets supplemented with amino acids had 20% of corn fermented protein.

Table 3.3. Ingredient composition of phase 2 experimental diets containing high protein corn co-product (FCP), as-fed basis[1,2,3,4,5]

Ingredient, %	Control diet	FCP, 10%	FCP, 20%	FCP + Ile	FCP + Trp	FCP + Val	FCP + Ile + Val	FCP + Ile + Trp	FCP + Val + Trp	FCP + Ile + Val + Trp
Ground corn	54.49	54.38	56.97	56.87	56.92	56.87	56.77	56.82	56.82	56.72
Soybean meal	29.00	19.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00
Fermented corn protein	-	10.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00
Soybean oil	2.80	2.80	2.80	2.80	2.80	2.80	2.80	2.80	2.80	2.80
Whey powder	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00
Limestone	0.95	1.10	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25
Dicalcium phosphate	0.88	0.77	0.73	0.73	0.73	0.73	0.73	0.73	0.73	0.73
L-Lysine	0.50	0.65	0.88	0.88	0.88	0.88	0.88	0.88	0.88	0.88
DL-Methionine	0.23	0.18	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
L-Threonine	0.17	0.16	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19
L-Tryptophan	-	0.02	0.06	0.06	0.11	0.06	0.06	0.11	0.11	0.11
L-Valine	0.08	0.02	0.02	0.02	0.02	0.12	0.12	0.02	0.12	0.12
L-Isoleucine	-	-	-	0.10	-	-	0.10	0.10	-	0.10

Table 3.3. (cont.)

L-Histidine	-	0.02	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Salt	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Vitamin-mineral premix[1]	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Calculated values										
ME, kcal/kg	3,382	3,399	3,409	3,406	3,408	3,406	3,402	3,404	3,404	3,401
Crude protein, %	17.68	18.38	17.89	17.89	17.89	17.89	17.88	17.88	17.88	17.88
Total Ca, %	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75
P[2], %	0.36	0.36	0.36	0.36	0.36	0.36	0.36	0.36	0.36	0.36
Amino acids[3], %										
Arg	1.06	0.96	0.77	0.77	0.77	0.77	0.77	0.77	0.77	0.77
His	0.44	0.44	0.44	0.44	0.44	0.44	0.44	0.44	0.44	0.44
Ile	0.69	0.70	0.66	0.76	0.66	0.66	0.76	0.76	0.66	0.76
Leu	1.31	1.60	1.80	1.80	1.80	1.80	1.80	1.80	1.80	1.80
Lys	1.29	1.29	1.29	1.29	1.29	1.29	1.29	1.29	1.29	1.29

Table 3.3. (cont.)

Met	0.46	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45
Met + Cys	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71
Phe	0.75	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80
Thr	0.76	0.76	0.76	0.76	0.76	0.76	0.76	0.76	0.76	0.76
Trp	0.21	0.21	0.21	0.21	0.26	0.21	0.21	0.26	0.26	0.26
Val	0.82	0.82	0.82	0.82	0.82	0.92	0.92	0.82	0.92	0.92

[1]The fermented corn protein was sourced from Green Plains Energy, Omaha, NE.

[2]The vitamin-mineral premix provided the following quantities of vitamins and micro-minerals per kilogram of complete diet: vitamin A as retinyl acetate, 11,150 IU; vitamin D3 as cholecalciferol, 2,210 IU; vitamin E as DL-alpha tocopheryl acetate, 66 IU; vitamin K as menadione nicotinamide bisulfate, 1.42 mg; thiamin as thiamine mononitrate, 1.10 mg; riboflavin, 6.59 mg; pyridoxine as pyridoxine hydrochloride, 1.00 mg; vitamin B12, 0.03 mg; D-pantothenic acid as D-calcium pantothenate, 23.6 mg; niacin, 44.1 mg; folic acid, 1.59 mg; biotin, 0.44 mg; Cu, 20 mg as copper chloride; Fe, 125 mg as iron sulfate; I, 1.26 mg as ethylenediamine dihydriodide; Mn, 60.2 mg as manganese hydroxychloride; Se, 0.30 mg as sodium selenite and selenium yeast; and Zn, 125.1 mg as zinc hydroxychloride.

[3]Standardized total tract digestible P.

[4]Amino acids are indicated as standardized ileal digestible amino acids.

Table 3.3. (cont.)

[5]Diets supplemented with amino acids had 20% of corn fermented protein.

Table 3.4. Analyzed nutrient composition of phase 1 diets[1,2]

Item	Control diet	FCP [1], 10%	FCP, 20%	FCP + Ile	FCP + Trp	FCP + Val	FCP + Ile + Val	FCP + Ile + Trp	FCP + Val + Trp	FCP + Ile + Val + Trp
Gross energy, kcal/kg	3,998	4,010	4,001	3,989	4,005	3,994	4,071	4,024	4,063	3,976
Crude protein, %	21.35	20.45	20.18	20.79	21.48	20.18	20.63	20.27	20.43	20.24
Dry matter, %	89.83	89.49	89.92	89.98	89.78	89.86	89.94	89.68	89.91	89.83
Ash, %	9.85	8.41	8.19	8.26	8.87	8.16	8.64	8.20	8.36	8.74
Acid hydrolyzed ether extract, %	4.59	4.63	4.74	4.41	4.85	4.25	4.16	4.34	4.95	4.77
Indispensable amino acids, %										
Arg	1.24	0.99	0.79	0.82	0.80	0.79	0.82	0.80	0.79	0.81
His	0.52	0.51	0.51	0.52	0.52	0.50	0.51	0.51	0.50	0.52
Ile	0.97	0.87	0.79	0.89	0.79	0.79	0.88	0.89	0.77	0.88
Leu	1.69	1.83	2.01	2.05	2.05	2.03	2.05	2.03	2.04	2.05
Lys	1.61	1.62	1.62	1.61	1.59	1.58	1.58	1.60	1.59	1.58
Met	0.4	0.45	0.45	0.45	0.47	0.44	0.47	0.45	0.45	0.45
Phe	0.97	0.92	0.88	0.89	0.89	0.87	0.89	0.89	0.86	0.86

Table 3.4. (cont.)

Thr	0.91	0.90	0.92	0.93	0.93	0.90	0.91	0.89	0.90	0.93
Trp	0.24	0.24	0.24	0.24	0.27	0.24	0.24	0.28	0.28	0.27
Val	1.02	1.01	1.00	1.01	1.01	1.10	1.11	1.00	1.10	1.10
Dispensable amino acids, %										
Ala	0.95	1.07	1.20	1.24	1.22	1.18	1.22	1.20	1.20	1.22
Asp	2.13	1.74	1.42	1.46	1.43	1.40	1.44	1.43	1.40	1.44
Cys	0.34	0.34	0.35	0.35	0.35	0.34	0.35	0.35	0.34	0.34
Glu	3.62	3.31	3.10	3.18	3.15	3.05	3.16	3.09	3.01	3.15
Gly	0.79	0.73	0.68	0.70	0.69	0.67	0.68	0.67	0.67	0.69
Pro	1.14	1.22	1.33	1.39	1.37	1.33	1.36	1.35	1.32	1.36
Ser	0.84	0.79	0.76	0.79	0.78	0.76	0.78	0.78	0.76	0.78

[1]FCP, fermented corn protein (Green Plains Energy, Omaha, NE).

[2]Diets supplemented with amino acids had 20% of corn fermented protein.

Table 3.5. Analyzed nutrient composition of phase 2 diets[1,2]

Item	Control diet	FCP[1], 10%	FCP, 20%	FCP + Ile	FCP + Trp	FCP + Val	FCP + Ile + Val	FCP + Ile + Trp	FCP + Val + Trp	FCP + Ile + Val + Trp
Gross energy, kcal/kg	4,004	4,059	4,087	4,105	4,136	4,104	4,091	4,047	4,112	4,103
Crude protein, %	19.21	19.90	19.18	20.01	19.42	20.81	20.26	20.54	19.55	19.99
Dry matter, %	89.87	89.70	89.89	89.91	89.97	89.84	90.04	89.65	89.10	89.66
Ash, %	10.04	9.37	8.42	9.13	8.61	8.11	8.85	8.59	8.24	8.86
Acid hydrolyzed ether extract, %	4.14	4.69	4.91	4.42	4.14	4.76	4.87	4.65	4.11	4.18
Indispensable amino acids, %										
Arg	1.12	1.08	0.88	0.88	0.87	0.82	0.89	0.89	0.89	0.84
His	0.50	0.51	0.51	0.50	0.54	0.54	0.52	0.54	0.52	0.53
Ile	0.90	0.90	0.82	0.93	0.83	0.83	0.95	0.95	0.85	0.94
Leu	1.63	1.90	2.02	2.04	2.02	2.05	2.09	2.11	2.04	2.08
Lys	1.53	1.52	1.53	1.40	1.47	1.52	1.51	1.44	1.44	1.48
Met	1.42	1.50	1.42	1.42	1.45	1.50	1.50	1.44	1.44	1.44
Phe	0.95	0.95	0.96	0.92	0.91	0.95	0.95	0.98	0.96	0.97

Table 3.5. (cont.)

Thr	0.88	0.87	0.86	0.89	0.89	0.89	0.85	0.89	0.89	0.88
Trp	0.22	0.23	0.23	0.23	0.28	0.23	0.23	0.28	0.28	0.28
Val	1.01	1.01	1.03	1.01	1.03	1.13	1.15	1.02	1.17	1.15
Dispensable amino acids, %										
Ala	0.93	1.20	1.20	1.20	1.21	1.24	1.25	1.28	1.27	1.28
Asp	1.93	1.79	1.46	1.47	1.44	1.54	1.50	1.53	1.53	1.51
Cys	0.29	0.33	0.31	0.33	0.32	0.35	0.34	0.36	0.36	0.35
Glu	3.41	3.42	3.15	3.11	3.13	3.25	3.21	3.33	3.28	3.31
Gly	0.75	0.77	0.71	0.71	0.71	0.74	0.72	0.74	0.73	0.74
Pro	1.09	1.26	1.33	1.32	1.38	1.33	1.35	1.36	1.34	1.35
Ser	0.79	0.80	0.77	0.78	0.78	0.80	0.79	0.80	0.80	0.80

[1]FCP, fermented corn protein (Green Plains Energy, Omaha, NE).

[2]Diets supplemented with amino acids had 20% of corn fermented protein.

Table 3.6. Growth performance of pigs fed experimental diets[1, 2,3,4,5]

Item	Control diet	HPCP, 10%	HPCP, 20%	HPCP + Ile	HPCP + Trp	HPCP + Val	HPCP + Ile + Val	HPCP + Ile + Trp	HPCP + Val + Trp	HPCP + Ile + Val + Trp	SEM	*P* value
Body weight, kg												
Initial body weight	6.33	6.29	6.34	6.33	6.32	6.30	6.29	6.31	6.31	6.29	0.23	0.154
d 14	7.42	7.26	7.35	7.21	7.20	7.35	7.34	6.97	7.40	7.43	0.28	0.398
d 28	14.22[a]	12.58[cde]	12.07[cdef]	11.80[ef]	11.93[def]	13.02[bcd]	13.08[bc]	11.44[f]	13.00[bcde]	13.77[ab]	0.54	0.001
ADG, g												
d 1 to 14	78	69	72	62	63	74	75	47	78	81	9.4	0.281
d 15 to 28	485[a]	379[cd]	337[d]	334[d]	337[d]	405[bc]	375[cd]	319[d]	399[bc]	453[ab]	23.7	0.001
d 1 to 28	281[a]	224[cde]	204[cdef]	198[ef]	200[def]	240[bc]	225[cde]	183[f]	238[bcd]	267[ab]	14.3	0.001
ADFI, g												
d 1 to 14	141	136	135	128	135	141	136	118	146	157	10.3	0.080

Table 3.6. (cont.)

d 15 to 28	662[a]	549[cd]	503[cde]	493[de]	499[cde]	572[bcd]	549[cd]	441[e]	577[bc]	646[ab]	31.84	0.001
d 1 to 28	401[a]	343[b]	319[bc]	313[bc]	317[bc]	356[ab]	349[b]	280[c]	361[ab]	402[a]	18.76	0.001
G:F												
d 1 to 14	0.55	0.44	0.52	0.44	0.45	0.51	0.49	0.35	0.52	0.51	0.05	0.119
d 15 to 28	0.73	0.69	0.66	0.68	0.67	0.70	0.68	0.73	0.69	0.70	0.37	0.349
d 1 to 28	0.70	0.65	0.63	0.63	0.62	0.67	0.64	0.65	0.66	0.66	0.01	0.154

[1] FCP, fermented corn protein (Green Plains Energy, Omaha, NE).

[2]Data are least square means of 8 observations per treatment.

[3]ADFI, average daily feed intake; ADG, average daily gain; BW, body weight; G:F, gain to feed ratio.

[4]All pigs were fed phase 1 diets for 14 d post-weaning, and they were then fed phase 2 diets from d 15 to 28 post-weaning.

[5]Diets supplemented with amino acids had 20% of corn fermented protein.

Table 3.7. Fecal scores of pigs fed the experimental diets[1,2,3,4]

Item	Control diet	FCP, 10%	FCP, 20%	FCP + Ile	FCP + Trp	FCP + Val	FCP + Ile + Val	FCP + Ile + Trp	FCP + Val + Trp	FCP + Ile + Val + Trp	SEM	*P* value
d 1 to 14	2.42	0.25	2.12	2.03	2.03	2.17	2.51	2.08	2.21	2.11	0.21	0.295
d 15 to 28	1.67[a]	1.44[b]	1.23[c]	1.23[c]	1.37[bc]	1.21[c]	1.26[bc]	1.26[bc]	1.21[c]	1.33[bc]	0.13	0.001
d 1 to 28	2.05[a]	1.84[abc]	1.67[bc]	1.63[c]	1.70[bc]	1.75[bc]	1.89[ab]	1.67[bc]	1.71[bc]	1.72[bc]	0.14	0.030

[1]FCP, fermented corn protein (Green Plains Energy, Omaha, NE).

[2]Data are least square means of 8 observations per treatment.

[3]Fecal scores were visually assessed every other day by one independent observer for 28 days. Fecal score: 1, normal feces; 2, moist feces; 3, mild diarrhea; 4, severe diarrhea; and 5, watery diarrhea.

[4]Diets supplemented with amino acids had 20% of corn fermented protein.

Table 3.8. Blood characteristics on d 14 of pigs fed experimental diets[1,2,3]

Item	Control diet	FCP, 10%	FCP, 20%	FCP + Ile	FCP + Trp	FCP + Val	FCP + Ile + Val	FCP + Ile + Trp	FCP + Val + Trp	FCP + Ile + Val + Trp	SEM	*P* value
Blood urea nitrogen, mg/dL	8.71	6.75	6.87	6.00	6.12	5.12	5.75	6.54	4.50	5.37	1.06	0.371
Total protein, mg/dL	4.53	4.50	4.31	4.38	4.47	4.42	4.38	4.40	4.41	4.38	0.11	0.858
Albumin, mg/dL	2.71	2.73	2.72	2.66	2.67	2.63	2.56	2.68	2.57	2.66	0.09	0.611
Peptide YY (ng/mL)	1.93	1.81	1.73	1.76	1.84	1.84	1.86	1.72	1.84	1.89	0.12	0.960
Immunoglobulin G (mg/mL)	5.35	3.71	3.92	4.78	3.07	3.17	3.05	4.06	4.29	3.60	0.84	0.290

[1] FCP, fermented corn protein (Green Plains Energy, Omaha, NE).

[2]Data are least square means of 8 observations per treatment.

[3]Diets supplemented with amino acids had 20% of corn fermented protein.

Table 3.9. Blood characteristics on d 28 of pigs fed experimental diets[1,2,3]

Item	Control diet	FCP, 10%	FCP, 20%	FCP + Ile	FCP + Trp	FCP + Val	FCP + Ile + Val	FCP + Ile + Trp	FCP + Val + Trp	FCP + Ile + Val + Trp	SEM	*P* value
Blood urea nitrogen, mg/dL	6.25[a]	4.62[abc]	4.50[abc]	4.00[bcd]	5.00[abc]	3.62[cd]	4.62[abc]	5.62[ab]	2.59[d]	3.37[cd]	0.66	0.006
Total protein, mg/dL	4.82	4.86	4.68	4.71	4.75	4.83	4.60	4.66	4.62	4.80	0.10	0.390
Albumin, mg/dL	3.18[a]	3.12[ab]	2.97[abcde]	2.85[cde]	2.83[de]	3.03[abcd]	2.72[e]	2.86[bcde]	2.82[de]	3.11[abc]	0.09	0.009
Peptide YY (ng/mL)	2.69	2.72	2.79	2.75	2.72	2.96	2.96	3.16	2.95	2.94	1.03	0.971
Immunoglobulin G (mg/mL)	6.59	4.59	4.61	5.64	4.93	4.59	4.68	4.70	4.71	3.91	0.91	0.679

[1] FCP, fermented corn protein (Green Plains Energy, Omaha, NE).

[2]Data are least square means of 8 observations per treatment.

[3]Diets supplemented with amino acids had 20% of corn fermented protein.

Table 3.10. Morphology measurements of ileal sampels on d 14 of pigs fed the three basal diets diets[1,2]

Item	Control diet	FCP, 10%	FCP, 20%	SEM	*P* value
Villus height, µm	283.15	260.95	285.18	35.13	0.866
Crypt depth, µm	241.28	224.23	206.91	11.17	0.119
Villus height:crypt depth ratio	1.24	1.33	1.50	0.19	0.534
Lamina propria thickness, µm	67.72	62.49	55.96	5.14	0.3370

[1] FCP, fermented corn protein (Green Plains Energy, Omaha, NE).

[2]Data are least square means of 8 observations per treatment.

Table 3.11. Intestinal microbial protein concentrations (mg/g, dry matter), rate of fermentation of volatile fatty acids, and ammonia concentration in feces on d 14 of pigs fed the three basal diets[1,2]

Item	Control diet	FCP, 10%	FCP, 20%	SEM	*P* value
Microbial protein, mg/g	2.87	3.74	3.43	0.34	0.199
Volatile fatty acids, µmol/100 µmol					
Acetate	291.79	279.63	259.89	29.73	0.160
Propionate	109.31	105.35	88.81	9.92	0.299
Butyrate	66.20	58.38	46.28	8.30	0.265
Isobutyrate	14.34	12.11	11.36	1.18	0.205
Isovalerate	23.62	18.61	20.51	2.05	0.221
Valerate	23.75	22.70	19.76	2.73	0.551
Ammonium (NH_4^+), µmol/g dry matter	139.24	98.00	128.31	13.69	0.123

[1] FCP, fermented corn protein (Green Plains Energy, Omaha, NE).

[2]Data are least square means of 8 observations per treatment.