THE NUTRITIONAL VALUE OF A NEW TYPE OF SOYBEAN MEAL FOR CHICKENS AND PIGS AND THE IMPACT OF REDUCING SOYBEAN MEAL INCLUSION IN DIETS FOR GROWING PIGS

BY

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DISSERTATION

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Abstract

Ten experiments were conducted with the objective of testing 3 hypotheses: (1) soybeans can be genetically improved without negatively affecting the nutritional value of the meal for poultry or pigs; (2) dietary protein can be reduced by reducing soybean meal (**SBM**) inclusion while supplementing with synthetic amino acids (**AA**) without affecting growth performance of pigs; (3) dietary protein does not influence net energy (**NE**) in corn-SBM diets fed to growing pigs. In experiments 1, 2, and 3, the objective was to compare the nitrogen-corrected true metabolizable energy (**TME_n**), standardized AA digestibility, and apparent ileal P digestibility between soybean expellers from high-oil soybeans (**SBE-HO**) and conventional soybeans (**SBE-CV**). Results demonstrated that SBE-HO had greater ($P < 0.05$) TME_n (3.261 kcal/g DM) compared with SBE-CV (3.162 kcal/g DM), but AA digestibility was not different. However, SBE-HO had greater ($P < 0.05$) concentrations of some digestible AA. In broiler chickens, the apparent ileal P digestibility was not different between SBE-CV (46.8%) and SBE-HO (40.6%). In experiments 4, 5, and 6 the objective was to test the hypothesis that standardized ileal digestibility (**SID**) of AA, metabolizable energy (**ME**), and standardized total tract digestibility (**STTD**) of P in SBE-HO are not different when compared with SBE-CV. Results indicated that whereas SID of some indispensable AA (Arg, Ile, and Lys) did not differ between the two types of soybean expellers, other AA had greater ($P < 0.05$) digestibility in SBE-CV than in SBE-HO, although SBE-HO had greater ($P < 0.05$) concentrations of digestible AA. No differences in ME were observed between SBE-HO and SBE-CV. Inclusion of microbial phytase improved P digestibility, but STTD of P was not different between SBE-CV and SBE-HO. It was concluded that SBE-HO, due to higher concentrations of digestible indispensable AA, requires slightly less inclusion than SBE-CV without affecting ME or STTD of P. In experiment 7, the objective was to test the

hypothesis that feeding intact protein from SBM to growing pigs instead of a combination of SBM and synthetic AA results in greater nitrogen retention and DE without affecting ME in the diet. Results indicated that reducing SBM inclusion and replacing with synthetic AA and corn resulted in a decrease (linear, $P < 0.05$) in apparent total tract digestibility of dry matter and gross energy, as well as a reduction (linear, $P < 0.05$) in absorbed and retained nitrogen. However, nitrogen retention efficiency improved (linear, $P < 0.05$) when synthetic AA were used. The DE in diets also decreased (linear, $P < 0.05$) with reduced SBM inclusion, whereas ME was unaffected. In experiment 8, the objective was to test the hypothesis that reducing dietary protein in corn-SBM diets will not increase diet NE and will not affect growth performance, carcass composition, nutrient deposition, intestinal morphology, blood cytokine concentrations, or the abundance of genes for intestinal AA transporters. Results indicated that reducing SBM inclusion and adding synthetic AA did not affect average daily gain, feed intake, carcass characteristics, or nutrient deposition. Whereas blood urea nitrogen decreased (linear, $P < 0.05$) with reduced protein, other blood components, intestinal morphology, and abundance of AA transporter genes were unaffected by dietary treatment. Net energy in diets tended to decrease (linear, $P < 0.10$) with reduced crude protein levels, and bacterial protein in the colon decreased (linear, $P < 0.05$). It was concluded from the experiment that reducing dietary protein did not impact growth, blood markers, intestinal morphology, or NE in diets. In experiments 9 and 10, two experiments were conducted to test the hypothesis that diet protein concentration does not affect apparent ileal digestibility (**AID**) of starch, SID of AA, or NE in diets fed to growing pigs. Results indicated that AID of starch was unaffected by dietary protein, whereas SID of protein and all AA increased (linear, $P < 0.05$) as dietary protein was reduced. Results also indicated that NE, which was measured using indirect calorimetry, did not change as dietary

SBM was reduced and inclusion of synthetic AA and corn increased, whereas DE and ME decreased (linear, $P < 0.05$) with reduced dietary protein. In conclusion, reducing protein in diets did not influence starch digestibility, but improved the SID of indispensable AA, and overall did not influence NE in diets fed to growing pigs. Overall, results of the ten experiments indicated that soybeans can be genetically improved without negatively affecting the nutritional value of the meal for poultry or pigs. It is also concluded that if dietary protein is reduced by reducing SBM inclusion and increasing the inclusion of corn and synthetic AA, growth performance of pigs and NE of diets are not influenced, but nitrogen retention is reduced indicating reduced protein synthesis in pigs fed diets with reduced protein.

Keywords: soybean meal, amino acids, pig, chicken, metabolizable energy, net energy

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CHAPTER 1: Introduction

Soybean meal (**SBM**) is a primary protein source in animal nutrition, and advancements in soybean cultivation have led to the development of new varieties that enhance both nutritional characteristics and yield (Baker et al., 2011; Alaswad et al., 2021). However, in some cases, the development of high-oil soybean varieties has resulted in a reduction in either protein concentration or crop yield (Tamagno et al., 2022). These changes impact the nutritional value of the meal that is obtained after oil extraction from soybeans.

Alongside the progress in soybean production, the synthetic amino acid (**AA**) industry has also advanced. Since the early 1900s, synthetic AA have been produced through various methods, including protein hydrolysis, chemical synthesis, fermentation, and enzymatic synthesis (Izumi et al., 1978). The commercial availability of synthetic AA has enabled their inclusion in animal diets, thereby reducing the need for protein ingredients like SBM and minimizing the excess of amino acids in these diets.

Reducing dietary protein while ensuring an adequate supply of indispensable AA can lower nitrogen excretion without negatively affecting growth performance, reduce feed costs, and potentially increase the net energy (**NE**) in diets (Kerr et al., 1995; Wang et al., 2018). However, low-protein diets carry the risk of an imbalance in indispensable AA, which can negatively affect growth performance, decrease daily nitrogen retention, and increase fat deposition while reducing muscle growth (Li et al., 2016; Wang et al., 2018).

Therefore, 10 experiments were conducted to test three hypotheses: 1) soybeans can be genetically improved without negatively impacting the nutritional value of the meal for poultry or pigs; 2) dietary protein can be reduced by decreasing SBM inclusion while supplementing

with synthetic AA without affecting growth performance of pigs; and 3) dietary protein does not influence NE in corn-SBM diets fed to growing pigs.

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CHAPTER 2: The nutritional value of soybean meal and use of synthetic amino acids in low protein diets for pigs and chickens: Review of literature

Introduction

Soybean meal (**SBM**) is the main protein source in animal nutrition, particularly for poultry and swine. Global production of SBM is primarily driven by the output of soybeans from the United States and Brazil, which together account for more than 80% of global soybean exports (Colussi et al., 2023). In 2023, Brazil achieved a record soybean production of 156 million metric tons, whereas the U.S. produced approximately 113 million metric tons (Colussi et al., 2024). These two countries play a central role in both the global supply of soybeans and the increasing demand for SBM in animal feed. As a result, SBM is expected to remain one of the most important feed ingredients worldwide, with its use in pig and poultry diets accounting for about 70% of domestic SBM consumption in the U.S. (Soybean Meal Info Center, 2022).

Soybean meal is renowned for its superior nutritional value compared to other protein sources. It is rich in indispensable amino acids (**AA**) such as Lys, Thr, and Trp, which are often limiting in other plant-based feeds (Stein et al., 2016). The digestibility of these AA in SBM is also high, with standardized ileal digestibility (**SID**) values typically ranging from 87% to 91% for Lys, and 84% to 93% for other indispensable AA (Lagos and Stein, 2017; Mathai et al., 2017). This high digestibility makes SBM an ideal protein source for growing pigs and broiler chickens, contributing to optimal growth performance and feed efficiency (Gonzalez-Vega and Stein, 2012). Additionally, SBM provides considerable energy, with its metabolizable energy

(**ME**) ranging from 3,500 to 4,000 kcal/kg on a dry matter (**DM**) basis, which further enhances its value as a primary feed ingredient (Li et al., 2015).

In recent years, the incorporation of synthetic AA into animal diets has become a significant development in animal nutrition. Synthetic AA, such as Lys, Met, and Thr, are increasingly used to supplement diets that contain lower levels of crude protein (**CP**). This allows for precise amino acid balancing while maintaining optimal growth performance and feed efficiency, especially in low-protein diets. By supplementing with synthetic AA, it is possible to reduce dietary CP by 3–4 percentage points without compromising animal performance (Toledo et al., 2014; Kerr et al., 2003). In fact, studies have shown that this approach can improve nitrogen utilization, reduce nitrogen excretion, and mitigate environmental pollution, all while sustaining productivity (Gloaguen et al., 2014). Furthermore, the use of synthetic AA has been associated with improved nitrogen retention and decreased ammonia emissions from livestock facilities, contributing to more sustainable agricultural practices (Portejoie et al., 2004; Wang et al., 2018).

This review aims to summarize the nutritional value of SBM in pig and poultry diets, highlighting its AA profile and energy concentration, as well as discussing the impact of supplementing SBM-based diets with synthetic AA. Advances in AA supplementation have resulted in improved growth performance and nitrogen efficiency in low-protein diets, presenting a strategy for optimizing animal nutrition while reducing environmental impact.

Soybean meal production

In 2023, the production of soybeans from the major global producers was as follows: Brazil achieved a record production of 156 million metric tons, the United States produced

approximately 113.3 million metric tons, and Argentina, severely impacted by drought, produced around 25 million metric tons (Colussi et al., 2023). Brazil and the U.S. account for approximately 80% of global soybeans exports (Colussi et al., 2024).

Brazilian soybean producers generally have lower production costs compared with U.S. producers. This cost advantage is attributed to lower land costs, greater yields, and less expensive labor in Brazil. Consequently, Brazilian producers tend to have greater national average net returns per acre than U.S. producers. For instance, in 2020, Brazilian producers earned an average of $118.48 per acre, whereas U.S. producers earned $92.35 per acre (Valdes et al., 2023). Brazilian producers also benefit from a depreciated currency, making their soybeans more competitively priced in international markets, including China. This advantage is reflected in the lower prices for Brazilian soybeans compared with U.S. soybeans. For instance, in August 2020, Brazilian free on-board prices were $358.96 per metric ton, whereas U.S. prices were $364.57 per metric ton (Valdes et al., 2023). There are also differences in government support programs between the two countries, with Brazil providing higher levels of support to its soybean producers compared with the U.S. This support helps Brazilian producers maintain their competitive edge in the global soybean market (Valdes et al., 2023).

Brazil exported around 97 million metric tons of soybeans, the U.S. exported approximately 55 million metric tons, and Argentina's exports were significantly reduced due to lower production, with an expected export volume of approximately 6 million metric tons (Colussi et al., 2023). China remains the largest importer of soybeans, projected to import around 100 million metric tons for the 2022/2023 marketing year, most of it sourced from Brazil and the U.S. (USDA, 2023).

Soybean meal production has been increasing steadily since the early 20th century, and SBM is one of the main ingredients in diets for pigs and poultry in the U.S. In 2023, the U.S. produced over 47 million metric tons of SBM for both domestic and export markets (Huang, 2023). From the domestic SBM used, around 70% was used to feed broilers and pigs (Soybean Meal Info Center, 2022). Most of the increase in SBM production is due to two main factors: The increased livestock production and the superior nutritional value compared with other protein sources (Ruiz et al., 2020).

Soybean meal processing

The primary product of the soybean harvest is full-fat soybeans (**FFSB**). Over the past 100 years, research has established the necessity of heat treatment for FFSB to eliminate anti-nutritional factors (**ANF**). As a result, FFSB is regularly extruded or roasted before use. Temperature control during processing is crucial for optimizing FFSB quality and improving animal performance. An extrusion temperature of around 125 °C yields optimal results in terms of reducing residual ANF (Van Eys and Ruiz, 2021).

A soybean consists of two cotyledons, which make up about 90% of its weight, along with a seed coat or hull (8% of weight), and smaller structures called the hypocotyl and the plumule (Van Eys and Ruiz, 2021). The cotyledons contain proteins and lipids, which are the primary nutritional components of soybean products. They also serve as the main storage for carbohydrates, enzymes, and anti-nutritional factors.

Various soybean products are obtained through the separation or extraction of different components of the soybean. Crushing soybeans yields crude oil as a major product, which is then refined and separated into lecithin and refined oil for use in human and animal diets (Shurtleff and Aoyagi, 2007). Soybean meal, the most significant product by volume, is obtained from de-

fatted flakes, which are an intermediate product requiring further treatment. Two main processes are used to extract oil and obtain de-fatted flakes: the expeller process (mechanical extraction) and solvent extraction, with the latter being the most efficient and widely used method (Van Eys and Ruiz, 2021). After extraction, the flakes are de-solventized and toasted to eliminate heat-labile anti-nutritional factors (Ruiz et al., 2020; Van Eys and Ruiz, 2021).

The energy content of SBM can vary depending on several factors, including processing methods, residual oil content, and the presence of anti-nutritional factors. For example, over-processing can reduce the energy value of SBM by damaging amino acids and lowering protein digestibility (Gonzalez-Vega et al., 2011). Conversely, under-processing can leave trypsin inhibitors active, impairing nutrient utilization and reducing energy availability.

The residual oil content in SBM also significantly impacts the energy value. SBM typically contains 1-2% residual oil, which contributes to its digestible energy (**DE**) and ME values. However, the oil content can vary depending on the extraction process. Mechanically extracted SBM contains higher oil levels (5-7%), which results in higher energy values (Powell et al., 2011; Pacheco et al., 2014). This difference is often reflected in mechanically-extracted soybeans, which have, on average, 275 kcal more ME for pigs and approximately 400 kcal more nitrogen-corrected true metabolizable energy (**TME_n**) for chickens compared with dehulled, solvent-extracted SBM on an as-fed basis (NRC, 1994; Zhang et al., 1993; NRC, 2012).

Energy concentration

Soybean meal generally consists of approximately 48% crude protein, 35-40% carbohydrates, with the majority of carbohydrates comprising non-starch polysaccharides (NSP), sucrose, and oligosaccharides (raffinose and stachyose; NRC, 2012). These oligosaccharides contribute to the

gross energy of SBM but are indigestible by monogastric animals due to the absence of specific enzymes, leading to fermentation in the hindgut by microbes (Ruiz et al., 2020).

According to NRC (1994), the TME_n of SBM is 2,760 kcal/kg DM, which is greater than that of canola meal (2,225 kcal/kg DM), but lower compared with corn (3,900 kcal/kg DM), barley (3,260 kcal/kg DM), distillers dried grains with solubles (DDGS, 3,330 kcal/kg DM), or fish meal (3,230 kcal/kg DM). The lower TME_n in SBM compared to these ingredients is primarily due to the poor digestibility and fermentability of NSP and oligosaccharides (Ruiz et al., 2020). Although oligosaccharides can promote beneficial bacterial populations as prebiotics, elevated levels in diets for chickens and pigs have been associated with adverse effects such as fluid retention and diarrhea, leading to impaired nutrient utilization and leg disorders (Ruiz et al., 2020; Teague et al., 2023). Heat processing of SBM inactivates heat-labile anti-nutritional components but leaves behind soy antigens, phytate, soluble NSP, and oligosaccharides, limiting its suitability for young poultry and swine diets (Van Eys and Ruiz, 2021).

Whereas soy protein concentrate may be used in diets for swine and poultry, its high cost limits practical application in poultry production. Alternative methods for removing oligosaccharides in SBM include enzymatic treatment and fermentation may provide a cost-efficient alternative to soy protein concentrate. Future advancements in soybean breeding aim to lower oligosaccharide concentrations, which, if successful, may result in greater protein and ME in SBM products used in animal nutrition.

The energy value of SBM has been extensively studied in pigs, with methodologies such as determining DE and ME employed (Zhang and Adeola, 2017). According to NRC (2012), SBM contains a DE of 4,066 kcal/kg DM, and a ME of 3,700 kcal/kg DM. Research indicates that the energy in SBM can vary based on factors such as processing methods and the origin of

soybeans. For instance, a study analyzing 22 SBM samples from different countries found that DE values ranged from 3,500 to 4,000 kcal/kg DM, and ME values ranged from 3,200 to 3,800 kcal/kg DM (Li et al., 2015). These variations underscore the importance of considering SBM source and processing when evaluating its energy content.

Another study estimated the DE and ME of SBM from different states in the U.S. to be approximately 4,261 and 4,044 kcal/kg, respectively, on a DM basis (Sotak-Peper et al., 2015). In a similar study, researchers indicated the ME in SBM to be 3,750 kcal/kg DM (Lopez et al., 2020). These findings were greater than the DE and ME values reported by NRC (2012), suggesting that the DE and ME values may have been underestimated. This underestimation could be due to changes in the composition of SBM over time, possibly due to changes in the composition of soybeans. Alternatively, the increased ME in SBM determined in recent years may reflect improved techniques for determining ME values in feed ingredients (Lopez et al., 2020).

Cereal grains, such as corn, wheat, and barley, are primary energy sources in swine diets due to their high starch content and energy density. Corn, the most commonly used cereal grain, has a DE value of approximately 3,800 kcal/kg DM and an ME value of 3,650 kcal/kg DM (NRC, 2012). These values are slightly higher than those of SBM, primarily due to corn's lower fiber and higher starch content. Similarly, wheat has a DE value of 3,908 kcal/kg DM and an ME value of 3,750 kcal/kg DM (NRC, 2012), making it a more energy-dense ingredient than SBM.

When compared with other protein sources, SBM generally provides more energy due to its relatively low fiber levels. For example, canola meal has a DE of approximately 3,273 kcal/kg DM and an ME value of 3,000 kcal/kg DM (NRC, 2012). These values are significantly lower than those of SBM, primarily due to canola meal having higher fiber and lower residual oil

content. However, corn DDGS typically have higher energy values than SBM due to their higher fat content. The DE in corn DDGS is reported to be around 4,140 kcal/kg DM, and ME around 3,900 kcal/kg DM (Stein and Shurson, 2009). It was indicated that corn DDGS contains approximately 8-10% higher ME content than SBM on a DM basis (Stein et al., 2006).

In contrast, animal-based protein sources, such as fish meal and poultry byproduct meal, often have greater energy values than SBM due to their higher fat concentration. Fish meal, for instance, has a DE of approximately 4,224 kcal/kg DM and an ME value of 3,765 kcal/kg DM (NRC, 2012). These values are greater than in SBM, making fish meal a valuable ingredient for high-energy diets, particularly for weanling pigs. However, the availability and often high costs limit the use of these ingredients.

Results of recent research contrasts with previously reported values regarding the energy concentration in SBM, indicating that the net energy (**NE**) in SBM may be equal to or close to that of corn (Li et al., 2017; Cemin et al., 2020; Lee et al., 2021). However, further investigations are needed to determine the NE value of SBM, given its economic significance in diet formulation (Cemin et al., 2020; Munoz, 2020).

Amino acid digestibility

Research to determine the digestibility of AA in SBM has advanced over the years, establishing SBM as a high-quality protein source for poultry. Early methods to assess AA digestibility, such as the T-cannula procedure developed in the 1970s (Ruiz et al., 2020), were critical in improving the understanding of AA digestibility in SBM fed to pigs. The introduction of SID has further underscored the superior AA profile of SBM compared with other plant protein sources (Gonzalez-Vega and Stein, 2012; Berrocoso et al., 2015; Liu et al., 2016). These advances in

digestibility measurement have helped establish SBM as a key ingredient in swine and poultry diets, thanks to its high digestibility of indispensable AA.

Determination of AA digestibility in poultry has also evolved over the past 50 years. The precision-fed rooster assay, introduced by Parsons (1985), remains a standard method for evaluating AA digestibility. Research by Parsons et al. (1992) revealed that heat processing, such as autoclaving, negatively affects lysine concentrations in SBM, emphasizing the importance of proper heat treatment to maintain protein quality. Soybean meal protein solubility, determined by KOH, correlates with improved in vivo performance in poultry (Araba and Dale, 1990), further highlighting the importance of processing methods.

The historical understanding of SBM's anti-nutritional factors, such as trypsin inhibitors and lectins, has also shaped poultry nutrition. Trypsin inhibitors, which were first isolated in 1945 (Kunitz), can negatively impact protein digestion by interfering with enzyme activity in the pancreas (Green and Lyman, 1972). Excess intake of trypsin inhibitors has been linked to pancreatic hypertrophy in poultry and rodents, although this response is less pronounced in swine (Liener, 1994). The residual presence of trypsin inhibitors in commercial SBM, particularly outside the U.S., has contributed to challenges like rapid feed passage syndrome (Aderibigbe et al., 2020). Recent efforts suggest that directly measuring residual trypsin inhibitors is critical for ensuring the quality of SBM and preventing adverse effects on poultry performance (Ruiz et al., 2020).

Soybean meal is widely recognized for its high SID of indispensable AA. It has been reported that the SID of lysine in conventional SBM typically ranges from 87% to 91% for growing pigs (Stein et al., 2016). For other indispensable AA, SID values in SBM generally range between 84% and 93%, with methionine at 86-91%, threonine at 82-87%, and tryptophan

at 85-91% (Lagos and Stein, 2017; Mathai et al., 2017). These consistently high SID values across studies highlight the superior quality of SBM as a protein source for swine diets.

The SID of AA in SBM can vary based on factors such as the origin of the soybeans and processing methods. Soybean meal from the United States, Brazil, and Argentina have slight variations in SID values, with U.S. SBM generally having 1-3 percentage points higher SID for most essential AA compared with SBM from other regions (Karr-Lilienthal et al., 2004). The SID of lysine in SBM from different geographical locations ranged from 86.2% to 91.3%, with these variations attributed to differences in growing conditions and soybean varieties (Ravindran et al., 2014).

In comparison to SBM, corn generally has lower SID of most AA. The SID of Lys in corn typically ranges from 74% to 78%, which is less than the 87-91% observed in SBM (NRC, 2012). For other indispensable AA, corn generally has SID values between 75% and 89%, with Met at 85-89%, Thr at 75-80%, and Trp at 77-83% (NRC, 2012).

Canola meal generally has lower SID of most AA compared with SBM. The SID of Lys in canola meal range from 75.2% to 78.1%, and the SID of Met ranges from 84.5% to 88.0% (Maison and Stein, 2014). The SID of Lys in canola meal average 76.2%, which is approximately 12 percentage points lower than in SBM (Adewole et al., 2017). This difference is primarily attributed to the greater fiber content and the presence of glucosinolates in canola meal, which can interfere with protein digestion.

Corn DDGS generally has lower and more variable SID values than SBM. The SID of lysine in corn DDGS ranges from 55.3% to 84.7%, with an average of approximately 63-70% (Stein and Shurson, 2009). This variation in SID values is primarily due to heat damage during

the drying process, which can lead to Maillard reactions between Lys and reducing sugars, thus reducing its digestibility (Parsons et al., 2023).

In contrast, high-quality fish meal typically has SID values comparable to or slightly greater than SBM for some AA. For instance, the SID of lysine in high-quality fish meal has been reported to be 91.3%, and the SID of methionine is 93.7% (Kim and Easter, 2001). These high SID values in fish meal are attributed to the highly digestible nature of animal proteins and the lower fiber content compared to plant protein sources. However, the digestibility of fish meal can vary depending on its source and processing methods (Rojas and Stein, 2012).

Meat and bone meal typically has lower and more variable SID of AA than SBM. The SID of Lys in meat and bone meal is around 65%, and the SID of Met around 79% and these values have a large variability (Kong et al., 2014). This variability is mainly attributed to differences in raw materials, processing conditions, and the presence of bone and connective tissue, which are less digestible than muscle proteins (Parsons et al., 1997).

Poultry by-product meal generally has lower SID of AA than SBM, but greater than in meat and bone meal. The SID of Lys in poultry by-product meal ranges from 68% to 82%, and the SID of Met ranges from 73% to 85% (Bandegan et al., 2015). The variability in SID of AA for poultry by-product meal depends on the quality of raw materials and processing conditions, with higher-quality products having SID values closer to those of SBM for some AA.

Processing techniques play a crucial role in the digestibility of AA in SBM. Moderate heat treatment during the solvent extraction process is beneficial because it inactivates trypsin inhibitors and other ANF, improving digestibility (Gonzalez-Vega et al., 2011). However, excessive heat treatment can significantly reduce the SID of AA, particularly Lys. For example,

autoclaving SBM at 125°C for 30 minutes reduces the SID of Lys from 93.0% to 84.2% (González-Vega et al., 2011).

Trypsin inhibitors are primary anti-nutritional factors in soybeans that affect protein digestibility. Raw soybeans contain high levels of trypsin inhibitors, which can significantly reduce the SID of AA by inhibiting proteolytic enzyme activity in the small intestine (Stein et al., 2008). Inadequately processed SBM retains trypsin inhibitor activity, which can reduce the SID of Lys and other indispensable AA (Stein et al., 2008). Proper heat treatment during processing inactivates most trypsin inhibitors, resulting in high SID values for SBM (Hoffmann et al., 2019). Soybean meal also contains lectins, which can bind to the intestinal mucosa and interfere with nutrient absorption (Gilani et al., 2012). Although SBM contains phytate, its impact on protein digestibility is less significant than in some other plant protein sources due to the higher overall digestibility of soybean proteins (Ravindran et al., 2014).

Phosphorus digestibility

Conventional SBM generally contains between 0.60% and 0.70% total P on an as-fed basis (NRC, 2012). A significant portion of this P (approximately 60-70%) is bound in phytate form, which is poorly digestible for monogastric animals (Raboy, 2009; Rojas and Stein, 2012; Sotak-Peper et al., 2016). The phytate-bound P content in SBM ranges from 0.38% to 0.46%, while the non-phytate P content typically ranges from 0.18% to 0.25% (Almeida and Stein, 2012; She et al., 2018).

The STTD of P in conventional SBM for growing pigs is approximately 48%, according to NRC (2012). This value is consistent with several studies, though some variation is observed depending on SBM source and experimental conditions. Almeida and Stein (2010) reported

STTD values for P in SBM ranging from 46.1% to 50.7%, with an average of 48.3%. Rodríguez et al. (2013) found STTD values for P in SBM from different origins ranging from 44.5% to 51.2%, with variations attributed to differences in processing conditions and soybean varieties. More recently, She et al. (2017a) established STTD values for P in conventional SBM at 47.6%, which closely aligns with NRC (2012) values.

In broiler chickens, phosphorus digestibility in SBM has traditionally been expressed as ATTD or AID. Ravindran et al. (2006) reported that the ATTD of P in conventional SBM for broiler chickens typically ranges from 32% to 38% without phytase supplementation. This lower digestibility compared to pigs is largely due to the shorter digestive tract and faster passage rate in poultry. Liu et al. (2014) found similar AID values ranging from 38.5% to 43.2% for conventional SBM in 21-day-old broiler chickens. More recently, Sommerfeld et al. (2018) reported ATTD values for P in SBM for broilers ranging from 35.6% to 40.2%, depending on bird age and SBM source. Rodehutscord et al. (2017) reviewed several studies and concluded that the average ATTD of P in conventional SBM for broiler chickens is approximately 36%, with variations due to bird age, diet composition, and methodology.

The primary factor limiting P digestibility in SBM is its phytate content. Phytate (myo-inositol hexakisphosphate) binds P strongly, making it largely unavailable to monogastric animals due to insufficient endogenous phytase production (Selle et al., 2009; Cowieson et al., 2017). Conventional SBM contains approximately 1.2-1.6% phytate, which binds approximately 60-70% of the total P (Rojas and Stein, 2012; She et al., 2018). The negative impact of phytate on P digestibility is well established, with higher phytate concentrations consistently linked to lower P digestibility (Raboy, 2009; Cowieson et al., 2017). Research has focused on developing low-phytate soybean varieties. Erdman (1979) reported that the STTD of P in low-phytate SBM

could reach 70-75%, which is significantly higher than conventional SBM. Similarly, Dilger and Adeola (2006) found that reducing phytate content in SBM increased P digestibility by up to 20 percentage points in both pigs and broiler chickens.

Processing conditions during SBM production can significantly affect P digestibility. Excessive heat treatment during the solvent extraction and desolventization processes can form insoluble complexes between phytate and proteins, further reducing P digestibility (Newkirk and Classen, 2001; González-Vega and Stein, 2012). Rojas and Stein (2012) demonstrated that moderate heat treatment (85-95°C) during SBM processing slightly improved P digestibility compared to lower temperatures, likely due to partial degradation of phytate. However, excessive heat treatment (>120°C) reduced P digestibility by 5-10 percentage points, primarily due to the formation of insoluble protein-phytate complexes. Particle size also influences P digestibility in SBM. Kim et al. (2005) reported that reducing the particle size of SBM from 900 to 600 μm increased the ATTD of P in broiler chickens by approximately 4 percentage points, likely due to the increased surface area for enzymatic action.

The calcium to phosphorus ratio (Ca:P) in the diet significantly affects P digestibility in SBM for both pigs and poultry. High dietary calcium levels can form insoluble calcium-phytate complexes in the gastrointestinal tract, further reducing P digestibility (Selle et al., 2009; González-Vega et al., 2015). González-Vega et al. (2015) demonstrated that increasing the Ca:P ratio from 1.0:1 to 2.0:1 reduced the STTD of P in SBM-based diets for pigs by approximately 10 percentage points. Similarly, Tamim et al. (2004) reported that increasing dietary calcium levels from 0.5% to 1.0% reduced the ATTD of P in SBM for broiler chickens by 6-8 percentage points. Maintaining an appropriate Ca:P ratio is therefore critical for maximizing P digestibility in SBM-based diets. For growing pigs, a Ca:P ratio between 1.1:1 and 1.5:1 is generally

recommended, while for broiler chickens, ratios between 1.8:1 and 2.2:1 are typically advised (NRC, 2012; Cowieson et al., 2017).

Phosphorus digestibility in SBM is generally higher than in cereal grains for both pigs and poultry. According to NRC (2012), the STTD of P in corn, wheat, and barley for pigs is approximately 26%, 50%, and 30%, respectively, compared to 48% for SBM. Almeida and Stein (2012) reported STTD values for P as follows: corn (25.2%), wheat (46.3%), barley (28.9%), and SBM (48.3%). The higher P digestibility in SBM compared to corn and barley is primarily attributed to differences in phytate concentration and structure (Almeida and Stein, 2012; Rojas and Stein, 2012). For broiler chickens, Ravindran et al. (2006) reported ATTD values for P of approximately 20% for corn, 46% for wheat, 30% for barley, and 36% for SBM.

Compared to other plant protein sources, SBM has moderate P digestibility. Rodríguez et al. (2013) reported STTD values for P in pigs as follows: SBM (48.3%), canola meal (31.0%), sunflower meal (35.6%), and pea protein concentrate (56.7%). The lower P digestibility in canola meal compared to SBM is attributed to its higher fiber content and the presence of glucosinolates that may interfere with mineral absorption (Maison and Stein, 2014; She et al., 2017b). Conversely, the higher P digestibility in pea protein concentrate is related to its lower phytate content and different phytate structure (Adeola and Sands, 2003; Rodríguez et al., 2013).

Animal protein sources generally have significantly higher P digestibility than SBM for both pigs and poultry due to the absence of phytate and the presence of P in more digestible forms. According to NRC (2012), the STTD of P in fish meal and meat and bone meal for pigs is approximately 80% and 70%, respectively, compared to 48% for SBM. The higher P digestibility in animal protein sources is primarily attributed to the absence of phytate and the presence of P in more digestible forms, including calcium phosphates (González-Vega and Stein, 2012).

Synthetic amino acids and low protein diets for pigs and chickens

Amino acids are essential for a wide range of applications, including in food, feed, pharmaceuticals, and cosmetics, which has led to significant growth in their global production. As of 2016, the AA market was valued at approximately US$13 billion, with lysine, methionine, threonine, and tryptophan dominating the feed market (Ikeda and Takeno, 2020). The majority of L-AA production is achieved through microbial fermentation, particularly using *Corynebacterium glutamicum* (Ikeda and Takeno, 2020), though other methods such as enzymatic, extraction, and synthetic processes are also employed depending on the specific AA and its applications (Izumi et al., 1978). These fermentation processes have become more efficient with advancements in genetic engineering, improving strain productivity and reducing by-products (Hirasawa and Wachi, 2016). Synthetic methods, though more reliant on chemical precursors, remain competitive for AA such as methionine, where no viable fermentation method exists (Izumi et al., 1978).

As the demand for specific AA continues to increase, improvements in production technologies are vital. Fermentation methods, while advantageous due to low raw material costs, are limited by product concentration and by-product formation. Synthetic and enzymatic methods offer advantages in large-scale production due to ease of isolation and purification, with continued innovations in immobilized enzyme systems providing further opportunities for efficiency (Izumi et al., 1978; Ikeda, 2016). These advances in AA production are critical for addressing the nutritional needs of livestock, especially in the context of low-protein diets for poultry and pigs, where the use of synthetic AA can optimize amino acid profiles while maintaining cost-effectiveness.

The concept of lowering CP levels in monogastric animal diets while supplementing with synthetic AA has gained considerable attention in recent decades. This approach aims to reduce nitrogen excretion while maintaining optimal growth performance and production efficiency. This review examines the effects of these dietary strategies on growth performance, energy utilization, and nitrogen retention in both pigs and chickens.

Research consistently demonstrates that CP can be reduced by 3-4 percentage points without compromising growth performance when diets are properly supplemented with indispensable amino acids. Toledo et al. (2014) reported that reducing CP from 19% to 15% in growing pigs maintained similar average daily gain and feed conversion ratio when supplemented with lysine, methionine, threonine, and tryptophan.

Kerr et al. (2003) demonstrated that reducing CP from 16% to 12% in growing-finishing pigs with supplemental Lys, Trp, Thr, and Met maintained growth performance while reducing nitrogen excretion by 28%. Similarly, Roux et al. (2011) published findings showing that reducing dietary CP from 17% to 13.5% in growing pigs, with appropriate essential amino acid supplementation, had no negative effects on growth rate or carcass quality.

Htoo et al. (2007) reported that supplementing low-CP diets with branched-chain amino acids (particularly isoleucine) allowed CP reduction from 18% to 14% without compromising growth performance in weaned pigs. More recently, Zhao et al. (2019) reduced dietary CP from 17% to 13.5% and observed no impact on growth performance, but lower N excretion and lower faecal *E. coli* count.

However, more aggressive protein reduction (more than 4 percentage units) often results in performance decreases even with amino acid supplementation. Gloaguen et al. (2014)

observed that reducing CP from 17% to 12% in weaner pigs resulted in decreased ADG despite supplementation with all essential amino acids. This suggests limitations beyond the commonly considered limiting amino acids.

Recent work by Millet et al. (2018) indicates that supplementation with branched-chain amino acids (valine, isoleucine, leucine) alongside the traditional limiting amino acids can permit CP reduction of up to 5 percentage units without performance losses.

In broilers, CP can generally be reduced by 2-3 percentage points with proper synthetic amino acid supplementation without negatively affecting growth. Belloir et al. (2017) demonstrated that broiler diets could be reduced from 22% to 19% CP with supplemental amino acids while maintaining body weight gain and feed efficiency.

Chrystal et al. (2020) reported that reducing dietary CP from 21% to 17% in broiler grower diets with balanced amino acid supplementation maintained growth performance while reducing nitrogen excretion. Additionally, Hilliar et al. (2020) documented that broiler diets could be formulated with CP levels as low as 16.5% (a reduction of approximately 4 percentage points from industry standards) without compromising growth when all limiting amino acids were adequately supplemented.

Kidd et al. (2013) observed that reducing CP from 21% to 19% in broiler starter diets with supplementation of Met, Lys, Thr, Val, Ile, and Arg maintained performance comparable to birds fed standard CP diets. More recently, Son et al. (2024) indicated that a moderate reduction of 1% of dietary CP does not affect growth performance but can reduce N excretion and improve litter quality.

However, further protein reduction typically leads to performance decreases. Ospina-Rojas et al. (2014) found that reducing CP below 18% in broiler starter diets resulted in decreased weight gain and feed efficiency despite supplementation with Lys, Met, Thr, Val, and Ile.

Layer hens appear more adaptable to low-CP diets. Heo et al. (2023) reported that CP could be reduced by up to 6 percentage points (from 19% to 13%) without affecting egg production when supplemented with essential amino acids.

One of the primary benefits of low-CP diets with synthetic amino acids is improved nitrogen utilization efficiency. Reducing CP by 3-4 percentage points typically results in 25-30% less nitrogen excretion. Portejoie et al. (2004) demonstrated that reducing CP from 20% to 16% in growing pigs decreased nitrogen excretion by 28% while maintaining similar nitrogen retention. Wang et al. (2018) found that for each percentage point reduction in dietary CP, total nitrogen excretion decreased by approximately 8-10%, with the greatest reductions observed in urinary nitrogen (10-12%) compared to fecal nitrogen (3-5%). Improved protein quality through synthetic amino acid supplementation enhances nitrogen retention efficiency. Kim et al. (2012) reported that pigs fed low-CP diets with balanced amino acid profiles retained approximately 69% of ingested nitrogen.

Similar benefits were observed in poultry. Hilliar et al. (2019) reported that reducing CP by 3 percentage points in broiler diets decreased nitrogen excretion by approximately 20% without compromising growth performance when essential amino acids were supplemented.

In laying hens, Mousavi et al. (2013) demonstrated that reducing dietary CP from 16.5% to 14.5% with supplemental essential amino acids maintained egg production while reducing nitrogen excretion by 18%. The environmental implications are significant. Reduced nitrogen

excretion leads to decreased ammonia emissions from animal facilities and manure. Ferguson et al. (1998) documented a 7-10% reduction in ammonia emissions for each percentage point reduction in dietary CP.

Several factors limit the practical implementation of very low-CP diets. Extremely low-CP diets may provide insufficient nitrogen for synthesis of dispensable amino acids. Hofmann et al. (2020) indicated that total CP should not drop below certain thresholds (approximately 16-17% for broilers) to avoid this limitation. Likewise, the NRC (2012) indicates a minimum of nitrogen besides the minimum requirements of standardized ileal digestible AA for optimum growth of pigs. Very low-CP diets may affect intestinal health and function. Qaisrani et al. (2014) reported increased incidence of digestive disorders in broilers fed diets below 17% CP despite amino acid supplementation. Dietary proteins contribute functional properties beyond amino acid supply, including buffering capacity and influence on gut microbiota. These aspects are not fully replicated by synthetic amino acids. Despite reductions in total protein costs, the increased expense of multiple synthetic amino acids can offset savings, particularly when expanding beyond the four primary limiting AA.

Conclusions

In conclusion, SBM remains the main protein source in pig and poultry nutrition due to its high protein content, superior AA profile, and consistent digestibility. Despite variations caused by origin and processing, SBM provides high SID values for indispensable AA and competitive ME levels compared to other plant and animal protein sources. However, limitations such as anti-nutritional factors, oligosaccharides, and phytate-bound phosphorus can affect nutrient availability, particularly in young animals. Advances in synthetic AA production have enabled

precise dietary formulations that allow for significant dietary protein reductions without compromising performance. When adequately supplemented, synthetic AA improve nitrogen efficiency and reduce environmental impacts such as nitrogen excretion and ammonia emissions. Nevertheless, the success of low-CP diets depends on proper AA balancing, minimum nitrogen thresholds, and consideration of non-nutritional functions of intact proteins. Overall, the integration of SBM with synthetic AA represents a sustainable and effective strategy for optimizing performance, reducing feed costs, and minimizing the environmental footprint of swine and poultry production.

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CHAPTER 3: True metabolizable energy, standardized amino acid digestibility, and digestibility of phosphorus in soybean expellers produced from conventional or high-oil varieties of soybeans fed to chickens

Abstract

The objective was to test the hypothesis that nitrogen-corrected true metabolizable energy (**TME_n**), standardized amino acid (**AA**) digestibility, and apparent ileal P digestibility are not different in soybean expellers produced from high-oil soybeans (**SBE-HO**) compared with expellers produced from conventional soybeans (**SBE-CV**). The two soybean expellers contained approximately 46.3 % crude protein (DM basis). In Experiments 1 and 2, 2 precision-fed rooster assays were conducted to determine TME_n and standardized AA digestibility in SBE-CV and SBE-HO using conventional and cecectomized roosters, respectively. For each experiment, 6 replicate White Leghorn roosters per treatment were fasted for 26 h prior to crop intubation with 25 g of sample and excreta were collected for 48 h post-feeding. Data were analyzed as a one-way ANOVA for a completely randomized design. The TME_n of SBE-HO (3.261 kcal/g DM) was greater than SBE-CV (3.162 kcal/g DM). Standardized digestibility of most AA was approximately 90%, and there were no differences between the two soybean expellers, but SBE-HO had greater ($P < 0.05$) concentration of some digestible AA compared with SBE-CV. In Experiment 3, an *ad-libitum*-fed broiler chicken assay was conducted to determine apparent ileal digestibility of P in SBE-HO and SBE-CV. Eighty commercial Ross 308 male chicks were fed a standard corn-SBM diet from 0 to 16 d of age, and experimental

diets from d 17 to 21. The 2 experimental diets had a total Ca:total P ratio of 1.4:1 and TiO_2 was used as a digesta marker. There were 5 chicks per pen and 8 replicate pens per treatment and the pen was the experimental unit. On d 21, chicks were euthanized and ileal digesta were collected. Data were analyzed as in Experiments 1 and 2. Apparent ileal P digestibility for SBE-CV (46.8%) was not different compared with SBE-HO (40.6%). Overall, data indicated that SBE-HO had greater TME_n, similar digestibility of AA and P, but greater digestible concentrations of some AA compared with SBE-CV for broiler chickens.

Keywords: amino acid, digestibility, energy, phosphorus, soybean expellers.

Introduction

Soybean meal (**SBM**) is an important ingredient in pig and poultry production in the U. S. because of the superior protein quality relative to other protein sources (Ruiz et al., 2020). Global soybean production has been increasing as pig and poultry production has increased, but in recent years, elevated biodiesel production is also increasing the demand for soybeans due to increased demand for soybean oil (Fousekis, 2023). The most common methods to extract oil from soybeans are by using solvent extraction or extrusion-expelling. Whereas the solvent extraction method results in a more complete oil extraction, use of extrusion-expelling has become an alternative because of its low capital and operational costs (Pacheco et al., 2014). The soybean expellers that are the co-product of extrusion-expelling of soybeans contain more metabolizable energy compared with solvent extracted SBM (NRC, 2012) because of the larger concentration of residual oil.

To improve soybean production efficiency, new varieties of soybeans have been developed (Liu et al., 2020), and taking advantages of new breeding techniques, improved

management practices, and atmospheric carbon dioxide, it has been possible to increase the yield of soybean seeds (Koester, 2014). However, the improvement of soybean seeds, mainly through genetic modification, has often resulted in a decrease in protein concentration (Tamagno et al., 2022). Nonetheless, a new genetic technology patented as PHOTOSEED by Zeakal Inc. (San Diego, CA, USA) was used to develop a new variety of soybeans. By modifying genes involved in biosynthesis and storage of lipids, it was possible to increase the accumulation of lipid droplets and increase carbon capture (Beechey-Gradwell et al., 2019). This resulted in soybeans that contain higher oil, without negatively affecting crude protein (**CP**) concentration, compared with conventional soybeans. There are, however, no data for the nutritional value of SBM or soybean expellers produced from the high-oil variety of soybeans. Therefore, the objective of this work was to test the hypothesis that nitrogen-corrected true metabolizable energy (**TME_n**), standardized amino acid (**AA**) digestibility, and apparent ileal **P** digestibility of soybean expellers produced from the high-oil soybeans (**SBE-HO**) are not different from expellers produced from conventional soybeans (**SBE-CV**) when fed to broiler chickens.

Materials and methods

The protocols for 3 experiments were reviewed and approved by the institutional Animal Care and Use Committee at the University of Illinois (protocol number 20131).

Ingredients and Analyses

Two samples of conventional and high-oil soybeans (PHOTOSEED) were procured from Zeakal Inc. (San Diego, CA, USA). The beans were extruded and expelled at Insta Pro (Grimes, IA, USA) to produce soybean expellers from the conventional and high-oil varieties of soybeans. Samples of SBE-HO and SBE-CV were analyzed for dry matter (**DM**; method 930.15; AOAC

Int., 2019), and N (method 990.03; AOAC Int., 2019) was determined on a FP628 protein analyzer (Leco Corporation, St. Joseph, MI). Crude protein was calculated as N × 6.25. Amino acids were analyzed on a Hitachi Amino Acid Analyzer (Model No. L8800; Hitachi High Technologies America, Inc., Pleasanton, CA) using ninhydrin for postcolumn derivatization and norleucine as the internal standard. Prior to analysis, samples were hydrolyzed with 6 *N* HCl for 24 h at 110 °C [method 982.30 E(a); AOAC Int., 2019]. Methionine and cysteine were determined as methionine sulfone and cysteic acid after cold performic acid oxidation overnight before acid hydrolysis [method 982.30 E(b); AOAC Int., 2019]. Tryptophan was determined after NaOH hydrolysis for 22 h at 110 °C [method 982.30 E(c); AOAC Int., 2019].

Samples were also analyzed for gross energy (**GE**) using bomb calorimetry (Model 6400; Parr Instruments, Moline, IL). Acid-hydrolyzed ether extract (**AEE**) was analyzed by crude fat extraction using petroleum ether (AnkomXT15, Ankom Technology, Macedon, NY, USA) following hydrolysis using 3 *N* HCl (AnkomHCl, Ankom Technology, Macedon, NY, USA). Soluble dietary fiber **(SDF)** and insoluble dietary fiber **(IDF)** were also analyzed in the 2 sources of SBE on an Ankom Total Dietary Fiber Analyzer (Ankom Technology, Macedon, NY, USA) using method 991.43 (AOAC Int., 2019). Total dietary fiber **(TDF)** was calculated as the sum of SDF and IDF. Trypsin inhibitor units (method Ba 12-75; AOCS, 2006) were determined in the 2 sources of soybean expellers and sugars including glucose, fructose, maltose, sucrose, stachyose, and raffinose were analyzed using high-performance liquid chromatography (Dionex App Notes 21 and 92). Ash was analyzed using method 942.05 (AOAC Int., 2019), and Ca and P (method 985.01 A, B and C; AOAC Int., 2019) were determined using inductively coupled plasma-optical emission spectrometry (ICP-OES; Avio 200, PerkinElmer, Waltham, MA, USA). Sample preparation included dry ashing at 600 °C for 4 h (method 942.05; AOAC Int., 2019) and wet

digestion with nitric acid (method 3050 B; Edgell, 2002). Phytate was analyzed as described by Ellis et al. (1977). Concentration of phytate-bound P in the 2 sources of soybean expellers was calculated as 28.2% of analyzed phytate (Tran and Sauvant, 2004).

Analyses for AA, sugars and oligosaccharides, Ca, P, and Ti were conducted at the Agricultural Experiment Station Chemical Laboratory (University of Missouri, Columbia, MO). Analyses for phytate and trypsin inhibitors were conducted at Eurofins Scientific, Inc. (Des Moines, IA), and all other analysis were conducted at the University of Illinois Urbana-Champaign.

Experiments 1 and 2: TME_n and standardized AA digestibility

Two experiments were conducted with Single Comb White Leghorn roosters using the precision-fed rooster assay (Sibbald, 1976; Parsons et al.,1982; Parsons, 1985). The TME_n was determined in conventional roosters and AA digestibility was determined in cecectomized roosters. Cecectomies were performed as described by Parsons (1985). Roosters were fasted for 26 h prior to being crop intubated with 25 g of sample. Each source of soybean expellers was fed to 6 individually caged roosters per treatment. All roosters were allowed free access to water throughout the experiment. Excreta (feces + urine) were quantitatively collected for 48 h post-feeding on trays placed underneath each cage, after which excreta were stored in a freezer prior to lyophilization. After lyophilization, dried excreta were weighed and ground. Excreta from conventional roosters were analyzed for GE and N, whereas excreta from cecectomized roosters were analyzed for AA as described for ingredients. Subsequently, TME_n and standardized digestibility of AA were calculated (Sibbald, 1976; Parsons et al., 1982).

Experiment 3: Apparent ileal P digestibility

This experiment was conducted using commercial broiler chickens (Ross 308 males) to determine apparent ileal P digestibility in SBE-HO and SBE-CV. Chickens were placed in heated Petersime starter batteries with raised wire floors in an environmentally controlled room, and fed a standard, nutritionally complete corn-SBM pretest diet for 17 d. Chickens had free access to water and feed, but on day 17, chicks were fasted overnight. On d 18, chicks were weighed, wingbanded, and allotted to 1 of 2 dietary treatments, ensuring consistency in average body weight between treatments in a completely randomized design. Average initial body weight at the start of the experimental period was 514 g and there were 8 replicate pens of 5 chickens for each dietary treatment, resulting in a total of 80 chicks in the experiment. Experimental diets were provided on an ad libitum basis from d 18 to 21. Based on analyzed Ca and P levels in SBE-CV and SBE-HO, diets containing SBE-CV or SBE-HO were formulated to contain 0.44 and 0.48% Ca, and 0.32 and 0.35 % total P with test ingredients serving as the sole source of P in each diet. Limestone was added to provide a 1.4:1 total Ca to total P ratio (Table 3.1) as recommended by Rodehutscord (2013). Titanium dioxide was added at 0.5% to each diet as an indigestible marker. Chickens were euthanized on the last day of the experimental period (d 21) via asphyxiation with carbon dioxide gas. Ileal digesta (from Meckel's diverticulum to ileal-cecal junction) were collected. Diets and ileal digesta were analyzed for Ca, P, and Ti as described for ingredients. Calculation of apparent ileal P digestibility followed the procedure by Mutucumarana et al. (2014).

Statistical analysis

Data from all 3 experiments were analyzed by ANOVA using the GLM procedure in SAS (SAS Institute. INC., 2010). Differences between treatments were considered to be significant at $P <$

0.05. For Exp. 1 and 2, the individual rooster was the experimental unit, whereas in Exp. 3, each pen containing 5 chickens was the experimental unit. Outliers were identified as values that deviated from the 1st and 3rd quartile by more than 3 times the interquartile range within treatment. However, no outliers were found.

Results and discussion

Chemical Analysis

Nutrient compositions of SBE-CV and SBE-HO were generally similar (Table 3.2). The CP in both sources were approximately 44%, which is in agreement with previous values (NRC, 2012; Espinosa et al., 2021). The AEE in SBE-CV and SBE-HO also agree with previous values for soybean expellers (Zhang et al., 1993; Karr-Lilienthal et al., 2006; Powell et al., 2011; Pacheco et al., 2014), and the SBE-HO contained slightly more AEE than SBE-CV. The SBE-HO contained slightly more P, non-phytate P, and less TDF compared with SBE-CV. Values for total Ca and total P in SBE-CV and SBE-HO agree with reported values for soybean expellers (Karr-Lilienthal et al., 2006; Pacheco et al., 2014). Gross energy was greater in SBE-HO compared with SBE-CV, which is likely due to the greater AEE in SBE-HO. Concentrations of most indispensable AA were slightly greater in SBE-HO compared with SBE-CV (Table 3.4), which is likely because of greater CP in SBE-HO. Concentrations of indispensable AA in general for both SBE ingredients were close to published values for soybean expellers (NRC, 2012; Espinosa et al., 2021).

TME_n

The TME_n for SBE-HO (3,261 kcal/kg DM) was greater ($P < 0.05$) than for SBE-CV (3,162 kcal/kg DM), and TME_n in both SBE-CV and SBE-HO are in agreement with the TMEn

(3,239 kcal/kg DM) reported for soybean expellers by Zhang et al. (1993). However, values from SBE-CV and SBE-HO were greater than the value of 2,761 kcal/kg DM for dehulled solvent extracted SBM (NRC, 1994). This is likely primarily due to greater AEE in both soybean expellers sources compared with solvent extracted SBM. The fact that SBE-HO had greater TME_n than SBE-CV is also likely partially due to the greater content of CP and slightly higher AEE. The lower TDF in SBE-HO compared with SBE-CV likely also contributed to TME_n differences as greater TDF can reduce TME_n (Parsons et al., 2023) or AME_n in ingredients (Sacranie et al., 2012).

Standardized AA Digestibility

Values for standardized digestibility of AA were not different ($P > 0.05$) between SBE-HO and SBE-CV (Table 3.3). Standardized digestibility of AA obtained for both SBE-CV and SBE-HO were in close agreement with values for soybean expellers obtained using cecectomized roosters (Zhang et al., 1993; Powell et al., 2011). Trypsin inhibitor content is one of the main factors that negatively affects digestibility of CP and AA in SBM (Han et al., 1991). Another factor that can impact digestibility of CP is TDF (Gutierrez et al., 2013). Even though SBE-HO had numerically greater content of trypsin inhibitors compared with SBE-CV, this difference was too small to cause a difference in standardized digestibility of AA. When comparing concentrations of digestible AA between SBE-HO and SBE-CV (Table 3.4), concentrations in SBE-HO were greater ($P < 0.05$) for Asp, Glu, Pro, Val, Phe, Lys, Arg, and Trp due to higher concentration of these AA in the SBE-HO.

Apparent ileal P digestibility

Feed intake of chicks fed the SBE-HO diet was less ($P < 0.05$) than that of chicks fed the SBE-CV diet (Table 3.5). Apparent ileal P digestibility for SBE-HO was 46.8%, which was not

different ($P > 0.05$) from 40.6% for SBE-CV (Table 3.5). Apparent ileal P digestibility values for both SBE-CV and SBE-HO were generally below many values previously reported for P digestibliity for SBM and corn-SBM diets. For example, studies by Nwokolo et al. (1976), Dilger and Adeloa (2006), and Mutucumarana et al. (2014; 2015) reported values of 70% or greater for ileal P digestibility or P retention for SBM. In addition, Munoz et al. (2020), using precision-fed chickens, obtained a value 64% for P digestibility or retention for SBM. Reducing total Ca to total P ratio from 0.80 to 0.56 increased apparent P retention from 33 to 54% in cornstarch-SBM diets fed to 15-d old broiler chicks, indicating that greater total Ca to total P reduces P digestibility or retention (Dilger and Adeola, 2006). In growing pigs, an increased Ca:P ratio also reduced the digestibility of P (Stein et al., 2011). Thus, a large part of the reason for variation in P digestibility and retention among studies may be associated with variation in dietary Ca levels.

Conclusions

In conclusion, SBE-HO contained more total AA and TME_n, but less TDF compared with SBE-CV. There were no differences in AA digestibility between SBE-HO and SBE-CV, but because SBE-HO contains more total AA concentration, the concentration of digestible AA was greater in SBE-HO. Apparent ileal digestibility of P was not different between SBE-HO and SBE-CV.

Tables

Table 3.1. Ingredient composition of experimental diets in Experiment 3 for determination of apparent ileal P digestibility in soybean expellers produced from conventional soybeans (SBE-CV) or from high-oil soybeans (SBE-HO)

	Dietary treatments	
Ingredient, %	SBE-CV	SBE-HO
Dextrose	33.92	33.81
SBE-CV	45.00	-
SBE-HO	-	45.00
Soybean oil	4.00	4.00
Cornstarch	10.00	10.00
Limestone	0.73	0.84
Solka floc[1]	5.00	5.00
Salt	0.40	0.40
Vitamin mix[2]	0.20	0.20
Mineral mix[3]	0.15	0.15
Titanium dioxide	0.50	0.50
Choline Cl 60%	0.10	0.10
Analyzed, %:		
Ca	0.54	0.59
P	0.32	0.29

[1]Powdered cellulose; Fiber Sales and Development Corp., Urbana, OH.

[2]Provided per kilogram of diet: retinyl acetate, 4,400 IU; cholecalciferol, 25 μg; DL-α-tocopheryl

Table 3.1 (cont.)

acetate, 11 IU; vitamin B12, 0.01 mg; riboflavin 4.41 mg; D-pantothenic acid, 10 mg; niacin, 22 mg; and menadione sodium bisulfite complex, 2.33 mg.

[3]Provided per kilogram of diet: manganese, 75 mg from $MnSO_4 \cdot H_2O$; iron, 75 mg from $FeSO_4 \cdot H_2O$; zinc, 75 mg from ZnO; copper, 5 mg from $CuSO_4 \cdot 5H_2O$; iodine, 75 mg from ethylene diamine dihydroiodide; selenium, 0.1 mg from Na_2SeO_3.

Table 3.2. Analyzed composition and nitrogen-corrected true metabolizable energy (TME_n) of soybean expellers produced from conventional soybeans (SBE-CV) or from high-oil soybeans (SBE-HO), as-fed basis

	SBE-CV	SBE-HO
Dry matter	94.56	94.63
Acid-hydrolyzed ether extract	6.57	7.60
Crude protein	43.15	44.56
Lys:CP	6.26	6.42
Total dietary fiber	24.10	22.90
Soluble dietary fiber	5.00	3.00
Insoluble dietary fiber	19.10	19.90
Ash	6.62	6.90
Ca	0.37	0.36
P	0.70	0.77
Phytate	1.60	1.71
Phytate-P[1]	0.45	0.48
Nonphytate-P[2]	0.25	0.28
Nonphytate-P, % of total P	35.71	36.36
Trypsin inhibitor (unit/mg)	5.30	6.00
Glucose	0.05	0.05
Sucrose	5.73	5.08
Maltose	0.28	0.27
Fructose	0.05	0.05

Table 3.2 (cont.)

Stachyose	5.68	5.05
Raffinose	1.62	1.34
Gross energy (kcal/kg)	4,760	4,820
TME_n[3] (kcal/kg DM)	3,162	3,261
SEM of TME_n	28	31

[1]Phytate-P was calculated by multiplying the analyzed phytate by 0.282 (Tran and Sauvant, 2004).

[2]Nonphytate-P was calculated as the difference between total P and phytate-P.

[3]TME_n values were significantly different ($P < 0.05$). TME_n values are means from 6 individually-caged conventional roosters (Experiment 1).

Table 3.3. Standardized amino acid digestibility values of soybean expellers produced from conventional soybeans (SBE-CV) or from high-oil soybeans (SBE-HO), Experiment 2[1]

Amino acid	SBE-CV	SBE-HO	Pooled SEM
Asp	89.3	89.6	0.69
Thr	86.5	85.4	1.30
Ser	88.9	87.3	1.02
Glu	92.3	92.0	0.90
Pro	88.1	87.4	1.05
Gly	65.3	66.4	8.18
Ala	85.8	85.4	1.18
Cys	80.4	80.4	1.69
Val	87.7	87.6	0.82
Met	93.2	92.2	1.33
Ile	91.2	90.7	0.59
Leu	90.6	89.7	0.78
Tyr	88.7	86.0	1.03
Phe	91.6	91.0	0.66
Lys	86.2	86.1	0.91
His	91.1	88.6	1.46
Arg	90.3	88.8	0.87
Trp	95.5	95.3	1.04

[1]Values are means of 6 individually-caged cecectomized roosters. There were no significant differences ($P > 0.05$) between the 2 soybean expellers for digestibility of any amino acids.

Table 3.4. Total and digestible concentrations of amino acids in soybean expellers produced from conventional soybeans (SBE-CV) or from high-oil soybeans (SBE-HO), as-fed basis, Experiment 2[1]

	Total amino acid concentration		Concentrations of digestible amino acids[2]		
Amino acid	SBE-CV	SBE-HO	SBE-CV	SBE-HO	Pooled SEM[3]
Asp	4.83	5.10	4.31	4.57	0.03
Thr	1.71	1.78	1.48	1.52	0.02
Ser	1.92	2.00	1.71	1.75	0.02
Glu	7.65	8.12	7.06	7.47	0.07
Pro	2.05	2.16	1.81	1.89	0.02
Gly	1.87	2.00	1.22	1.33	0.15
Ala	1.87	1.96	1.60	1.67	0.02
Cys	0.70	0.71	0.56	0.57	0.01
Val	1.97	2.06	1.73	1.80	0.02
Met	0.62	0.65	0.58	0.60	0.01
Ile	1.93	1.97	1.76	1.79	0.01
Leu	3.22	3.31	2.92	2.97	0.03
Tyr	1.58	1.67	1.40	1.44	0.02
Phe	2.12	2.19	1.94	2.00	0.01
Lys	2.70	2.86	2.33	2.46	0.03
His	1.09	1.15	0.99	1.02	0.02
Arg	2.94	3.22	2.66	2.86	0.03
Trp	0.57	0.61	0.54	0.58	0.01

Table 3.4 (cont.)

[1]Values are means of 6 individually-caged cecectomized roosters. Digestible concentrations of Asp, Glu, Pro, Val, Phe, Lys, Arg, and Trp were greater ($P < 0.05$) in SBE-HO compared with SBE-CV.

[2]Digestible concentration = (total amino acid concentration × standardized digestibility)/100.

[3]SEM for digestible concentrations.

Table 3.5. Apparent ileal P digestibility of soybean expellers produced from conventional soybeans (SBE-CV) or high-oil soybeans (SBE-HO) and fed to broiler chicks, Experiment 3[1]

Soybean expellers type	Weight gain (g/chicken)	Feed intake (g/chicken)	Gain:feed (g/kg)	Ileal P digestibility (%)
SBE-CV	187	247	755	46.8
SBE-HO	177	236	750	40.6
Pooled SEM	3.1	2.6	8.4	3.01
P-value	0.053	0.012	0.657	0.169

[1]Values are means of 8 pens of 5 chickens from 18 to 21 days of age for weight gain, feed intake, and gain: feed ratio. Ileal P digestibility values are at 21 days of age.

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CHAPTER 4: Digestibility of amino acids, energy, and phosphorus and metabolizable energy in soybean expellers produced from conventional or high-oil varieties of soybeans fed to growing pigs

Abstract

The objective was to test the hypothesis that standardized ileal digestibility (**SID**) of amino acids (**AA**), metabolizable energy (**ME**), and standardized total tract digestibility (**STTD**) of P in soybean expellers produced from a new variety of high-oil soybeans (**SBE-HO**) are not different when compared with expellers produced from conventional soybeans (**SBE-CV**). In Exp. 1, nine barrows (30.0 ± 1.5 kg) that had a T-cannula installed in the distal ileum were allotted to a triplicated 3 × 3 Latin Square design with three diets and three periods in each square. An N-free diet and two diets containing SBE-CV or SBE-HO were used. Pigs were housed individually in fully slatted pens and ileal digesta were collected on d 6 and 7 of each period. Ileal digesta and diets were analyzed for AA, and SID of AA was calculated. Results indicated that the SID of Arg, Ile, and Lys was not different between the two sources of soybean expellers, but the SID of other indispensable AA were greater ($P < 0.05$) in SBE-CV compared with SBE-HO. However, because of greater AA concentration, SBE-HO had greater concentrations of digestible Arg, Lys, Met, and Trp compared SBE-CV. In Exp. 2, thirty pigs (18.3 ± 1.3 kg) were randomly allotted to three diets containing corn, corn and SBE-CV, or corn and SBE-HO as energy sources. Pigs were housed in metabolism crates and feces and urine were separately collected for 4 d after 5 d of adaptation. Feces, urine, and diets were analyzed for gross energy and ME was calculated. Results indicated that ME in SBE-HO was not different from ME in SBE-CV. In Exp. 3, forty-

eight barrows (12.0 ± 1.6 kg) were allotted to six diets. The SBE-CV and SBE-HO were included in diets with three levels of microbial phytase (i.e., 0, 500, or 1,000 units/kg). Pigs were housed in metabolism crates and feces were collected quantitatively for 4 d after 5 d of adaptation. Feces and diets were analyzed for P and the STTD of P was calculated. Results indicated that inclusion of phytase in the diets linearly ($P < 0.001$) increased the STTD of P regardless of source of soybean expellers, but STTD of P was not different between SBE-HO and SBE-CV. It is concluded that if SBE-HO is included in diets for pigs instead of SBE-CV, slightly less soybean expellers is needed due to greater concentration of limiting AA, but ME and STTD of P will not be changed.

Key Words: amino acids, digestibility, energy, phosphorus, pig, soybean expellers

Introduction

Soybean oil demand has increased in recent years due to increased use in food and biofuel applications (Santeramo and Searle, 2019). As the soybean oil production increases, production of the co-product, which is soybean meal or soybean expellers, also increases. Soybean meal or soybean expellers is the principal amino acid (**AA**) source in diets for pigs, and it is recognized that the AA profile and digestibility of soybean meal is superior to that of other oilseed meals (Ruiz et al., 2020). In the last 20 years, new varieties of soybeans have been developed to increase oil yield, but a reduced protein concentration is often the trade-off (Tamagno et al., 2022). Recently a new variety of high-oil soybeans based on a genetic technology patented as PHOTOSEED has been developed. This technology creates more oil bodies in the green tissue by preventing degradation of oleosin shell from leaf proteases (Beechey-Gradwell et al., 2019).

The continuous and increased capture and storage of carbon as lipids also increases carbon fixing cycles (Wu et al., 2019). The increased carbon storage creates a high demand for photosynthate, which sustains and elevates photosynthesis (Beechey-Gradwell et al., 2019). Enhanced photosynthesis may also promote higher nitrogen fixation by rhizobia, and thereby increase protein concentration in the seed (Unkovich and Pate, 2000). There is, however, no information about the nutritional value of soybean meal or soybean expellers produced from the new high-oil variety of soybeans. Therefore, the objective of this research was to test the hypothesis that standardized ileal digestibility (**SID**) of crude protein (**CP**) and AA, concentrations of digestible energy (**DE**) and metabolizable energy (**ME**), apparent total tract digestibility (**ATTD**) of gross energy (**GE**), and the standardized total tract digestibility (**STTD**) of P are not different in soybean expellers produced from the new high-oil soybeans compared with soybean expellers produced from conventional soybeans when fed to growing pigs.

Materials and methods

Three experiments were conducted, and the Institutional Animal Care and Use Committee at the University of Illinois reviewed and approved the protocols for each experiment before animal work was initiated. Pigs were the offspring of Line 800 boars and Camborough females (Pig Improvement Company, Hendersonville, TN, USA). Conventional and high-oil soybeans (PHOTOSEED) were procured from Zeakal Inc. (San Diego, CA, USA), and beans were expelled and extruded at Insta Pro (Grimes, IA, USA) to produce soybean expellers from conventional soybeans (**SBE-CV**) and soybean expellers from high-oil soybeans (**SBE-HO**). Composition of whole conventional and high-oil soybeans were obtained from Zeakal Inc. (Table S4.1; San Diego, CA, USA). Composition of non-dehulled soybean meal produced from

conventional or high-oil soybeans were calculated by projecting expected composition of hexane extracted soybean meal containing 1.4% fat at 12% moisture from soybean expellers composition (Table S4.2).

Exp. 1: Digestibility of AA

Nine barrows (average initial body weight: 30.0 ± 1.5 kg) that had a T-cannula installed at the distal ileum were used. Pigs were housed individually in 1.2 × 1.5 m pens equipped with a self-feeder, a nipple waterer, and fully slatted tri-bar floors. Pigs were allotted to a triplicated 3 × 3 Latin square design with three diets and three periods of 7 d in each square. There were three pigs per diet in each period for a total of nine observations per treatment. Two diets were formulated using SBE-CV or SBE-HO as the only AA-contributing ingredient in the diet, and a N-free diet that was used to determine the basal endogenous losses of AA, was formulated as well (Table 4.1). Vitamins and minerals were included in all diets to meet or exceed the estimated nutrient requirements for growing pigs (NRC, 2012). All diets contained 0.40% chromic oxide as an indigestible marker. A sample of each diet was collected at the time of diet mixing.

Pigs were fed their respective diets at 3.0 times the maintenance requirement for ME (i.e., 197 kcal ME per kg weight$^{0.60}$; NRC, 2012) and water was available at all times. Pig weights were recorded at the beginning of each period and at the conclusion of the experiment. Each experimental period lasted 7 d. The initial 5 d of each period was considered the adaptation period, but ileal digesta were collected on d 6 and 7 for 9 h using standard procedures (Stein et al., 1998). Pigs were fed experimental diets each day at 0700 and ileal digesta samples were collected from 0700 to 1600. Cannulas were opened at the beginning of collection and a 225-mL plastic bag was attached to the cannula barrel using a cable tie. Digesta flowing into the bag were

collected and bags were replaced whenever they were full or at least once every 30 min. All samples were stored at –20 °C after collection. At the conclusion of the experiment, ileal digesta samples were thawed, mixed within animal and diet, and a subsample was lyophilized and finely ground (Lagos and Stein, 2019).

Samples of the SBE-CV and SBE-HO, ileal digesta, and diets, were analyzed for dry matter (**DM**; method 930.15; AOAC Int., 2019) and N was also analyzed in these samples (method 990.03; AOAC Int., 2019) using a FP628 protein analyzer (Leco Corporation, St. Joseph, MI, USA). Crude protein was calculated as N × 6.25. Amino acids in the two sources of soybean expellers, diets, and ileal digesta samples were analyzed on a Hitachi Amino Acid Analyzer (Model No. L8800; Hitachi High Technologies America, Inc., Pleasanton, CA, USA) using ninhydrin for postcolumn derivatization and norleucine as the internal standard. Prior to analysis, samples were hydrolyzed with 6 *N* HCl for 24 h at 110 °C [method 982.30 E(a); AOAC Int., 2019]. Methionine and Cys were determined as Met sulfone and cysteic acid after cold performic acid oxidation overnight before hydrolysis [method 982.30 E(b); AOAC Int., 2019]. Tryptophan was determined after NaOH hydrolysis for 22 h at 110 °C [method 982.30 E(c); AOAC Int., 2019]. Chromium in diets and ileal digesta samples was analyzed using the Inductive Coupled Plasma Atomic Emission Spectrometric method (method 990.08; AOAC Int., 2019).

The apparent ileal digestibility (**AID**) and SID of CP and AA were calculated using the analyzed CP, AA, and Cr concentrations in the diet and ileal digesta samples (Stein et al., 2007). Basal endogenous losses of CP and AA were calculated from pigs fed the N-free diet as previously described (Stein et al., 2007). Values for AID and SID of CP and AA calculated for each diet also represented the AID and SID of CP and AA in SBE-CV and SBE-HO,

respectively, because these ingredients were the only AA-containing ingredients in the diets. Values for concentrations of standardized ileal digestible AA were calculated by multiplying the analyzed CP and AA in each source of soybean expellers by the digestibility value for CP and each AA.

Data were analyzed using the PROC MIXED procedure (SAS Inst. Inc., Cary, NC, USA). The model included diet as the fixed effect and square, period, and animal as the random effects. Homogeneity of the variances among treatments was confirmed using the UNIVARIATE procedure, and outliers were identified as values that deviated from 1st and 3rd quartile by more than 3 times the interquartile range within treatment. However, no outliers were found. Mean values were calculated using the LSMeans statement. The pig was the experimental unit for all analyses. Statistical significance and tendency were considered at $P < 0.05$ and $0.05 \leq P < 0.10$, respectively.

Exp. 2: Concentrations of DE and ME

Thirty barrows and gilts (average initial body weight: 18.3 ± 1.3 kg) were allotted to a completely randomized design with three diets and 10 replicate pigs per diet. Pigs were housed individually in metabolism crates (0.71 × 0.84 m) equipped with a self-feeder, a nipple waterer, and a fully slatted floor. A screen and a urine pan were placed under the slatted floor to allow for the total, but separate, collection of urine and fecal samples.

A basal diet containing corn as the sole source of energy and two diets containing corn and SBE-CV or corn and SBE-HO were formulated; thus, a total of three diets were used (Table 4.2). Vitamins and minerals were included in all diets to meet or exceed current requirement estimates (NRC, 2012). A sample of each diet was collected at the time of diet mixing. Pigs were limit fed at 3.2 times the ME requirement for maintenance (i.e., 197 kcal ME per $kg^{0.60}$; NRC,

2012); feed was provided each day in two equal meals at 0800 and 1600 hours. The ME in the diets was calculated based on the ME in corn and soybean expellers that were reported previously (NRC, 2012). Water was available at all times. The initial 5 days were considered the adaptation period to the diets. Indigestible markers were included in the morning meals on d 6 (chromic oxide) and d 10 (ferric oxide). Fecal collections were initiated when chromic oxide appeared in the feces and ceased when ferric oxide appeared according to standard procedures using the marker-to-marker approach (Adeola, 2001). Feces were collected twice daily and stored at –20 °C immediately after collection. Urine collections were initiated on d 6 at 0900 and ceased on d 10 at 0900. Urine was collected in buckets placed under the crates. The collected urine was weighed daily, and a 10% subsample was stored at –20 °C. Urine buckets were emptied every morning, and a preservative of 50 mL of 6*N* HCl was added to the urine buckets before the beginning of urine collection each day.

At the conclusion of the experiment, urine samples were thawed, and a sub-sample was lyophilized before analysis (Kim et al., 2009). For this procedure, 10 mL of urine was dripped on a cotton ball that was placed in a plastic bag, the bag with the urine and cotton ball was lyophilized, and GE was analyzed using bomb calorimetry (Model 6400; Parr Instruments, Moline, IL, USA). Fecal samples were dried in a 55 °C forced air drying oven for 7 d to reach less than 10% moisture and samples were ground using a swing-type grain mill (model: RRH-500, Zhejiang Winki Plastic Industry Co., Ltd., Zhejiang, China) prior to analysis. Diet and fecal samples were analyzed for DM as described for Exp. 1. Fecal, diet, and ingredient samples were also analyzed for GE.

The two sources of soybean expellers were also analyzed for acid-hydrolyzed ether extract by crude fat extraction using petroleum ether (Ankom^{XT15}, Ankom Technology,

Macedon, NY, USA) following hydrolysis with 3*N* HCl (Ankom^{HCl}, Ankom Technology, Macedon, NY, USA). Insoluble dietary fiber and soluble dietary fiber were also analyzed in the two sources of soybean expellers on an Ankom Total Dietary Fiber Analyzer (Ankom Technology, Macedon, NY, USA) using method 991.43 (AOAC Int., 2019). Total dietary fiber was calculated as the sum of insoluble and soluble dietary fiber. Trypsin inhibitor units were also analyzed (method Ba 12-75; AOCS, 2006) and both sources of soybean expellers were analyzed for glucose, fructose, maltose, sucrose, stachyose, and raffinose using high-performance liquid chromatography (method 977.2, AOAC Int., 2007).

The ATTD of GE and DM was calculated for each diet, and the DE and ME in each diet were calculated as well (NRC, 2012). The DE and ME in corn were calculated by dividing the DE and ME of the basal diet by the inclusion rate of corn in that diet. The contribution of DE and ME from corn to the DE and ME in the diets containing corn and SBE-CV or corn and SBE-HO were subtracted from the DE and ME of each diet, and the DE and ME in SBE-CV and SBE-HO were calculated by difference (Adeola, 2001).

Data were analyzed using the PROC MIXED in SAS (SAS Institute Inc., Cary, NC, USA). Homogeneity of the variances among treatments was confirmed using the UNIVARIATE procedure. Outliers were identified as values that deviated from 1st and 3rd quartile by more than 3 times the interquartile range within treatment. However, no outliers were found. Diet was the fixed effect, and replicate was the random effect. Least squares means were calculated and separated using the PDIFF statement with Tukey's adjustment if the model was significant. The pig was the experimental unit for all analyses. Statistical significance and tendency were considered at $P < 0.05$ and $0.05 \leq P < 0.10$, respectively.

Exp. 3: STTD of P

Forty-eight barrows (average initial weight: 12.0 ± 1.6 kg) were allotted to six diets using a randomized complete block design with weaning group being the blocks. There were 4 replicate pigs per diet in each block for a total of eight replicate pigs per diet in the two blocks. Pigs were housed individually in the same metabolism crates as used in Exp. 2. Six diets were arranged in a 2 × 3 factorial with two sources of soybean expellers, (SBE-CV and SBE-HO) and three levels of microbial phytase (0, 500, or 1,000 phytase units per kg; Quantum Blue, AB Vista, Marlborough, UK). Cornstarch and sucrose were also included in the diets, and SBE-CV or SBE-HO were the only sources of P in the diets (Table 4.3). Limestone was added to the diets to satisfy a Ca to P ratio of 1.3:1. Vitamins and minerals other than Ca and P were included in all diets to meet or exceed the estimated nutrient requirements for weanling pigs (NRC, 2012). Feed and water were provided as in Exp. 2. Indigo blue was used as the marker and was provided in the morning meals on d 6 and 10. Fecal collection started when the initial marker appeared in the feces after d 6 and ceased when the second marker appeared after d 10 (Adeola, 2001). A sample of each diet was collected at the time of diet mixing.

Fecal samples were thawed at the conclusion of the experiment and mixed within pig and diet and dried and ground as described for Exp. 2. Ash in ingredient and diet samples was analyzed (method 942.05; AOAC Int., 2019). Total Ca and P in ingredient, diet, and fecal samples were analyzed (method 985.01 A, B and C; AOAC Int., 2019) using inductively coupled plasma-optical emission spectrometry (ICP-OES; Avio 200, PerkinElmer, Waltham, MA, USA). Sample preparation included dry ashing at 600 °C for 4 h (method 942.05; AOAC Int., 2019) and wet digestion with nitric acids (method 3050 B; U.S. Environmental Protection Agency, 2000). Phytase activity in diet samples was analyzed (method 2000.12; AOAC Int., 2019), and DM in

diets and fecal samples was analyzed as described for Exp. 1. Ingredients were analyzed for phytate (Ellis et al., 1977). The concentration of phytate-bound P in the two sources of soybean expellers was calculated as 28.2% of analyzed phytate (Lee et al., 2023; Tran and Sauvant, 2004).

The ATTD of P and Ca in each diet was calculated (NRC, 2012). By correcting values of ATTD of P for basal endogenous loss of P (i.e., 190 mg per kg DM intake; NRC, 2012), the STTD of P in each ingredient without and with phytase was calculated. Because each source of soybean expellers were the only source of P in the diets, the ATTD of P and STTD of P also represented the ATTD of P and STTD of P in each soybean expeller source.

Data were analyzed using the PROC MIXED in SAS (SAS Institute Inc., Cary, NC, USA). Homogeneity of the variances among treatments was confirmed using the UNIVARIATE procedure, and outliers were identified as values that deviated from 1st and 3rd quartile by more than 3 times the interquartile range within treatment. An outlier was found for the diet containing SBE-HO and 500 phytase unit/kg, and in the diet containing SBE-HO and 1,000 phytase unit/kg. Two outliers were found in the diet containing SBE-CV and 500 phytase unit/kg. The statistical model included diet as the fixed effect and block and replicate within block as the random effects. Least squares means were calculated using the LSMEANS statement in SAS, and outliers were not included in the means. Contrasts were used to analyze effects of source of soybean expellers, linear effect of increasing phytase, and the interaction between source of soybean expellers and phytase. The pig was the experimental unit for all analyses. Statistical significance and tendency were considered at $P < 0.05$ and $0.05 \leq P < 0.10$, respectively.

Results

Concentrations of GE, CP, acid-hydrolyzed ether extract, P, and Ca were greater, and concentration of total dietary fiber was less in SBE-HO than in SBE-CV (Table 4.4). Concentrations of phytate and trypsin inhibitors were greater in SBE-HO compared with SBE-CV. Even though the concentration of phytate was greater, the non-phytate P as a percentage of total P was greater in SBE-HO due to the greater total P compared with SBE-CV. Concentrations of most indispensable AA were greater in SBE-HO than in SBE-CV. Pigs remained healthy during each of the three experiments and very little feed refusals were observed. The concentration of crude fat and crude protein was greater in whole high-oil soybeans compared with whole conventional soybeans (Table S4.1). Values for non-dehulled solvent extracted soybean meal produced from high-oil soybeans are expected to have greater CP, AA, and less total dietary fiber compared with expected values for non-dehulled conventional solvent extracted soybean meal (Table S4.2).

Exp. 1: Digestibility of AA

The AID and SID of CP and Lys were not different between SBE-CV and SBE-HO, but the AID and SID of all other indispensable AA were greater ($P < 0.05$) or tended to be greater ($P < 0.10$) in SBE-CV compared with SBE-HO (Table 4.5). The AID and SID of all dispensable AA except Cys and Pro were greater ($P < 0.05$) or tended to be greater ($P < 0.10$) in SBE-CV compared with SBE-HO. However, the concentration of standardized ileal digestible AA was not different between SBE-CV and SBE-HO with the exception that SBE-HO had greater ($P < 0.05$) concentrations of standardized ileal digestible Arg, Lys, Met, Trp, Asp, and Glu compared with SBE-CV (Table 4.6).

Exp. 2: Concentrations of DE and ME

Feed intake and GE intake were not different between pigs fed the diets containing SBE-CV and SBE-HO, but feed intake and GE intake of pigs fed the corn diet were less ($P < 0.05$) compared with the two diets containing soybean expellers (Table 4.7). Weight of dried feces and fecal excretion of GE were less ($P < 0.05$) from pigs fed the corn diet compared with pigs fed the diets containing SBE-CV and SBE-HO. Weight of urine and urine excretion of GE from pigs fed the corn diet were less ($P < 0.05$) than from pigs fed diets containing SBE-CV SBE-HO, but the ATTD of GE was not different among the three diets. Concentrations of DE and ME were greater ($P < 0.05$) in the two diets containing SBE-CV or SBE-HO than in the corn diet, but DE and ME were not different between the two diets containing soybean expellers. On an as-fed basis, concentrations of DE and ME were not different between SBE-CV or SBE-HO. However, on a DM basis, concentration of DE tended to be greater ($P = 0.076$) in SBE-HO compared with SBE-CV, but no difference in ME between the two sources of soybean expellers was observed.

Exp. 3: STTD of P

As phytase increased in diets, P intake of pigs linearly increased in both SBE-CV and SBE-HO diets, but the increase was greater in the diets containing SBE-HO than SBE-CV (interaction; $P = 0.041$; Table 4.8). There were no interactions between soybean expellers source and phytase for fecal excretion of P and Ca, the ATTD of P and Ca, the STTD of P, or for Ca intake. Regardless of phytase level, fecal P excretion from pigs was not different between the two sources of soybean expellers. The ATTD of P was greater ($P < 0.05$) and the STTD of P tended to be greater ($P = 0.055$) in SBE-HO than in SBE-CV. Calcium intake was greater ($P = 0.001$) in pigs fed SBE-CV compared with SBE-HO, but fecal Ca excretion and the ATTD of Ca were not different between the two sources of soybean expellers. Fecal P excretion was reduced (linear, *P*

< 0.001) by phytase, which resulted in increases (linear, $P < 0.001$) in the ATTD and STTD of P. Increasing phytase in diets did not affect Ca intake, but fecal Ca excretion was reduced (linear, $P < 0.001$) by increasing phytase, and ATTD of Ca was increased (linear, $P < 0.001$) with increasing phytase.

Discussion

The SBE-HO contained more trypsin inhibitors than SBE-CV. It is possible that the high-oil soybeans originally contain more trypsin inhibitors compared with the conventional soybeans, but the level of trypsin inhibitor in soybean meal or soybean expellers depends on the heat treatment applied to soybeans during processing (Vagadia et al., 2017). Even though both sources of soybean expellers were processed in the same facility, it is possible that different heat treatments were applied to the two sources of soybeans, and because trypsin inhibitors are heat labile (Palacios et al., 2004), it is possible that SBE-CV was exposed to higher temperatures or for longer time, which may be the reason for the difference in analyzed trypsin inhibitors.

The SID of indispensable AA in the two sources of soybean expellers agreed with previous values (NRC, 2012; Kiarie et al., 2020; Rodriguez et al., 2020). The SID of AA is reduced by increasing levels of trypsin inhibitors in diets fed to pigs (Batterham et al., 1993; Li et al., 1998; Chen et al., 2020). Indeed, increasing trypsin inhibitor units by one percentage unit reduced SID of most indispensable AA by two to four percentage units (Goebel and Stein, 2011). Therefore, the observation that SBE-CV had greater SID of most indispensable AA compared with SBE-HO may be a result of the lower trypsin inhibitors. Nevertheless, because SBE-HO had greater concentration of total AA compared with SBE-CV, concentrations of standardized ileal digestible Arg, Lys, Met, and Trp were greater in SBE-HO compared with SBE-CV.

Addition of oil to diets increased the AID of AA in the diets (Li and Sauer, 1994; Cervantes-Pahm and Stein, 2008; Kil and Stein, 2011). Therefore, it was expected that the SID of AA in SBE-HO was greater than in SBE-CV, but that was not the case. However, the difference in the concentration of fat between the two sources of soybean expellers was approximately one percent unit and because the inclusion of soybean expellers was 40% in the diets, the difference in fat between the two diets was minimal, and likely did not impact measured values for SID of AA.

The Lys:CP ratio for a high-quality soybean meal is between 6.2 to 6.6% and a ratio below 6.0% indicates heat damage in the soybean meal (Stein et al., 2008). When subjected to excessive heating, both concentration and digestibility of Lys are reduced due to the Maillard reaction (Hurrell and Carpenter, 1981). The Lys:CP ratio in both SBE-CV and SBE-HO was in agreement with previous values for high quality soybean expellers (Webster et al., 2003; Rodriguez et al., 2020, Espinosa et al., 2021), which indicates that the two sources were not heat-damaged.

Values for the ATTD of GE and concentrations of DE and ME in corn were in agreement with previous values (NRC, 2012). However, DE and ME in SBE-CV and SBE-HO were slightly greater than previous values (Rodriguez et al., 2020), which is likely due to the greater concentration of acid-hydrolyzed ether extract in the soybean expellers used in this experiment compared with the soybean expellers used in previous experiments. The DE and ME in SBE-CV used in this experiment were close to values obtained for dry extruded-expelled soybean expellers that had lower concentration of ether extract, but contained more CP (Woodworth et al., 2001). It was expected that SBE-HO generates more energy than SBE-CV because SBE-HO contained more fat and CP, and less total dietary fiber. However, the lack of differences in DE

and ME between SBE-CV and SBE-HO indicates that the differences in nutrient concentrations between the two ingredients were too small to result in a difference in DE and ME. A lack of differences in DE and ME despite small differences in nutritional composition was previously reported when comparing dry extruded-expelled soybean expellers without and with hulls (Woodworth et al., 2001).

Values for the ATTD and STTD of P in SBE-HO were slightly greater than values reported for soybean meal (NRC, 2012). The observation that addition of microbial phytase to both sources of soybean expellers increased the STTD of P, is in agreement with results from previous experiments in which soybean meal was used (Almeida et al., 2010; Rojas and Stein, 2012; Almaguer et al., 2014; Sotak-Peper et al., 2016). The tendency for greater STTD of P in SBE-HO compared with SBM-CV is likely a result of the slightly greater concentration of non-phytate P in SBE-HO than in SBE-CV. In general, however, the STTD of P in soybean expellers seems to be in agreement with STTD of P in soybean meal.

The majority of Ca in all diets was from limestone, and the ATTD of Ca in the diets, therefore represents the ATTD of Ca in the blend of limestone and soybean expellers. The ATTD of Ca that was determined was within the range of values for ATTD of Ca that has been reported for limestone (González-Vega et al., 2015; Lee et al., 2019). To our knowledge, the ATTD of Ca in soybean expellers has not been reported, but the current data indicate that the ATTD of Ca in soybean expellers is not different from the ATTD of Ca in limestone.

The observation that the use of microbial phytase in diets increased the STTD of P in the two sources of soybean expellers indicates that both SBE-CV and SBE-HO have sufficient substrate (i.e., phytate) for microbial phytase. In previous experiments, quadratic increases in STTD of P were observed as phytase levels increased in the diets (Kerr et al., 2010; Almeida et

al., 2013; She, et al., 2018), but due to the limited number of phytase levels used in this experiment, only linearity was tested. It is likely that greater levels of phytase is needed to obtain a quadratic response to phytase (Arredondo et al., 2019).

The observation that the ATTD of Ca in diets was increased by phytase agrees with results of previous experiments (González-Vega et al., 2015; She et al., 2018; Lee et al., 2019). Phytate forms a Ca-phytate complex, which reduces Ca digestibility (Walk, 2016), but addition of microbial phytase releases Ca from the complex, which leads to an increase in the digestibility of Ca (Almeida and Stein, 2012; González-Vega et al., 2015; Lee et al., 2019).

Conclusions

The SBE-HO contained more nutrients and less fiber compared with SBE-CV. Concentrations (g/kg) of standardized ileal digestible AA were not different between SBE-HO an SBE-CV for most AA, but concentrations of Arg, Lys, Met, and Trp were greater in SBE-HO compared with SBE-CV. It will, therefore, be possible to reduce inclusion of soybean expellers in diets based on SBE-HO compared with SBE-CV. The SBE-HO also tended to contain more DE and to have greater STTD of P than SBE-CV. It is concluded that if SBE-HO is included in diets for pigs instead of SBE-CV, the nutritional value of the diet will not be compromised.

Tables

Table 4.1. Ingredient and analyzed nutrient compositions of experimental diets containing soybean expellers from conventional soybeans (SBE-CV) or soybean expellers from high-oil soybeans (SBE-HO), as-fed basis (Exp. 1)

Item	SBE-CV	SBE-HO	N-free
Ingredient, %			
SBE-CV	40.00	-	-
SBE-HO	-	40.00	-
Soybean oil	3.00	3.00	4.00
Ground limestone	1.00	1.00	0.37
Dicalcium phosphate	0.75	0.75	2.10
Sucrose	15.00	15.00	20.00
Cornstarch	38.95	38.95	67.73
Solka floc[1]	-	-	4.00
Magnesium oxide	-	-	0.10
Potassium carbonate	-	-	0.40
Sodium chloride	0.40	0.40	0.40
Chromic oxide	0.40	0.40	0.40
Vitamin-mineral premix[2]	0.50	0.50	0.50
Analyzed composition, %			
Dry matter	95.65	95.66	94.16
Crude protein	16.40	18.09	0.25
Indispensable amino acids			

Table 4.1 (cont.)

Arg	1.18	1.19	0.01
His	0.45	0.44	0.00
Ile	0.79	0.76	0.01
Leu	1.33	1.29	0.03
Lys	1.11	1.10	0.02
Met	0.26	0.25	0.01
Phe	0.87	0.84	0.02
Thr	0.70	0.68	0.01
Trp	0.27	0.23	0.02
Val	0.81	0.79	0.01
Dispensable amino acids			
Ala	0.78	0.76	0.01
Asp	2.00	1.97	0.02
Cys	0.28	0.27	0.01
Glu	3.25	3.21	0.04
Gly	0.77	0.77	0.01
Pro	0.84	0.84	0.01
Ser	0.81	0.78	0.01
Tyr	0.58	0.54	0.01

[1]Fiber Sales and Development Corp., Urbana, OH, USA.

[2]The vitamin-micromineral premix provided the following quantities of vitamins and micro minerals per kg of complete diet: vitamin A as retinyl acetate, 10,622 IU; vitamin D_3 as

Table 4.1 (cont.)

cholecalciferol, 1,660 IU; vitamin E as $_{DL}$-alpha-tocopheryl acetate, 66 IU; vitamin K as menadione nicotinamide bisulfate, 1.40 mg; thiamin as thiamine mononitrate, 1.08 mg; riboflavin, 6.49 mg; pyridoxine as pyridoxine hydrochloride, 0.98 mg; vitamin B_{12}, 0.03 mg; $_{D}$-pantothenic acid as $_{D}$-calcium pantothenate, 23.2 mg; niacin, 43.4 mg; folic acid, 1.56 mg; biotin, 0.44 mg; Cu, 20 mg as copper chloride; Fe, 123 mg as iron sulfate; I, 1.24 mg as ethylenediamine dihydriodide; Mn, 59.4 mg as manganese hydroxychloride; Se, 0.27 mg as sodium selenite and selenium yeast; and Zn, 124.7 mg as zinc hydroxychloride.

Table 4.2. Ingredient and analyzed nutrient compositions of experimental diets containing soybean expellers from conventional soybeans (SBE-CV) or soybean expellers from high-oil soybeans (SBE-HO), as-fed basis (Exp. 2)

Item	Corn	SBE-CV	SBE-HO
Ingredient composition, %			
Ground corn	97.05	57.45	57.45
SBE-CV	-	40.00	-
SBE-HO	-	-	40.00
Dicalcium phosphate	1.35	0.95	0.85
Ground limestone	0.70	0.70	0.80
Sodium cloride	0.40	0.40	0.40
Vitamin micromineral premix [1]	0.50	0.50	0.50
Total	100.00	100.00	100.00
Analyzed composition			
Dry matter, %	87.36	91.28	91.02
Gross energy, kcal/kg	3,722	4,178	4,186

[1]The vitamin-micromineral premix provided the following quantities of vitamins and micro minerals per kg of complete diet: vitamin A as retinyl acetate, 10,622 IU; vitamin D_3 as cholecalciferol, 1,660 IU; vitamin E as $_{DL}$-alpha-tocopheryl acetate, 66 IU; vitamin K as menadione nicotinamide bisulfate, 1.40 mg; thiamin as thiamine mononitrate, 1.08 mg; riboflavin, 6.49 mg; pyridoxine as pyridoxine hydrochloride, 0.98 mg; vitamin B_{12}, 0.03 mg; $_{D}$-pantothenic acid as $_{D}$-calcium pantothenate, 23.2 mg; niacin, 43.4 mg; folic acid, 1.56 mg; biotin, 0.44 mg; Cu, 20 mg as copper chloride; Fe, 123 mg as iron sulfate; I, 1.24 mg as

Table 4.2 (cont.)

ethylenediamine dihydriodide; Mn, 59.4 mg as manganese hydroxychloride; Se, 0.27 mg as sodium selenite and selenium yeast; and Zn, 124.7 mg as zinc hydroxychloride.

Table 4.3. Ingredient and analyzed nutrient compositions of experimental diets containing soybean expellers from conventional soybeans (SBE-CV) or soybean expellers from high-oil soybeans (SBE-HO), as-fed basis (Exp. 3)[1,2]

Item	SBE-CV	SBE-HO
Ingredient composition, %		
SBE-CV	40.00	-
SBE-HO	-	40.00
Soybean oil	3.00	3.00
Sucrose	10.00	10.00
Cornstarch	45.40	45.40
Ground limestone	0.70	0.70
Sodium chloride	0.40	0.40
Vitamin-micromineral premix[3]	0.50	0.50
Total	100.00	100.00
Analyzed composition, %		
Dry matter	95.33	95.18
Ash	4.18	4.54
Ca	0.37	0.46
P	0.30	0.32

[1]Four additional diets were formulated by adding 500 or 1,000 of phytase units per kg of diet to each of diet (Quantum Blue®, AB Vista, Marlborough, UK).

[2]Analyzed phytase activity in diets formulated to contain 0, 500, or 1000 phytase units/kg and SBE-CV was 70, 440, and 1100 phytase units/kg, respectively; and SBE-HO diets analyzed

Table 4.3 (cont.)

values were 70, 600, and 760 phytase units/kg, respectively.

[3]The vitamin-micromineral premix provided the following quantities of vitamins and micro minerals per kg of complete diet: vitamin A as retinyl acetate, 10,622 IU; vitamin D_3 as cholecalciferol, 1,660 IU; vitamin E as $_{DL}$-alpha-tocopheryl acetate, 66 IU; vitamin K as menadione nicotinamide bisulfate, 1.40 mg; thiamin as thiamine mononitrate, 1.08 mg; riboflavin, 6.49 mg; pyridoxine as pyridoxine hydrochloride, 0.98 mg; vitamin B_{12}, 0.03 mg; $_{D}$-pantothenic acid as $_{D}$-calcium pantothenate, 23.2 mg; niacin, 43.4 mg; folic acid, 1.56 mg; biotin, 0.44 mg; Cu, 20 mg as copper chloride; Fe, 123 mg as iron sulfate; I, 1.24 mg as ethylenediamine dihydriodide; Mn, 59.4 mg as manganese hydroxychloride; Se, 0.27 mg as sodium selenite and selenium yeast; and Zn, 124.7 mg as zinc hydroxychloride.

Table 4.4. Analyzed composition of soybean expellers from conventional soybeans (SBE-CV) or soybean expellers from high-oil soybeans (SBE-HO), as-fed basis

Item, %	SBE-CV	SBE-HO
Dry matter	94.56	94.63
Gross energy, kcal/kg	4,760	4,820
Acid-hydrolyzed ether extract	6.57	7.60
Crude protein	43.15	44.56
Lys:crude protein	6.26	6.42
Total dietary fiber	24.10	22.90
Soluble dietary fiber	5.00	3.00
Insoluble dietary fiber	19.10	19.90
Ash	6.62	6.90
Ca	0.37	0.36
Total P	0.70	0.77
Phytate	1.60	1.71
Phytate-P[1]	0.45	0.48
Nonphytate-P[2]	0.25	0.28
Nonphytate-P, % of total P	35.77	37.10
Indispensable amino acids		
Arg	2.94	3.22
His	1.09	1.15
Ile	1.93	1.97
Leu	3.22	3.31

Table 4.4 (cont.)

Lys	2.70	2.86
Met	0.62	0.65
Phe	2.12	2.19
Thr	1.71	1.78
Trp	0.57	0.61
Val	1.97	2.06
Total	18.87	19.80
Dispensable amino acids		
Ala	1.87	1.96
Asp	4.83	5.10
Cys	0.70	0.71
Glu	7.65	8.12
Gly	1.87	2.00
Pro	2.05	2.16
Ser	1.92	2.00
Tyr	1.58	1.67
Total	22.47	23.72
Total amino acids	41.69	43.89
Trypsin inhibitor, unit/mg	5.30	6.00
Sugar profile		
Glucose	0.05	0.05
Sucrose	5.73	5.08

Table 4.4 (cont.)

Maltose	0.28	0.27
Fructose	0.05	0.05
Stachyose	5.68	5.05
Raffinose	1.62	1.34

[2]Phytate-P was calculated by multiplying the analyzed phytate by 0.282 (Tran and Sauvant, 2004).

2Nonphytate-P was calculated as the difference between total P and phytate-P.

Table 4.5. Apparent ileal digestibility (AID) and standardized ileal digestibility (SID) of crude protein and amino acids (AA) in soybean expellers from conventional soybeans (SBE-CV) or soybean expellers from high-oil soybeans (SBE-HO), fed to growing pigs (Exp. 1)[1,2]

Item, %	AID				SID			
	SBE-CV	SBE-HO	SEM	*P*-value	SBE-CV	SBE-HO	SEM	*P*-value
Crude protein	79.1	78.0	1.18	0.467	91.8	89.5	1.18	0.146
Indispensable AA								
Arg	92.2	90.6	0.60	0.086	98.6	96.9	0.60	0.078
His	87.5	84.5	1.05	0.032	92.9	90.1	1.05	0.038
Ile	87.1	84.9	0.74	0.046	91.9	89.8	0.74	0.064
Leu	86.6	83.8	1.04	0.028	91.4	88.7	1.04	0.036
Lys	85.8	84.6	1.08	0.333	91.7	90.6	1.08	0.355
Met	89.2	86.8	0.91	0.018	94.2	91.9	0.91	0.028
Phe	87.4	84.5	0.95	0.020	92.3	89.6	0.95	0.028
Thr	78.0	74.1	1.24	0.026	87.4	83.8	1.24	0.037
Trp	88.0	84.0	0.83	0.003	94.5	91.6	0.83	0.017
Val	82.9	79.4	1.15	0.014	90.0	86.7	1.15	0.019
Total	86.5	84.0	0.88	0.029	93.5	91.2	0.88	0.040
Dispensable AA								
Ala	80.6	77.1	1.21	0.039	90.6	87.3	1.21	0.054
Asp	84.0	81.8	0.81	0.076	88.6	86.5	0.81	0.085
Cys	73.8	70.5	1.57	0.153	85.1	82.2	1.57	0.203
Glu	87.9	85.8	1.24	0.030	92.1	90.0	1.24	0.033

Table 4.5 (cont.)

Gly	69.7	61.3	2.39	0.002	103.9	95.5	2.39	0.002
Pro	59.5	50.2	8.99	0.175	155.9	146.5	8.99	0.175
Ser	83.9	80.3	0.80	0.006	92.0	88.8	0.80	0.010
Tyr	84.9	80.8	0.91	0.004	97.6	94.3	0.91	0.019
Total	81.4	77.6	1.31	0.016	98.2	94.7	1.31	0.024
Total AA	83.4	80.1	0.98	0.026	94.0	91.0	0.98	0.036

[1]Each least squares mean represents nine observations.

[2]Values for SID were calculated by correcting the values for AID for the basal ileal endogenous losses. The basal ileal endogenous losses were determined (g/kg dry matter intake) as crude protein, 23.04; Arg, 0.83; His, 0.27; Ile, 0.42; Leu, 0.71; Lys, 0.73; Met, 0.14; Phe, 0.47; Thr, 0.73; Trp, 0.19; Val, 0.64; Ala, 0.87; Asp, 1.02; Cys, 0.35; Glu, 1.50; Gly, 2.93; Pro, 8.99; Ser, 0.73; and Tyr, 0.81.

Table 4.6. Concentrations of standardized ileal digestible crude protein (CP) and amino acids (AA) in soybean expellers from conventional soybeans (SBE-CV) or soybean expellers from high-oil soybeans (SBE-HO) fed to growing pigs, as-fed basis (Exp. 1)[1,2]

Item, g/kg	SBE-CV	SBE-HO	SEM	*P*-value
CP	396.1	398.8	5.17	0.682
Indispensable AA				
Arg	29.0	31.2	0.19	< 0.001
His	10.1	10.4	0.12	0.114
Ile	17.7	17.7	0.14	0.858
Leu	29.4	29.4	0.34	0.880
Lys	24.8	25.9	0.30	0.008
Met	5.8	6.0	0.06	0.037
Phe	19.6	19.6	0.20	0.836
Thr	14.9	14.9	0.21	0.901
Trp	5.4	5.6	0.05	0.007
Val	17.7	17.9	0.23	0.612
Total	176.5	180.5	1.69	0.059
Dispensable AA				
Ala	16.9	17.1	0.23	0.562
Asp	42.8	44.1	0.40	0.033
Cys	6.0	5.8	0.11	0.429
Glu	70.5	73.1	0.99	0.005
Gly	19.4	19.1	0.47	0.339

Table 4.6 (cont.)

Pro	31.9	31.7	1.91	0.832
Ser	17.7	17.8	0.16	0.667
Tyr	15.4	15.8	0.15	0.110
Total	220.6	224.6	3.02	0.200
Total AA	392.1	399.2	4.17	0.207

[1]Each least squares mean represents nine observations.

[2]Values for standardized ileal digestible AA were calculated by multiplying analyzed CP and AA in each source of soybean expellers by the corresponding digestibility value.

Table 4.7. Digestible energy (DE) and metabolizable energy (ME) and apparent total tract digestibility (ATTD) of gross energy (GE) in experimental diets and in soybean expellers produced from conventional soybeans (SBE-CV) or from high-oil soybeans (SBE-HO), as-fed basis (Exp. 2)[1]

Item	Corn	SBE-CV	SBE-HO	SEM	*P*-value
Intake					
Feed, g/d	733[b]	974[a]	950[a]	36.08	< 0.001
GE, kcal/d	2,728[b]	4,069[a]	3,976[a]	155	< 0.001
Fecal excretion					
Dry feces output, g/d	61[b]	98[a]	87[a]	5	< 0.001
GE, kcal/d	280[b]	434[a]	388[a]	21	< 0.001
Urine excretion					
Urine output, g/d	2,599[b]	5,389[a]	4,435[a]	571	0.004
GE, kcal/d	75[b]	154[a]	170[a]	18	0.002
ATTD of GE, %	89.1	89.3	90.3	0.8	0.500
Energy in diets, kcal/kg					
DE	3,314[b]	3,732[a]	3,779[a]	29	< 0.001
ME	3,205[b]	3,574[a]	3,624[a]	39	< 0.001
Energy in ingredients[2], kcal/kg					
As-fed basis					
DE	-	4,366	4,483	56	0.141
ME	-	4,176	4,301	64	0.168
Dry matter basis					

Table 4.7 (cont.)

DE	-	4,497	4,647	57	0.076
ME	-	4,301	4,458	70	0.101

[a-b]Means within a row that do not have a common superscript are different ($P < 0.05$).

[1]Each least squares mean represents nine observations.

[2]Concentrations of DE and ME in corn on an as-fed basis were 3,420 and 3,308 kcal/kg, respectively.

Table 4.8. Effects of increasing phytase levels on apparent total tract digestibility (ATTD) and standardized total tract digestibility (STTD) of P in soybean expellers from conventional soybeans (SBE-CV) or soybean expellers from high-oil soybeans (SBE-HO) and ATTD of Ca in diets fed to growing pigs, as-fed basis (Exp. 3)[1,2]

Item	Source	SBE-CV			SBE-HO				Contrast *P*-value		
Microbial phytase, unit/kg		0	500	1,000	0	500	1,000	SEM	Source	Linear[3]	Interaction
P digestibility											
P intake, g/d		2.19	2.22	2.43	2.41	2.29	2.41	0.07	0.032	0.020	0.041
P in feces, %		2.11	1.26	1.43	2.23	1.12	1.21	0.07	0.221	< 0.001	0.513
P excretion in feces, g/d		0.98	0.65	0.53	1.08	0.45	0.51	0.06	0.373	< 0.001	0.467
ATTD of P, %		55.21	70.65	78.31	55.43	80.27	78.89	2.08	0.044	< 0.001	0.840
STTD[4] of P, %		61.34	76.77	83.76	61.09	86.12	84.56	2.08	0.055	< 0.001	0.891
Ca digestibility											
Ca intake, g/d		2.76	3.17	3.71	3.49	2.78	2.75	0.10	0.001	0.101	0.085
Ca in feces, %		2.29	1.72	1.98	2.63	1.90	1.83	0.16	0.361	0.002	0.558
Ca excretion in feces, g/d		1.07	0.86	0.75	1.28	0.77	0.75	0.09	0.566	< 0.001	0.197
ATTD of Ca, %		61.27	72.71	80.09	63.76	72.46	72.45	2.47	0.385	< 0.001	0.296

[1]Each least squares mean represents eight observations except for the two diets containing SBE-HO with 500 and 1,000 phytase

Table 4.8 (cont.)

unit/kg (n = 7) and the diet containing SBE-CV with 500 phytase unit/kg (n = 6).

[2]Analyzed phytase activity in diets formulated to contain 0, 500, or 1000 phytase units/kg and SBE-CV was 70, 440, and 1100 phytase units/kg, respectively. The SBE-HO diets analyzed values were 70, 600, and 760 phytase units/kg, respectively.

[3]Linear effects of increasing phytase in diets.

[4]Values for STTD of P were calculated by correcting values for the ATTD of P with the basal endogenous loss (i.e., 190 mg/kg DM intake, NRC, 2012).

Supplementary tables

Supplementary Table 4.1. Composition of conventional and high-oil soybean seeds[1]

Item, %	Conventional soybeans	High-oil soybeans
Moisture	9.9	10.4
Crude fat	20.8	23.0
Crude protein	32.9	33.8
Crude fiber	6.5	6.6

[1]Values obtained from Zeakal Inc. (San Diego, CA, USA)

Supplementary Table 4.2. Calculated composition of non-dehulled soybean meal produced from conventional or high-oil varieties of soybeans, as-fed basis (Table 4).

Item, %	Conventional soybean meal	High-oil soybean meal
Dry matter	88.00	88.00
Acid-hydrolyzed ether extract	1.40	1.40
Crude protein	42.48	44.28
Lys:crude protein	6.26	6.42
Total dietary fiber	23.73	22.76
Soluble dietary fiber	4.92	2.98
Insoluble dietary fiber	18.80	19.78
Ash	6.52	6.86
Ca	0.36	0.36
Total P	0.69	0.77
Phytate	1.58	1.70
Phytate-P[1]	0.44	0.48
Nonphytate-P[2]	0.24	0.29
Nonphytate-P, % of total P	35.54	37.37
Indispensable amino acids		
Arg	2.89	3.20
His	1.07	1.14
Ile	1.90	1.96
Leu	3.17	3.29
Lys	2.66	2.84

Supplementary Table 4.2 (cont.)

Met	0.61	0.65
Phe	2.09	2.18
Thr	1.68	1.77
Trp	0.56	0.61
Val	1.94	2.05
Total	18.58	19.68
Dispensable amino acids		
Asp	1.84	1.95
Cys	4.76	5.07
Glu	0.69	0.71
Gly	7.53	8.07
Pro	1.84	1.99
Ser	2.02	2.15
Tyr	1.89	1.99
Total	1.56	1.66
Total amino acids	22.12	23.57
Trypsin inhibitor, unit/mg	5.22	5.96
Sugar profile		
Glucose	0.05	0.05
Sucrose	5.64	5.05
Maltose	0.28	0.27
Fructose	0.05	0.05

Supplementary Table 4.2 (cont.)

Stachyose	5.59	5.02
Raffinose	1.59	1.33

[1]Phytate-P was calculated by multiplying the analyzed phytate by 0.282 (Tran and Sauvant, 2004).

[2]Non-phytate-P was calculated as the difference between total P and phytate-P.

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CHAPTER 5: Diets based on soybean meal instead of synthetic amino acids increase energy and nitrogen balances when fed to growing pigs

Abstract

The objective was to test the hypothesis that feeding intact protein from soybean meal (**SBM**) to growing pigs instead of a combination of SBM and synthetic amino acids (**AA**) results in greater nitrogen retention and digestible energy (**DE**) without affecting metabolizable energy (**ME**) in the diet. A control diet based on corn and SBM and three additional diets were formulated. In the three additional diets, SBM inclusion was reduced and supplemented with either three, four, or five synthetic AA (i.e. Lys, Met, Thr, Trp, and Val). The concentration of standardized ileal digestible indispensable AA among diets was at or above current requirements for growing pigs, but dietary crude protein was reduced as synthetic AA were added. Forty pigs (initial body weight: 20.5 ± 2.4 kg) were allotted to the four diets using a randomized complete block design. Two blocks of 20 pigs were used with five pigs per diet in each block. Pigs were housed in metabolism crates containing fully slatted floors, screens, and urine pans that allowed for quantitative collection of feces and urine for 4 days after 5 days of adaptation. Samples of diets, feces, and urine were analyzed for dry matter (**DM**), gross energy (**GE**), and nitrogen. The statistical model included diet as a fixed effect and block and replicate within block as random effects, and pig was the experimental unit. Contrast coefficients were used to determine linear and quadratic effects of reducing dietary protein. Results indicated that apparent total tract digestibility (**ATTD**) of DM was reduced (quadratic, $P = 0.027$), and ATTD of GE also was

reduced (linear, $P = 0.046$) as SBM inclusion was reduced in diets. Absorbed nitrogen, retained nitrogen (g/day), and ATTD of nitrogen were reduced (linear, $P < 0.001$) as SBM inclusion decreased, but retention of nitrogen, calculated as percent of intake or percent of absorbed nitrogen, increased (linear, $P < 0.001$). The DE in diets decreased (linear, $P = 0.007$) as SBM inclusion was reduced, whereas SBM inclusion had no effect on ME. Reducing SBM inclusion tended to increase (quadratic, $P = 0.096$) ME to GE ratio and increased (linear, $P = 0.008$) ME to DE ratio. In conclusion, diets containing intact protein from SBM had greater ATTD of GE and nitrogen, and greater DE, whereas ME was not changed when compared with diets containing synthetic AA. Daily protein retention decreased when synthetic AA rather than SBM were used to furnish the digestible AA in the diets, potentially impacting whole-body protein synthesis and carcass composition.

Key words: amino acids, energy, low protein diets, nitrogen retention, pigs, soybean meal.

Introduction

Diets based on soybean meal (**SBM**) and cereal grains usually meet requirements for amino acids (**AA**) by growing pigs, and therefore, maximize growth performance and protein synthesis. However, synthetic AA are often included in diets fed to pigs at the expense of SBM or other protein sources to provide a portion of the indispensable AA needed by pigs. Replacing SBM with synthetic AA may support pig growth performance (Che et al., 2017) but may reduce daily nitrogen retention by pigs (Kerr and Easter, 1995). This indicates that there may either be an AA deficiency in diets with synthetic AA, or there are factors in SBM other than AA that are needed to maximize protein synthesis in pigs.

It is believed that pigs have a genetically predetermined ability to deposit protein in the body, and if AA are provided in quantities less than needed to support maximum protein synthesis, more fat and less skeletal muscle will be deposited (Wang et al., 2018). However, protein synthesis takes place only if all needed AA are present in the same cell at the same time, and it has been speculated that synthetic AA are absorbed more rapidly than AA from intact protein, which may impair protein synthesis because the AA that arrive first will be oxidized before all other AA arrive in the cell (Eugenio et al., 2022; Zhang et al., 2022). It is therefore possible that, if protein from SBM is replaced by synthetic AA, some of the synthetic AA will not be used for protein synthesis, which may result in reduced nitrogen retention by the pigs.

Diets with reduced concentrations of SBM should theoretically have the same digestible energy (**DE**) and metabolizable energy (**ME**) as diets based only on corn and SBM because the DE and ME in SBM are close to DE and ME in corn (NRC, 2012). However, results of recent research indicate that the DE in SBM is greater than in corn (Sotak-Peper et al., 2015), and therefore, dietary DE may be reduced if SBM in the diet is replaced by synthetic AA and corn. However, at this time, no research has been conducted to confirm this assumption. Therefore, the objective of this experiment was to test the hypothesis that feeding intact protein from SBM to growing pigs instead of a combination of SBM and synthetic AA results in greater nitrogen retention and greater DE in the diet without affecting ME in the diet.

Materials and methods

The Institutional Animal Care and Use Committee at the University of Illinois reviewed and approved the protocols for the experiment before animal work was initiated. Pigs were the

offspring of Line 800 boars and Camborough females (Pig Improvement Company, Hendersonville, TN, USA).

Dietary treatment

A control diet was formulated based on corn and SBM without synthetic AA. Three additional diets were formulated by reducing the inclusion rate of SBM and adding more corn and three synthetic AA (i.e., Lys, Met, Thr); four synthetic AA (i.e., Lys, Met, Thr, Trp); or five synthetic AA (i.e., Lys, Met, Thr, Trp, Val) to the diet. Therefore, a total of four diets were used (Tables 5.1 and 5.2). Concentrations of standardized ileal digestible indispensable AA and all other nutrients were at or above requirements for growing pigs from 25 to 50 kg (NRC, 2012) in all diets, but the concentration of crude protein (**CP**) was reduced from 20.0% to 16.4%, 15.4%, or 13.4% in diets containing three, four, or five synthetic AA.

Animals, housing, feeding and sample collection

Forty growing pigs (average initial body weight: 20.5 ± 2.4 kg) were allotted to a randomized complete block design with four diets and 10 replicate pigs per diet. There were two blocks of 20 pigs, with five pigs per diet in each block. Pigs were housed individually in metabolism crates (0.81 × 1.40 m) equipped with a self-feeder, a nipple waterer, and a slatted floor. A screen and a urine pan were placed under the slatted floor to allow for the total, but separate, collection of urine and fecal materials. Pigs were limit-fed at 3.2 times the ME requirement for maintenance (i.e., 197 kcal/kg × body weight$^{0.60}$; NRC, 2012), and feed was provided each day in two equal meals at 0800 and 1600 h. Throughout the experiment, pigs had free access to water. Pigs were fed experimental diets for 12 days. The initial five days were considered the adaptation period to the diet, with urine and fecal material collected during the following four days according to standard procedures using the marker-to-marker approach (Adeola, 2001). Urine was collected in

urine buckets over a preservative of 50 mL of hydrochloric acid. Fecal samples and 10% of the collected urine were stored at −20 °C immediately after collection.

Chemical analysis

At the conclusion of the experiment, urine samples were thawed and mixed within animal and diet, and two sub-samples were collected. One subsample was lyophilized, whereas the other subsample was not lyophilized. Fecal samples were thawed and mixed within pig and diet and then dried in a forced-air oven at 65 °C prior to analysis. All diet, ingredient, and fecal samples were ground using a swing-type grain mill (model: RRH-500, Zhejiang Winki Plastic Industry Co., Ltd., Zhejiang, China) prior to chemical analyses.

Diet, ingredient, and fecal samples were analyzed for dry matter (**DM**; method 930.15; AOAC Int., 2019), and nitrogen was analyzed using the combustion procedure (method 990.03; AOAC Int., 2019) on a LECO FP628 (LECO Corp., Saint Joseph, MI, USA). Crude protein in diet and ingredient samples was calculated as analyzed nitrogen × 6.25. Filtered urine samples that were not lyophilized were analyzed for nitrogen using the Kjeldahl method on a Kjeltec™ 8400 apparatus (FOSS Inc., Eden Prairie, MN, USA). Diet, ingredient, fecal, and lyophilized urine samples were analyzed for gross energy (**GE**) using bomb calorimetry (Model 6400; Parr Instruments, Moline, IL, USA). Diet and ingredient samples were analyzed for acid hydrolyzed ether extract by acid hydrolysis using 3 *N* HCl (Ankom HCl Hydrolysis System, Ankom Technology, Macedon, NY, USA) followed by fat extraction (Ankom XT-15 Extractor, Ankom Technology, Macedon, NY, USA). Diet and ingredient samples were also analyzed for ash (method 942.05; AOAC Int., 2019) and insoluble and soluble dietary fiber on an Ankom Total Dietary Fiber Analyzer (Ankom Technology, Macedon, NY, USA) according to method 991.43 (AOAC Int., 2019). Total dietary fiber was calculated as the sum of insoluble and soluble dietary

fiber. Amino acids in diet and ingredient samples were analyzed at the Agricultural Experiment Station Chemical Laboratories at the University of Missouri (Columbia, MO, USA) on a Hitachi Amino Acid analyzer (Model No. L8800; Hitachi High Technologies America, Inc., Pleasanton, CA, USA) using ninhydrin for postcolumn derivatization and norleucine as the internal standard. Prior to analysis, samples were hydrolyzed with 6 *N* HCl for 24 h at 110 °C [method 982.30 E(a); AOAC Int., 2019]. Methionine and Cys were determined as Met sulfone and cysteic acid after cold performic acid oxidation overnight before hydrolysis [method 982.30 E(b); AOAC Int., 2019]. Tryptophan was determined after NaOH hydrolysis for 22 h at 110 °C [method 982.30 E(c); AOAC Int., 2019]. Total starch was analyzed in corn and diet samples using the glucoamylase procedure (method 979.10; AOAC Int., 2019), whereas glucose, sucrose, maltose, fructose, stachyose, and raffinose were analyzed in SBM using high-performance liquid chromatography (method 977.2, AOAC Int., 2019). Soybean meal was also analyzed for trypsin inhibitors (method Ba 12-75; AOCS, 2006).

Calculations and statistical analysis

The apparent total tract digestibility (**ATTD**) of DM, nitrogen, and GE, retention of nitrogen, and biological value of CP were calculated as described (NRC, 2012; Rojas and Stein, 2013). Digestible energy and ME for each diet were calculated as well (NRC, 2012). Data were analyzed using the MIXED procedure of SAS (version 9.4; SAS Inst. Inc., Cary, NC, USA). Homogeneity of the variances among treatments was confirmed, and outliers were identified as values that deviated from the 1st and 3rd quartile by more than 3 times the interquartile range within treatment, however, no outliers were detected. Pig was the experimental unit for all analyses. The statistical model included diet as fixed effect and block and replicate within block were random effects. Least square means were calculated, and contrast coefficients were

generated from analyzed dietary CP using the Interactive Matrix Language procedure of SAS (version 9.4; SAS Inst. Inc., Cary, NC, USA). These coefficients were used to determine linear and quadratic effects of reducing dietary CP. Statistical significance and tendencies were considered at $P < 0.05$ and $0.05 \leq P < 0.10$, respectively.

Results

Pigs remained healthy during the experiment and no feed refusals were observed.

Daily feed intake and daily GE intake were not affected by dietary CP (Table 5.3). Daily nitrogen intake decreased (linear, $P < 0.001$) with decreasing CP in diets. Daily dry feces output and daily fecal GE output increased (quadratic, $P < 0.05$) as CP decreased in diets, but daily nitrogen in feces tended to decrease (quadratic, $P = 0.082$) as CP decreased in diets. Daily urine output also tended to decrease (linear, $P = 0.096$) as CP was reduced in diets, and daily GE in urine and daily nitrogen excretion in urine were reduced (linear, $P < 0.05$) as CP decreased in diets. The ATTD of DM decreased (quadratic, $P = 0.027$), and the ATTD of GE also decreased (linear, $P < 0.05$) as CP was reduced in diets.

Absorbed nitrogen (g/day), retained nitrogen (g/day), and the ATTD of nitrogen decreased (linear, $P < 0.001$) as CP decreased in diets, but retention of nitrogen, calculated as a percent of intake, and the biological value of CP, increased (linear, $P < 0.001$) as dietary CP was reduced. The DE was reduced (linear, $P = 0.007$) as CP decreased in diets, but dietary protein concentration had no effect on ME in diets. The ME to GE ratio tended to increase (quadratic, $P = 0.096$) and the ME to DE ratio increased (linear, $P = 0.008$) as dietary protein was reduced.

Discussion

The analyzed values for CP and total AA in experimental diets were in close agreement with formulated values, which indicates that diets were mixed correctly. Likewise, analyzed concentration of nutrients in corn and SBM were in agreement with previous data (NRC, 2012).

The lack of an effect of diet CP on daily feed intake was in agreement with previous data (Kerr and Easter, 1995; Le Bellego et al., 2001), although weanling pigs fed diets with synthetic branched chain AA may have increased feed intake (Zheng et al., 2016). Reduced urine nitrogen excretion by pigs fed diets with lower CP was also previously observed (Le Bellego et al., 2001; Wang et al., 2018), which is likely a result of a more balanced AA supply in diets with lower CP, and therefore, reduced deamination of AA with a subsequent reduced elimination of nitrogen in the urine via the urea cycle.

Because the inclusion of corn was increased in diets with reduced SBM, the reduced digestibility of nitrogen in diets with low CP is likely a result of the fact that the digestibility of AA in corn is less than in SBM (NRC, 2012). However, corn is believed to have greater energy digestibility than SBM (NRC, 2012), but the observation that the ATTD of GE was reduced as dietary SBM was reduced indicates that this may not always be the case. The implication of this observation is that DE in SBM may be greater than previously thought, which has also been reported by others (Rojas and Stein, 2013; Sotak-Peper et al., 2015). The observed decrease in DE in lower-CP diets is, therefore, a result of the greater DE in SBM compared with corn (NRC, 2012), which is also in agreement with reported data (Le Bellego et al., 2001). The lack of an effect of dietary CP on ME in diets is likely because there is no difference in ME between corn and SBM (Sotak-Peper et al., 2015; Li et al., 2017). However, the current data for ME differ from reports that stated that SBM contains less ME than corn (NRC, 2012). The average DE and

ME obtained among diets were in agreement with data for corn-SBM diets fed to growing pigs (Rojas and Stein, 2013; Sotak-Peper et al., 2015; Li et al., 2017), and there appear to be no differences in ME among different sources of SBM produced in the U.S. (Lopez et al., 2020).

The reduced daily absorbed nitrogen by pigs fed diets with reduced CP were mainly due to the reduction in daily nitrogen intake as CP was reduced in diets, which is in agreement with previous data (Kerr and Easter, 1995). The reduced retention of nitrogen measured as g per day by pigs fed diets with reduced CP may be due to a more rapid absorption of the synthetic AA compared with the AA from corn and SBM (Eugenio et al., 2022), and it is possible that the synthetic AA were metabolized before the AA from corn and SBM arrived in the cells and protein synthesis was, therefore, reduced. If this hypothesis is correct, the implication may be that there is a limit to the quantities of synthetic AA that can be used in diets for growing pigs. However, additional research is required to fully understand the relationship between dietary SBM and body protein synthesis and it is possible that SBM contain anabolic substances other than AA that positively impacts nitrogen retention (White et al., 2024). The observation that retention of nitrogen, measured as percent of intake and percent of absorbed nitrogen, increased by reducing dietary CP was in agreement with previous data (Kerr and Easter, 1995; Wang et al., 2018), and is likely a result of the fact that low-CP diets contain more balanced indispensable AA and less excess AA than diets based on only corn and SBM. Fewer AA, therefore, need to be deaminated and oxidized in pigs fed low-CP diets compared with pigs fed the corn-SBM diet.

The nitrogen retention obtained for all diets in this experiment was greater than some reported data (Carr et al., 1977; Kerr and Easter, 1995; Rojas and Stein, 2013; Ochoa et al., 2024). It has been estimated that nitrogen retention by growing pigs is between 40 and 50% of consumed nitrogen (Noblet et al., 2004), which is in agreement with nitrogen retention data

published between 1971 and 1992 (McConnell et al., 1971; Carr et al., 1977; Campbell and Dunkin, 1983; Gatel and Grosjean, 1992). However, in later experiments, greater efficiency of nitrogen retention by growing pigs was observed, indicating retention values between 50 and 60% of nitrogen (Kerr and Easter, 1995; Lenis et al., 1999; Le Bellego et al., 2001; Zervas and Zijlstra, 2002; Otto et al., 2003; Shriver et al., 2003; Lovatto et al., 2006; Pedersen et al., 2007; Patrás et al., 2012). More recent data indicate that nitrogen retention in modern genotypes of pigs is greater than 60% of nitrogen intake in diets containing corn and SBM when fed to growing pigs (Rojas and Stein, 2013; Li et al., 2017; Corassa et al., 2024; Ochoa et al., 2024). Thus, it appears that nitrogen retention in growing pigs has increased over the years, which may be due to a greater efficiency of converting dietary protein into body protein by modern genotypes of pigs compared with older genotypes. A possible explanation for this change may be the greater emphasis on selection for lean deposition by pig genetic companies during recent decades. Nevertheless, the greater nitrogen retention by modern pigs indicates a greater capacity for utilizing dietary AA for protein synthesis, and modern pigs, therefore, likely have less excess AA that need to be deaminated, which reduces the amount of nitrogen that needs to be excreted via the urea cycle. Because AA deamination and urea cycle activity are energy consuming processes, reduced deamination of excess AA may have a positive effect on the energy contribution from dietary protein.

Conclusions

Reducing SBM inclusion and supplementing diets with synthetic AA reduced the ATTD of GE and the DE concentration, but did not affect ME in diets fed to growing pigs. Reducing SBM inclusion and supplementing with synthetic AA in diets also reduced ATTD of nitrogen and the

daily nitrogen retention (g/day), whereas the biological value increased as diet CP decreased. The implication of these observations is that AA from SBM appears to be better utilized than synthetic AA. However, diets supplemented with synthetic AA may result in reduced carcass value due to reduced protein synthesis. It was also demonstrated that nitrogen retention in modern genotypes of pigs is much greater than what was observed in older genotypes, which may have implications for equations used to calculate net energy of diets for pigs.

Tables

Table 5.1. Ingredient composition of experimental diets formulated to different concentrations of crude protein (CP)

Item	Diets			
	20.0% CP	16.4% CP	15.4% CP	13.4% CP
Ingredient, %				
Ground corn	60.40	70.27	71.40	75.11
Soybean meal	34.50	24.00	22.80	18.75
Soybean oil	2.50	2.50	2.50	2.50
Dicalcium phosphate	0.90	1.10	1.10	1.15
Ground limestone	0.80	0.75	0.75	0.75
$_{L}$-Lys-HCl	-	0.32	0.35	0.47
$_{DL}$-Met	-	0.08	0.09	0.12
$_{L}$-Thr	-	0.08	0.10	0.15
$_{L}$-Trp	-	-	0.01	0.03
$_{L}$-Val	-	-	-	0.07
Sodium chloride	0.40	0.40	0.40	0.40
Vitamin-mineral premix[1]	0.50	0.50	0.50	0.50

[1]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 D_3 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 B_{12}, 0.03 mg; $_{D}$-

Table 5.1 (cont.)

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.

Table 5.2. Analyzed nutrient composition of ingredients and experimental diets formulated to different crude protein (CP) concentrations, as-fed basis

Item, %	Ingredients		Diets			
	Corn	Soybean meal[1,2]	20.0% CP	16.4% CP	15.4% CP	13.4% CP
Dry matter	86.95	89.80	88.49	89.73	88.16	88.55
Crude protein	7.80	45.18	20.02	16.36	15.38	13.40
Gross energy, kcal/kg	3,819	4,204	3,976	3,936	3,924	3,963
Acid-hydrolyzed ether extract	2.53	1.76	5.17	4.49	4.15	4.62
Starch	64.50	-	39.75	45.88	46.58	48.88
Total dietary fiber	15.10	23.10	17.70	18.20	18.50	17.00
Soluble dietary fiber	2.50	3.4	2.40	2.10	2.00	2.80
Insoluble dietary fiber	12.6	19.7	15.30	16.10	16.50	14.20
Ash	1.32	6.19	4.65	4.27	4.21	4.05
Indispensable amino acids, %						
Arg	0.35	3.31	1.25	0.94	0.98	0.93
His	0.23	1.23	0.50	0.40	0.41	0.39
Ile	0.27	2.05	0.85	0.69	0.69	0.65
Leu	0.91	3.48	1.58	1.35	1.39	1.32
Lys	0.24	2.84	1.07	1.09	1.08	1.11
Met	0.17	0.63	0.28	0.28	0.31	0.36
Phe	0.37	2.30	0.95	0.77	0.79	0.75
Thr	0.27	1.79	0.70	0.63	0.67	0.64
Trp	0.06	0.63	0.34	0.23	0.26	0.25

Table 5.2 (cont.)

Val	0.36	2.14	0.92	0.75	0.75	0.80
Dispensable amino acids, %						
Ala	0.57	1.98	0.87	0.82	0.82	0.75
Asp	0.51	5.19	1.81	1.56	1.56	1.35
Cys	0.18	0.67	0.27	0.26	0.27	0.26
Glu	1.40	8.20	3.16	2.82	2.83	2.54
Gly	0.29	1.91	0.73	0.65	0.65	0.58
Pro	0.67	2.43	1.03	0.97	0.97	0.90
Ser	0.36	2.27	0.75	0.68	0.69	0.61
Tyr	0.25	1.53	0.58	0.52	0.52	0.47
Total amino acids, %	7.46	44.58	17.64	15.41	15.40	14.66

[1]Sugar composition (%): glucose, 0.05; sucrose, 6.27; maltose, 0.13; fructose, 0.07; stachyose, 5.61; and raffinose, 1.64.

[2]Trypsin inhibitor units per mg: 3.42.

Table 5.3. Apparent total tract digestibility (ATTD) of energy, nitrogen balance, and concentrations of digestible energy (DE) and metabolizable energy (ME) in experimental diets fed to growing pigs, as-fed basis[1]

Item	Dietary protein, %					Contrast *P*-value	
	20.0% CP	16.4% CP	15.4% CP	13.4% CP	SEM	Linear	Quadratic
Intake							
Feed, kg/day	1.07	1.07	1.08	1.11	0.04	0.375	0.523
Gross energy, kcal/day	4,270	4,231	4,249	4,381	146	0.490	0.290
Nitrogen, g/day	34.39	28.13	26.65	23.70	0.93	< 0.001	0.622
Fecal excretion							
Dry feces output, g/day	106.50	102.40	108.70	122.20	4.65	0.040	0.024
Gross energy, kcal/day	489	470	503	564	21	0.030	0.024
Nitrogen, g/day	4.57	3.79	4.23	4.23	0.22	0.241	0.082
Urine excretion							
Urine output, g/day	5.92	3.87	2.48	3.48	1.28	0.096	0.379
Gross energy, kcal/day	150	116	84	86	19	0.008	0.700
Nitrogen, g/day	6.76	4.04	3.46	1.93	0.47	< 0.001	0.999
ATTD of dry matter, %	89.63	90.02	89.31	88.21	0.42	0.032	0.027
ATTD of gross energy, %	88.58	88.81	88.18	87.09	0.50	0.046	0.076
Nitrogen balance							
Nitrogen absorbed, g/day	29.83	24.33	22.43	19.47	0.88	< 0.001	0.895
Nitrogen retained, g/day	23.06	20.30	18.96	17.54	0.59	< 0.001	0.892

Table 5.3 (cont.)

ATTD of nitrogen, %	86.77	86.38	84.14	82.11	0.77	< 0.001	0.054
Nitrogen retention, % of intake	67.09	72.05	71.26	73.89	1.31	< 0.001	0.686
Biological value, %[2]	77.28	83.41	84.73	90.02	1.53	< 0.001	0.336
Energy in diets, kcal/kg							
Digestible energy	3,522	3,496	3,460	3,451	20	0.007	0.898
Metabolizable energy	3,382	3,389	3,382	3,372	24	0.811	0.679
Energy efficiency, %							
ME to GE	85.05	86.10	86.19	85.10	0.61	0.713	0.096
ME to DE	96.02	96.96	97.75	97.72	0.48	0.008	0.660

[1]Data are least square means of 10 observations for all treatments.

[2]Calculated according to Rojas and Stein (2013).

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CHAPTER 6: Feeding intact protein from soybean meal instead of synthetic amino acids does not affect growth performance, carcass composition, energy deposition, blood cytokines or abundance of intestinal amino acid transporters in growing pigs

Abstract

The objective was to test the hypothesis that reducing dietary crude protein in corn-soybean meal (**SBM**) diets will not increase diet net energy (**NE**) and will not affect growth performance, carcass composition, nutrient deposition, intestinal morphology, blood cytokine concentrations, or the abundance of genes for intestinal AA transporters. A control corn-SBM diet and three additional diets were formulated by reducing the SBM inclusion and increasing corn and adding three, four, or five synthetic amino acids (**AA**; Lys, Met, Thr, Trp, and Val) to the diets, resulting in protein in diets of 20%, 16.4%, 15.4%, or 13.4%. All diets were formulated to meet current requirements. A total of 176 pigs (initial weight: 32.2 ± 4.2 kg) were used. On d 1, 16 pigs were euthanized, and body nutrient composition was determined. The remaining 160 pigs were allotted to the four experimental diets using a randomized complete block design with four pigs per pen and 10 replicate pens per diet. Diets were provided on an *ad libitum* basis for 28 days. One pig per pen was slaughtered and blood, carcass, and viscera were collected and analyzed for nitrogen, fat, and energy to calculate nutrient deposition. Samples of blood were also analyzed for total protein, albumin, plasma urea nitrogen, and cytokines. Samples of ileal mucosa, ileum and colon tissue, and ileum and colon digesta were collected. Tissue morphology, abundance of genes for amino acid transporters, and ammonia concentration were determined. Contrasts

coefficients were used to determine linear and quadratic effects of reducing dietary protein. Results indicated that average daily gain, average daily feed intake, gain to feed ratio, carcass characteristics, and protein, lipid, and energy depositions were not affected by reducing dietary crude protein. However, net energy in diets tended to decrease (linear, $P = 0.051$) as dietary protein was reduced. Blood urea nitrogen was reduced (linear, $P < 0.001$) as dietary protein was reduced, but blood total protein or albumin were not affected by dietary protein. Cytokines, ileal and jejunal morphology, ammonia in ileal or in colon digesta, and abundance of AA transporters in the ileal mucosa were also not affected by dietary protein. Bacterial protein in colon digesta decreased (linear, $P = 0.030$) by reducing dietary protein. In conclusion, reducing dietary crude protein in corn-SBM diets did not affect growth performance, carcass composition, nutrient deposition, intestinal morphology, blood cytokines, or abundance of AA transporters in growing pigs, but NE of diets tended to be reduced as diet crude protein was reduced.

Key words: growing pigs, carcass composition, low protein, soybean meal, net energy

Introduction

Diets for growing pigs based on soybean meal (**SBM**) and cereal grains typically meet amino acid (**AA**) requirements and maximize growth performance and protein synthesis. However, the growing demand for more precise nutrition has led to increased use of synthetic AA in pig diets. As a result, over the past few decades, the use of synthetic AA has increased, while the inclusion of SBM has declined (Pope et al., 2023). Regardless of wether AA are supplied by SBM or synthetic sources, it is generally assumed that protein and energy deposition occur at similar rates as long as AA requirements are met (Che et al., 2017; Wang et al., 2018). However, results

of recent research indicate that daily nitrogen retention may decrease when pigs are fed diets with less SBM and more synthetic AA, compared with diets containing more SBM (Zhao et al., 2019; Cristobal et al., 2024b). This may indicate either an AA deficiency in diets with synthetic AA or that there are other factors in SBM, beyond AA, that are beneficial for pigs. In addition to AA, SBM provides other functional factors, such as polyphenols, terpenoids, bioactive peptides, dietary fiber, oligosaccharides, and functional lipids, that may offer anti-inflammatory, antimicrobial, antioxidant, or immunomodulatory effects for pigs (Smith and Dilger, 2018; Boyd et al., 2023; Petry et al., 2024; White and Dilger, 2024).

The shift from intact protein to synthetic AA may also result in a compensatory increase in AA transporter expression or changes in the intestinal morphology to facilitate the absorption of free AA (Morales et al., 2015). However, there is a lack of data for the abundance of genes for AA transporters or morphology in different sections of the small intestine of pigs fed diets based on either intact protein or synthetic AA (Morales et al., 2015; Wang et al., 2018).

Reducing crude protein in corn-SBM diets by decreasing SBM inclusion and increasing corn, while supplementing with synthetic AA, is believed to increase net energy (**NE**) in diets because corn contains more NE than SBM (NRC, 2012). However, results of recent experiments indicate that the NE in SBM may have been underestimated and is close to the NE in corn (Cemin et al., 2020; Cristobal et al., 2024a). Therefore, if the NE in SBM is close to that of corn, it is expected that diet NE will not increase if more corn and less SBM is used in diets, but validation of this hypothesis is lacking.

Therefore, the objective of this experiment was to test the hypothesis that reducing crude protein by reducing the inclusion of SBM in corn-SBM diets will not increase diet NE and will not affect growth performance, carcass composition, protein and energy deposition, intestinal

morphology, blood cytokine concentrations, or the abundance of genes for intestinal AA transporters.

Materials and methods

The Institutional Animal Care and Use Committee at the University of Illinois reviewed and approved the protocol for the experiment before animal work was initiated. Pigs were the offspring of Line 800 boars and Camborough females (Pig Improvement Company, Hendersonville, TN, USA).

Dietary treatment

A control diet was formulated based on corn and SBM without synthetic AA. Three additional diets were formulated by reducing the inclusion rate of SBM and adding more corn and 3 synthetic AA (i.e., Lys, Met, Thr); 4 synthetic AA (i.e., Lys, Met, Thr, Trp); or 5 synthetic AA (i.e., Lys, Met, Thr, Trp, Val) to the diet. Therefore, a total of four diets were used (Table 6.1). Concentrations of standardized ileal digestible indispensable AA and all other nutrients were at or above requirements for growing pigs (NRC, 2012) in all diets, but the concentration of CP was reduced from 20.0% in the control diet to 16.4%, 15.4%, or 13.4% in diets containing three, four, or five synthetic AA.

Animals, housing, feeding and growth performance

A total of 176 growing pigs (average initial body weight: 32.2 ± 4.2 kg) were used in the experiment. Among these, 16 pigs (eight gilts and eight barrows) were randomly selected at the start of the experiment and designated as an initial slaughter group (**ISG**) to determine nutrient deposition. The remaining 160 pigs were allotted to the four diets using a randomized complete block design. Thus, there were four pigs per pen (two gilts and two barrows) and 10 replicate

pens per diet. Starting weight was used as the blocking factor. Diets were provided to pigs on an *ad libitum* basis for 28 d. Individual pig weights were recorded at the beginning of the experiment and at the conclusion of the experiment on day 29. Feed additions were recorded daily and the weight of feed left in the feeder was recorded on day 29. Data were summarized to calculate final body weight, average daily feed intake (**ADFI**), average daily gain (**ADG**), and gain:feed ratio (**G:F**) within each pen and treatment group.

Sample collection and analysis

At the conclusion of the experiment, one gilt or barrow (average final body weight: 61.1 ± 8.4 kg) was randomly selected from each pen, for a total of 20 gilts and 20 barrows. In each pen, the selected pig was the one whose body weight was closest to the pen average. Two blood samples were then collected from the jugular vein of each selected pig. One blood sample was collected in heparinized vacutainers, whereas the other sample was collected in vacutainers containing ethylenediaminetetraacetic acid (**EDTA**). After sample collection, the selected pigs were transported to the Meat Science Laboratory at the University of Illinois and slaughtered after an overnight fast. Pigs were euthanized via head to heart electric stunning followed by exsanguination. Blood was quantitatively collected from each pig. A sample of 50 mL of the total blood was collected for compositional analysis.

Euthanized pigs were scalded, dehaired, and singed to remove all hair from the carcass; toenails, tail, and head were then removed. Weights of organs (i.e., heart, kidneys, liver, gall bladder, spleen, lungs, and gastrointestinal tract) were recorded. The gastrointestinal tract was emptied and cleaned with water and empty weight of stomach, small intestine, and large intestine was recorded. Before emptying the intestinal tract, digesta samples from the small intestines and colon were collected for ammonia analysis. For each sample, 3 mL of digesta were collected in

25-mL tubes, and 3 mL of HCl were added as a preservative. Concentration of protein was also analyzed in colon digesta.

The carcass was split down the midline from the groin to the chest cavity. One half of each carcass was used to determine hot carcass weight, dressing percentage, and chilled carcass weight. For body composition analysis, the body was partitioned into three components: carcass, blood, and viscera. The carcass consisted of skin, lean tissue, and fat tissue, without all bones. Blood included all blood collected during exsanguination. Viscera comprised the liver, heart, kidneys, lungs, spleen, and the emptied stomach, small intestine, and large intestine.

Jejunal and ileal tissue samples were collected (about 5 cm in length) approximately 200 cm distal to the pylorus and 80 cm caudal to the ileal-cecal junction, respectively. The jejunal and ileal tissue samples were opened longitudinally along the mesenteric attachment, rinsed with phosphate buffered saline, pinned serosa side down on a piece of cardboard (Nabuurs et al., 1993), and then fixed by immersion in 10% neutral buffered formalin. These tissue samples were delivered to Veterinary Diagnostic Laboratory at the University of Illinois (Urbana, IL, USA) within 24 h post fixation to be sectioned and transferred to slides. After fixation, the intestinal tissues were embedded in paraffin, sectioned at five μm, and stained with hematoxylin and eosin (Pluske et al., 1996). Villus height and crypt depth of the jejunum and ileum were measured from 10 straight and integrated villi and their associated crypts of each sample were also measured using Nanozoomer Digital Pathology View2 (Hammatsu, Bridgewater, NJ, USA) as described by others (Liu et al., 2018).

Ileal mucosa samples were also collected and gene abundance of AA transporters including solute carrier family 3 member 2 (***SLC3A2***; rBAT), solute carrier family 6 member 14 (***SLC6A14***; $ATB^{\circ,+}$), and solute carrier family 6 member 19 (***SLC6A19***; B°AT) were determined

in these samples. Samples were washed with phosphate-buffered saline, scraped gently, snap-frozen in liquid nitrogen, and stored at -80ºC until used for ribonucleic acid (**RNA**) extraction and quantitative reverse-transcription polymerase chain reaction (**qRT-PCR**) as described by others (Espinosa et al., 2021).

Weights of muscle and fat tissues and skin from the left side of the carcass were recorded the day after pigs were slaughtered. Because one side was used, the weights of muscle and fat tissues and skin were calculated by multiplying the recorded weights from the left half of the carcass by two, and the sum was assumed to represent the weight of the total carcass.

Samples of viscera, muscle, fat, skin, and blood from exsanguination were stored at −20 °C until processing. Prior to lyophilization, samples were frozen at −80 °C for 8 h and then lyophilized for 70 h. Lyophilized samples were ground using a swing-type grain mill (model: RRH-500, Zhejiang Winki Plastic Industry Co., Ltd., Zhejiang, China). Samples were analyzed for dry matter (method 930.15; AOAC Int., 2019), and for nitrogen using the combustion procedure (method 990.03; AOAC Int., 2019) on a LECO FP628 (LECO Corp., Saint Joseph, MI, USA). Crude protein was calculated as analyzed nitrogen × 6.25. Gross energy in these samples was analyzed using bomb calorimetry (Model 6400; Parr Instruments, Moline, IL, USA), and acid-hydrolyzed ether extract was analyzed by acid hydrolysis using 3 *N* HCl (Ankom HCl Hydrolysis System, Ankom Technology, Macedon, NY, USA) followed by fat extraction (Ankom XT-15 Extractor, Ankom Technology, Macedon, NY, USA).

The blood samples in the vacutainer with EDTA were centrifuged at 4,000 × *g* for 13 min to recover the plasma, which was then stored at –20 ºC until analyzed. Heparinized plasma samples were analyzed for plasma urea nitrogen, total protein, albumin, and plasma AA, whereas

plasma that contained EDTA were analyzed for cytokines [e.g., interleukin (**IL**) 1β, IL-10, IL-4, and tumor necrosis factor alpha (**TNF-α**)].

Plasma urea nitrogen, total protein, and albumin were analyzed using a Beckman Coulter Clinical Chemistry AU analyzer (Beckman Coulter Inc., Brea, CA, USA). Concentrations of AA in plasma were analyzed by liquid chromatography-mass spectrometry analysis using a Sciex 5500 QTrap with Agilent 1200 LC (AB Sciex, Framingham, MA, USA) according to the protocol described by others (Beals et al., 2016). Cytokines in plasma samples were analyzed using a sandwich enzyme-linked immunosorbent assay kit according to manufacturer's instructions (R&D Systems Minneapolis, MN, USA; Invitrogen, MA, USA).

Ammonia in digesta samples from the small intestines and colon were analyzed by gas chromatography using a Hewlett-Packard 5890A Series II gas-liquid chromatograph (Agilent, Santa Clara, CA) and a glass column (180 cm by 64 mm i.d.). Digesta samples from the colon were also analyzed for microbial protein. Samples were fractionated using differential centrifugation (Metges et al., 1999) and centrifuged at 250 relative centrifugal force for 15 min at 4 °C, which separated fractions that contained undigested feed particles in the precipitate, and porcine cells in the supernatant (Miner-Williams et al., 2009). The supernatant was centrifuged at 14,500 relative centrifugal force for 30 min at 4 °C, which resulted in a precipitate that contained microbial cells (Miner-Williams et al., 2009). This precipitate was then subjected to a lysis buffer, which contained 100 mM of tris(hydroxymethyl)aminomethane at pH 7.2, 0.5% sodium dodecyl sulfate, and 0.5% sodium deoxycholate. The protein concentration of the lysed microbial cells was analyzed using Pierce Bicinchoninic Acid Assay Kit (ThermoFisher Scientific, Waltham, MA).

The RNA was extracted from 30 ± 0.2 mg of frozen ileal mucosa using β-mercaptoethanol (Sigma-Aldrich, St. Louis, MO, USA) according to the RNeasy Mini Kit (QIAGEN, Germantown, MD, USA) manufacturer's instructions and following the procedure described by others (Espinosa et al., 2021). Total RNA was quantified using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). The RNA quality was determined using a Fragment Analyzer Automated CE System (method DNF-471-33-SS Total RNA 15nt; Advanced Analytical, Ankeny, IA, USA). The RNA samples with an RNA quality number greater than 7 were diluted to 100 ng/μL with DNase/RNase free water and used for complementary deoxyribonucleic acid (**cDNA**) synthesis. The cDNA was then diluted 1:4 with DNase/RNase-free water to conduct qRT-PCR analysis which was performed using 4 μL of diluted cDNA and 6 μL of a mixture including forward and reverse primers, SYBR Green master mix (Quanta Biosciences Inc., Gaithersburg, MD, USA), and DNase/RNase free water in a MicroAmpTM Optical 384-Well Reaction Plate (Applied Biosystems, Foster City, CA, USA). Two internal control genes, glyceraldehyde 3-phosphate dehydrogenase and beta-actin (Nygard et al., 2007), were used to normalize the abundance of tested genes. Tested genes (i.e., *SLC3A2*, *SLC6A14*, and *SLC6A19*) were analyzed to determine if dietary crude protein influences regulation of AA absorption and transport in the small intestine.

Chemical analysis of diets and ingredients

All diet and ingredient samples were ground using a swing-type grain mill (model: RRH-500, Zhejiang Winki Plastic Industry Co., Ltd., Zhejiang, China) prior to chemical analyses. Diet and ingredient samples were analyzed for dry matter, nitrogen, acid-hydrolyzed ether extract, and gross energy as described for viscera, muscle, fat, skin, and blood. Crude protein in diet and ingredient samples was also calculated as described for viscera, muscle, fat, skin, and blood. Diet

and ingredient samples were also analyzed for ash (method 942.05; AOAC Int., 2019) and insoluble and soluble dietary fiber on an Ankom Total Dietary Fiber Analyzer (Ankom Technology, Macedon, NY, USA) according to method 991.43 (AOAC Int., 2019). Total dietary fiber was calculated as the sum of insoluble and soluble dietary fiber. Amino acids in diet and ingredient samples were analyzed at the Agricultural Experiment Station Chemical Laboratories at the University of Missouri (Columbia, MO, USA) on a Hitachi Amino Acid analyzer (Model No. L8800; Hitachi High Technologies America, Inc., Pleasanton, CA, USA) using ninhydrin for postcolumn derivatization and norleucine as the internal standard [method 982.30 E(a, b, and c); AOAC Int., 2019]. Total starch was analyzed in corn and in diet samples using the glucoamylase procedure (method 979.10; AOAC Int., 2019), whereas glucose, sucrose, maltose, fructose, stachyose, and raffinose were analyzed in SBM using high-performance liquid chromatography (method 977.2, AOAC Int., 2019). Soybean meal was also analyzed for trypsin inhibitors (method Ba 12-75; AOCS, 2006).

Calculations

The analyzed energy, protein, and lipids in blood, viscera, muscle, fat, and skin samples was used to estimate total energy, protein, and lipids of viscera and blood, and total energy, protein, and lipids in the carcass was calculated as the sum of these components in muscle, fat, and skin. The total amount of energy, protein, and lipids in each pig at the conclusion of the experiment was calculated from the sum of the energy, protein, and lipids in the blood, viscera, and carcass. All values were calculated on a dry matter basis. Retention of energy, protein, and lipids was calculated from the difference between the average quantity of energy, protein, and lipids in the 16 pigs from the ISG and the quantity of energy, protein, and fat in the treatment pigs at the end of the 28 day experimental period.

Net energy in protein and lipids was calculated by multiplying retained protein and lipids by 5.66 and 9.46 kcal/g, respectively (Ewan, 2001). Net energy for growth was calculated as the sum of NE from retained protein and lipids. Daily NE for maintenance was calculated by multiplying the mean metabolic body weight ($kg^{0.60}$) by 179 kcal (Noblet et al., 1994). Net energy per kg of diet was calculated by dividing the sum of NE for growth and NE for maintenance by daily feed intake.

To calculate energy efficiency, daily energy intake was calculated by multiplying ADFI of pigs by analyzed gross energy in diets. Energy efficiency for growth was then calculated by dividing retained energy in the total body by energy intake and multiplying by 100.

Statistical analysis

Data were analyzed using the MIXED Procedure in SAS (SAS Inst. Inc., Cary, NC, USA). Homogeneity of the variances among treatments was confirmed using the UNIVARIATE procedure. The MIXED procedure in SAS was used to generate studentized residuals and outliers were defined as means with residuals greater than 3 or less than -3. Outliers that were identified and removed included 1 pig fed the diet with 13.4% protein for the carcass composition analysis, 1 pig fed the diet with 20.0% protein for the IL-1β analysis, 1 pig fed the diet with 16.4% protein for the TNF-α analysis, 1 pig fed the diet with 15.4% for the ileal morphology analysis, and 1 pig fed the diet with 13.4% protein for the jejunal morphology analysis. Pen was the experimental unit for all analyses. The statistical model included diet as fixed effect and block as random effect. Least square means were calculated, and contrast coefficients were generated from analyzed dietary crude protein using the Interactive Matrix Language Procedure in SAS. These coefficients were used to determine linear and quadratic

effects of reducing dietary crude protein. Statistical significance and tendencies were considered at $P < 0.05$ and $0.05 \leq P < 0.10$, respectively.

Results

Pigs remained healthy during the experiment and no mortality or feed refusals were observed. Final body weight of pigs was not affected by dietary treatment (Table 6.2). Average daily gain and ADFI of pigs were not affected by reducing SBM and increasing synthetic AA in diets, which resulted in no differences in G:F. Live weight, hot carcass weight, dressing percentage, viscera weights, and digesta-free body weight were also not different among treatments (Table 6.3). However, chilled carcass weight tended to decrease (quadratic, $P = 0.099$) as dietary protein was reduced.

Retention of nutrients and energy

Weights of carcass, viscera, and blood were not affected by dietary treatments (Table 6.4). Concentrations of protein and fat in carcass and viscera were also not affected by dietary treatments, but concentration of protein in blood showed a quadratic response ($P < 0.034$). Concentration of energy in carcass, blood, and bone-free total body either significantly (quadratic, $P = 0.034$) or tended (quadratic, $P < 0.10$) to increase and then decrease as dietary protein was reduced. Retained protein, lipid, and energy were not affected by dietary treatment, but energy efficiency tended to decrease (quadratic, $P = 0.087$) as dietary protein was reduced. Net energy in diets also tended to decrease (linear, $P = 0.051$) as dietary protein was reduced.

Blood characteristics, metabolites in digesta, and AA transporters

Plasma urea nitrogen was reduced (linear, $P < 0.001$) as dietary protein was reduced, but blood total protein was not affected by dietary treatment (Table 6.5). Albumin in blood tended to

increase and then decrease (quadratic, $P = 0.072$) as dietary protein was reduced, but concentrations of cytokines were not affected by dietary protein. Ileal and jejunal morphologies were also not affected by dietary protein (Table 6.6). Ammonia concentrations in ileal digesta increased and then decreased (quadratic, $P = 0.043$) and ammonia in colon digesta tended to increase and then decrease (quadratic, $P = 0.074$) as dietary protein was reduced. Bacterial protein in colon digesta was reduced (linear, $P = 0.030$) as dietary protein was reduced, but abundance of genes related to AA transporters in the ileal mucosa were not affected by dietary protein (Table 6.7).

Discussion

The analyzed crude protein and AA in experimental diets were in close agreement with formulated values, which indicates that diets were mixed correctly. Likewise, analyzed concentration of nutrients in corn and SBM were in agreement with previous data (NRC, 2012).

It was expected that on average, all indispensable AA in diets, except for Met, would decrease by reducing the inclusion of SBM and increasing corn and synthetic AA in diets because of the increased digestibility of synthetic AA compared with AA in SBM, and this was also observed. However, the analyzed Met increased in the diets as SBM was reduced and synthetic AA were added, which is likely because synthetic $_{DL}$-Met was added to diets to meet the requirements for both Met and Cys.

Growth performance, carcass weights, and retained nutrients and energy

The lack of differences among treatments in final body weight, ADG, ADFI, and G:F is in agreement with results from previous experiments (Kerr et al., 1995; Le Bellego et al., 2001; Wang et al., 2018). The lack of differences in growth performance is likely a result of the fact

that reduced protein diets supplemented with synthetic AA can provide enough nutrients to meet minimum requirements by pigs to maximize growth. However, reducing crude protein in diets by more than 4% may result in reduced growth performance due to a potential AA deficiency (Wang et al., 2018). Feeding reduced protein diets may also result in increased fat deposition of pigs and reduced protein deposition (Ruusunen et al., 2006; Morazan et al., 2015; Ruiz-Ascacibar et al., 2016). It is, however, possible that there are fewer AA provided in excess of the requirement in reduced protein diets, which requires less energy for deamination of AA and excretion of nitrogen, and low-protein diets, therefore, may provide more NE, which can result in increased fat deposition (Smith et al., 1999). However, despite a reduction in diet protein by more than six percent units in this experiment, neither growth performance nor carcass weights were affected by treatment. It is, therefore, likely that the AA profile in the diets used in the present experiment was closer to the requirements of the pigs than in some previous experiments where reduced growth performance was observed in pigs fed low-protein diets (Ruusunen et al., 2006; Ruiz-Ascacibar et al., 2016). However, the current data are in agreement with data demonstrating that it is possible to feed reduced protein diets without negatively impacting growth performance or carcass characteristics (Kerr et al., 1995; Li et al., 2016; Suarez-Belloch et al., 2016).

The observed values for hot carcass weight and digesta-free body weight in diets with 20.0% or 16.4% crude protein were in agreement with data from previous experiments in which corn-SBM diets with approximately 20% crude protein were fed to growing pigs (Kil et al., 2011; 2013; Stewart et al., 2013). The observed values for chilled carcass and the full viscera weight also agreed with previous data. However, empty viscera weight in kg or as percent of live weight was less in this experiment than previously reported (Stewart et al., 2013), but that is

likely because diets high in fiber were used by Stewart et al. (2013), and dietary fiber will increase intestinal mass (Henry, 1985; Pond et al., 1988), which may be the reason for this difference between the two experiments.

The observed values for total protein, lipids, and energy in carcass, viscera, and blood were slightly greater than values reported with a 20% crude protein diet (Stewart et al., 2013). Likewise, retained protein, lipid, and energy were in agreement with previous data (Ruiz-Ascacibar et al., 2016). Compared with results of other experiments (Kil et al., 2011; 2013), retained protein and lipid were greater, but the lipid:protein ratio aligned closely. The lack of differences in retained protein and lipid among pigs fed experimental diets contrasts with results indicating a reduced protein retention and increased lipid retention in pigs fed low-protein diets compared with pigs fed diets with greater protein concentraiton (Ruiz-Ascacibar et al., 2016). Results from the present experiment also were different from data indicating that energy retention is increased in pigs fed low-protein diets (Le Bellego et al., 2001). It is not clear if these differences are caused by differences in diet composition or by changes in the ability of pigs to retain energy.

The observed NE in diets were on average greater than values determined in corn-SBM diets (Kil et al., 2011; Kil et al., 2013), but that is likely because less soybean oil was used in those experiments, and the observed NE in the current experiment was close to the determined NE in corn-SBM diets with a similar soybean oil inclusion (Stewart et al., 2013). It was expected that NE would increase as dietary crude protein decreased because the NE in SBM is believed to be less than in corn (Sauvant et al., 2004; NRC, 2012). Therefore, reducing SBM and increasing corn concentration should have increased diet NE. However, the observation that no increase of NE in diets was observed as crude protein was reduced in diets indicates that NE in SBM is close

to or greater than the NE of corn. In fact, the tendency for a reduction in NE as SBM in the diets was reduced indicates that NE in SBM may be greater than in corn, which has also been reported in other experiments in which NE in SBM was estimated from growth performance of pigs (Cemin et al., 2020). In other experiments in which NE in SBM was determined using indirect calorimetry, the NE in SBM was close to the NE in corn (Li et al., 2017; Cristobal et al., 2024a). These observations imply that current book values (Sauvant et al., 2004; NRC, 2012) may underestimate the NE in SBM. There is, therefore, a need for generating new prediction equations for NE in diets for pigs. The determined greater NE in SBM compared with previous values also implies that SBM can offer a greater nutritional value for pigs than previously thought, which has also been demonstrated in the past (Che et al., 2017).

The observed plasma urea nitrogen concentrations were within the range of values determined in previous experiments (Kerr and Easter, 1995; Che et al., 2017) and the observed decrease in plasma urea nitrogen as crude protein in diets was reduced, was also observed by others (Kerr and easter., 1995; Che et al., 2017; Wang et al., 2018; Limbach et al., 2021). Plasma urea nitrogen is generated during AA metabolism and reflects the amount of excess nitrogen that pigs are consuming (Wang et al., 2018). In pigs fed high protein diets, some AA are fed in excess of the requirement and these AA are deaminated, producing ammonia that is then converted to urea prior to excretion, which increases plasma urea nitrogen. Pigs fed diets with reduced protein are expected to have less excess AA, and therefore, less deamination, less ammonia production, and less urea synthesis, which reduces plasma urea nitrogen. Thus, the observed reduction in plasma urea nitrogen in pigs fed diets with reduced crude protein was expected.

The observed values for total protein and albumin in blood agreed with the values obtained in a previous experiment (Che et al., 2017). The lack of differences in total protein and

albumin in blood indicate that the provision of indispensable AA was close to the requirements regardless of the diet being fed. Albumin synthesis relies on indispensable AA, and stable levels of albumin in blood indicates that sufficient AA were available for protein synthesis. Maintaining stable levels of total protein in blood along with the observed reduction in blood urea nitrogen also support efficient nitrogen utilization.

The observed lack of effects on pro-inflammatory or anti-inflammatory cytokine levels, indicates that reducing dietary crude protein does not confer immune advantages compared with high-protein diets in healthy non-disease-challenged pigs. This observation contrasts data indicating that low-protein diets reduced post-weaning diarrhea in pigs, potentially due to improved intestinal health (Limbach et al., 2021). However, results from the present experiment indicate that reducing protein in diets may not necessarily modulate systemic immune responses as measured by cytokine levels. Therefore, growing pigs appear to tolerate high-protein diets without adverse effects on their immune status, and reducing crude protein does not provide immunological benefits. The observed lack of effects on villi height or crypt depth in ileal and jejunal morphology also supports this conclusion, and this lack of effect was also observed in a previous experiment with low protein diets (Opapeju et al., 2008).

Reducing dietary crude protein intake decreases substrate availability for bacterial fermentation, potentially lowering ammonia production. However, low-protein diets do not always result in significant changes in intestinal ammonia levels (Tao et al., 2021). This may be due to compensatory mechanisms in nitrogen metabolism or variations in gut microbiota composition (Liu and Fan, 2022). Reducing dietary protein may result in a decrease in bacteria that thrive on protein (Liu and Fan, 2022), which may reduce bacterial protein content in the colon, which was also observed in the present experiment. It is, however, acknowledged that the

data presented for intestinal ammonia and bacterial protein are based on the concentration in intestinal contents. If dietary treatments affected total fecal mass, concentrations in intestinal contents would not necessarily represent daily synthesis.

It was hypothesized that a reduction in dietary protein may result in adaptative responses for AA transporters, which may increase the abundance of genes related to AA transporters to increase the uptake of more free AA (Morales et al., 2015; Li et al., 2024). The observed lack of effects of dietary treatments on SLC6A14 differs from a previous experiment (Wang et al., 2017), where it was observed that the abundance of this gene was reduced in pigs fed diets containing 14% crude protein compared with pigs fed a 20% crude protein diet, but the lack of differences observed for SLC3A2 does agree with the previous experiment (Wang et al., 2017). The overall lack of effects observed of dietary treatments on abundance of genes for AA transports indicates that these specific transporters may not be sensitive to dietary protein levels. This observation agrees with results of other experiments where it was observed that the abundance of AA transporters is not impacted by crude protein level in diets (Morales et al., 2015).

Conclusions

The hypothesis that reducing diet protein concentration by reducing dietary SBM and increasing corn and synthetic AA would not increase diet NE was confirmed as results demonstrated that there was a tendency for a reduction in NE as crude protein was reduced. Likewise, the hypothesis that reducing diet crude protein would not impact growth performance or carcass composition of pigs was confirmed. Intestinal morphology, blood cytokine concentrations and abundance of genes related to AA transporters were also not impacted by dietary treatments,

which was also in agreement with the hypothesis. Overall, these results indicate no advantage of reducing diet SBM concentrations, but the observation that the NE in SBM is likely greater than current book values deserves further investigation.

Tables

Table 6.1. Ingredient composition of experimental diets and analyzed nutrient composition of ingredients and experimental diets formulated to different concentrations of crude protein, as-fed basis

Item	Ingredients		Diets			
	Corn	SBM[1,2]	20.0% crude protein	16.4% crude protein	15.4% crude protein	13.4% crude protein
Ingredient, %						
Ground corn	-	-	60.40	70.27	71.40	75.11
Soybean meal	-	-	34.50	24.00	22.80	18.75
Soybean oil	-	-	2.50	2.50	2.50	2.50
Dicalcium phosphate	-	-	0.90	1.10	1.10	1.15
Ground limestone	-	-	0.80	0.75	0.75	0.75
$_{L}$-Lys-HCl	-	-	-	0.32	0.35	0.47
$_{DL}$-Met	-	-	-	0.08	0.09	0.12
$_{L}$-Thr	-	-	-	0.08	0.10	0.15
$_{L}$-Trp	-	-	-	-	0.01	0.03
$_{L}$-Val	-	-	-	-	-	0.07
Sodium chloride	-	-	0.40	0.40	0.40	0.40
Vitamin-mineral premix[3]	-	-	0.50	0.50	0.50	0.50
Analyzed nutrient						
Dry matter, %	86.95	89.80	88.49	89.73	88.16	88.55

Table 6.1 (cont.)

Crude protein, %	7.80	45.18	20.02	16.36	15.38	13.40
Gross energy, kcal/kg	3,819	4,204	3,976	3,936	3,924	3,963
Acid-hydrolyzed ether extract, %	2.53	1.76	5.17	4.49	4.15	4.62
Starch, %	64.50	-	39.75	45.88	46.58	48.88
Total dietary fiber, %	15.10	23.10	17.70	18.20	18.50	17.00
Soluble dietary fiber, %	2.50	3.4	2.40	2.10	2.00	2.80
Insoluble dietary fiber, %	12.6	19.7	15.30	16.10	16.50	14.20
Ash, %	1.32	6.19	4.65	4.27	4.21	4.05
Indispensable amino acids, %						
Arg	0.35	3.31	1.25	0.94	0.98	0.93
His	0.23	1.23	0.50	0.40	0.41	0.39
Ile	0.27	2.05	0.85	0.69	0.69	0.65
Leu	0.91	3.48	1.58	1.35	1.39	1.32
Lys	0.24	2.84	1.07	1.09	1.08	1.11
Met	0.17	0.63	0.28	0.28	0.31	0.36
Phe	0.37	2.30	0.95	0.77	0.79	0.75
Thr	0.27	1.79	0.70	0.63	0.67	0.64
Trp	0.06	0.63	0.34	0.23	0.26	0.25
Val	0.36	2.14	0.92	0.75	0.75	0.80
Dispensable amino acids, %						
Ala	0.57	1.98	0.87	0.82	0.82	0.75

Table 6.1 (cont.)

Asp	0.51	5.19	1.81	1.56	1.56	1.35
Cys	0.18	0.67	0.27	0.26	0.27	0.26
Glu	1.40	8.20	3.16	2.82	2.83	2.54
Gly	0.29	1.91	0.73	0.65	0.65	0.58
Pro	0.67	2.43	1.03	0.97	0.97	0.90
Ser	0.36	2.27	0.75	0.68	0.69	0.61
Tyr	0.25	1.53	0.58	0.52	0.52	0.47
Total amino acids, %	7.46	44.58	17.64	15.41	15.40	14.66

[1]Sugar composition (%): glucose, 0.05; sucrose, 6.27; maltose, 0.13; fructose, 0.07; stachyose, 5.61; raffinose, 1.64.

[2]Trypsin inhibitor units per mg: 3.42.

[3]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 D_3 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 B_{12}, 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.

Table 6.2. Growth performance of growing pigs fed experimental diets[1]

Item	Dietary crude protein, %					Contrast *P*-value	
	20.0	16.4	15.4	13.4	SEM	Linear	Quadratic
Initial body weight, kg	32.17	32.29	32.20	32.18	-	-	-
Final body weight, kg	61.31	60.64	60.62	61.70	2.20	0.908	0.378
ADG[2], kg/d	1.040	1.012	1.015	1.055	0.041	0.912	0.339
ADFI[2], kg/d	2.116	2.207	2.147	2.234	0.072	0.133	0.914
G:F[2]	0.493	0.460	0.473	0.474	0.014	0.296	0.275

[1]Least squares means represent 10 observations per dietary treatment.

[2]ADG = average daily gain; ADFI = average daily feed intake; G:F = gain to feed ratio.

Table 6.3. Weights of carcass and viscera of growing pigs fed experimental diets[1]

Item	Dietary crude protein, %					Contrast *P*-value	
	20.0	16.4	15.4	13.4	SEM	Linear	Quadratic
Live weight, kg	58.85	59.47	58.47	59.80	1.96	0.542	0.658
Hot carcass weight[2], kg	42.50	43.11	43.66	42.34	1.60	0.896	0.305
Dressing percentage, %	72.10	72.42	73.32	72.02	0.63	0.721	0.172
Chilled carcass, kg	42.23	43.41	43.91	41.82	1.71	0.953	0.099
Full viscera[3], kg	8.28	8.47	8.29	8.51	0.25	0.514	0.920
Full viscera, % of live weight	14.14	14.28	14.22	13.97	0.35	0.790	0.548
Empty viscera, kg	7.09	6.99	7.00	6.97	0.20	0.562	0.881
Empty viscera, % of live weight	12.08	11.79	11.87	11.70	0.23	0.195	0.883
Digesta-free body weight[4], kg	52.32	53.27	53.61	51.54	1.98	0.825	0.171

[1]Least squares means represent 10 observations per each dietary treatment except that means for dressing percentage and empty viscera of diet containing 15.4% protein represent 9 observations and that means for dressing percentage and full viscera of diet containing 13.4% protein represent 9 observations.

[2]Hot carcass weight does not include leaf fat.

[3]Full viscera include the combined weights of the liver, gall bladder, heart, kidneys, lungs, spleen, and the stomach, small intestine, and large intestine with their contents.

[3]Calculated as the sum of the weights of chilled carcass, empty viscera, and blood.

Table 6.4. Analyzed composition of carcass, retention of energy, protein, and lipids in pigs from the initial slaughter group (ISG) and pigs fed experimental diets, and net energy in experimental diets[1,2], dry matter basis

Item		Dietary crude protein, %					Contrast *P*-value	
	ISG	20.0	16.4	15.4	13.4	SEM	Linear	Quadratic
Carcass[3]								
Weight, kg	8.82	21.60	21.91	20.99	20.33	1.14	0.163	0.261
Protein, g/kg	558	481	457	496	486	13	0.670	0.401
Lipids, g/kg	365	479	474	419	463	24	0.378	0.483
Energy, Mcal/kg	6.65	7.15	7.22	7.30	7.02	0.1	0.640	0.096
Viscera[4]								
Weight, kg	0.68	1.58	1.63	1.61	1.63	0.06	0.438	0.784
Protein, g/kg	696	626	632	598	609	19	0.357	0.866
Lipids, g/kg	194	237	245	244	250	13	0.482	0.976
Energy, Mcal/kg	5.52	5.60	5.76	5.72	5.70	0.08	0.346	0.311
Blood								
Weight, kg	0.25	0.53	0.50	0.46	0.49	0.03	0.120	0.544
Protein, g/kg	994	1,006	1,022	1,025	1,009	7	0.454	0.034
Lipids, g/kg	10	21	14	14	12	2	0.005	0.645
Energy, Mcal/kg	5.41	5.26	5.58	5.41	5.26	0.11	0.837	0.025
Total body[5]								
Weight, kg	9.74	23.71	24.04	23.07	22.45	1.19	0.167	0.270
Protein, g/kg	579	503	481	514	506	13	0.747	0.399

Table 6.4 (cont.)

Lipids, g/kg	344	452	449	399	438	22	0.391	0.513
Energy, Mcal/kg	6.54	7.00	7.09	7.15	6.89	0.10	0.664	0.069
Retained protein[6], g/d	–	223.23	209.74	220.83	200.98	18.84	0.285	0.725
Retained lipid[6], g/d	–	265.35	266.44	234.07	236.17	29.71	0.213	0.751
Retained lipid:protein	–	1.06	1.09	0.94	1.01	0.07	0.491	0.976
Retained energy[6], Mcal/d	–	3.66	3.82	3.52	3.25	0.33	0.135	0.120
Energy intake[7], Mcal/d	–	8.41	8.68	8.42	8.85	0.29	0.184	0.609
Energy efficiency for growth[7], %	–	42.79	43.86	41.68	36.40	3.03	0.083	0.087
NE in diets								
NE from retained protein[8], kcal/d	-	1,263	1,187	1,250	1,138	107	0.285	0.725
NE from retained lipids[8], kcal/d	-	2,510	2,521	2,214	2,234	281	0.213	0.751
NE for growth[9], kcal/d	-	3,774	3,708	3,464	3,372	357	0.124	0.671
NE for maintenance[10], kcal/d	-	1,795	1,788	1,787	1,800	40	0.883	0.411
NE in diets[11], kcal/kg	-	2,605	2,488	2,436	2,305	134	0.051	0.667

[1]Least squares means represent 10 observations per dietary treatment.

[2]Concentrations of protein, lipid, and energy represent analyzed nitrogen × 6.25, acid hydrolyzed ether extract, and gross energy in each body part, respectively.

Table 6.4 (cont.)

[3]Carcass includes skin, lean tissue, and fat tissue, excluding all bones.

[4]Viscera include the liver, heart, kidneys, lungs, spleen, and the empty stomach, small intestine, and large intestine.

[5]Total body includes bone-free carcass, viscera, and blood.

[6]Retained nutrients and energy in the body were calculated using the difference in body composition between ISG (n = 16) and body composition of pigs fed experimental diets for 28 d.

[7]Energy intake was calculated as multiplying average daily feed intake of pigs by analyzed gross energy in respective diets; energy efficiency for growth was calculated as dividing retained energy by the energy intake and multiplying by 100.

[8]NE in protein and lipids was calculated by multiplying retained protein and lipids by 5.66 and 9.46 kcal/g, respectively (Ewan, 2001).

[9]NE for growth was calculated as the sum of NE from retained protein and lipids.

[10]Daily NE for maintenance was calculated by multiplying the mean metabolic body weight ($kg^{0.60}$) by 179 kcal (Noblet et al., 1994).

[11]NE in diets was calculated by dividing the sum of NE for growth and NE for maintenance by daily feed intake.

Table 6.5. Concentrations of plasma urea nitrogen, total protein, albumin, and cytokines in plasma of pigs fed experimental diets[1]

Item	Dietary crude protein, %					Contrast *P*-value	
	20.0	16.4	15.4	13.4	SEM	Linear	Quadratic
Plasma urea nitrogen, mg/dL	16.10	10.30	8.10	5.00	0.93	<0.001	0.886
Total protein, g/dL	5.59	5.88	5.67	5.64	0.12	0.742	0.175
Albumin, g/dL	3.70	3.86	3.84	3.66	0.10	0.999	0.072
Cytokines, pg/mL							
IL[2]-1β	33.22	13.87	18.75	20.77	7.70	0.197	0.211
IL-4	0.23	0.22	0.22	0.22	0.00	0.142	0.347
IL-10	0.09	0.09	0.09	0.09	0.00	0.996	0.236
TNF-α[2]	118.14	92.87	105.26	96.58	11.20	0.182	0.539

[1]Least squares means represent 10 observations per each dietary treatment except that means for IL-1β of diet containing 20.0% protein represent 9 observations and that means for IL-1β and TNF-α of diet containing 16.4% protein represent 9 observations.

[2]IL, interleukin; TNF, tumor necrosis factor alpha.

Table 6.6. Morphology of jejunal and ileal tissues and ammonia concentrations in ileal and colon digesta and bacteria in colon digesta of pigs fed experimental diets[1]

Item	Dietary crude protein, %					Contrast *P*-value	
	20.0	16.4	15.4	13.4	SEM	Linear	Quadratic
Ileal morphology							
Villi height, μm	511.06	488.14	477.42	495.06	15.84	0.324	0.287
Crypt depth, μm	259.51	246.24	259.30	251.83	12.79	0.704	0.781
Villi height to crypt depth	2.02	2.02	1.87	1.97	0.08	0.401	0.673
Jejunal morphology							
Villi height, μm	496.45	514.81	510.86	502.58	16.60	0.715	0.450
Crypt depth, μm	275.46	290.24	271.93	289.37	12.15	0.525	0.931
Villi height to crypt depth	1.83	1.79	1.90	1.75	0.07	0.631	0.426
Ammonia in ileum, mg/g	0.52	0.84	0.78	0.60	0.12	0.421	0.043
Ammonia in colon, mg/g	2.14	2.32	2.49	2.15	0.13	0.559	0.074
Bacteria protein in colon, μg/g	963.14	817.07	868.43	710.05	74.30	0.030	0.700

[1]Least squares means represent 10 observations per each dietary treatment except that means for ileal morphology of pigs fed diet containing 15.4% protein represent 9 observations and that means for jejunal morphology of pigs fed diet containing 13.4% protein represent 9 observations.

Table 6.7. Abundance of genes for amino acid transporters in the ileal mucosa of pigs fed experimental diets[1,2]

Item	Dietary crude protein, %					Contrast *P*-value	
	20.0	16.4	15.4	13.4	SEM	Linear	Quadratic
SLC3A2	1.85	1.10	2.19	1.41	1.29	0.629	0.762
SLC6A14	2.02	2.11	1.26	1.30	1.46	0.269	0.759
SLC6A19	0.62	0.58	0.54	0.94	1.35	0.454	0.265

[1]Data are least squares means of 6 observations per treatment.

[2]Least squares means and SEM were log2-backtransformed after the statistical analysis.

[3]*SLC3A2*, solute carrier family 3 member 2; *SLC6A14*, solute carrier family 6 member 14; and *SLC6A19*, solute carrier family 6 member 19.

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CHAPTER 7: Effects of protein concentration on net energy, digestibility of starch, crude protein, and amino acids in diets fed to growing pigs

Abstract

Two experiments were conducted with the objective of testing the hypothesis that diet protein concentration does not affect apparent ileal digestibility (**AID**) of starch, standardized ileal digestibility (**SID**) of amino acids (**AA**), or net energy (**NE**) in diets fed to growing pigs. In experiment 1, two diets with 17 or 14% crude protein (**CP**) were formulated. One diet was based on corn, soybean meal (**SBM**), and no synthetic AA and the other diet was based on corn, SBM, and synthetic Lys, Met, and Thr. Four additional diets containing 13, 12, 11, or 10% CP were formulated by reducing SBM and increasing corn and synthetic Lys, Met, and Thr and adding synthetic Trp, Val, Ile, Phe, and His. A nitrogen-free diet was also used to determine basal endogenous losses of AA, and all diets also contained Cr_2O_3 as an indigestible marker. Seven barrows (initial body weight: 38.2 ± 1.5 kg) that had a T-cannula installed in the distal ileum were allotted to a 7 × 7 Latin square design with 7 diets and 7 periods and ileal digesta were collected for 2 days of each period. Results demonstrated that AID of starch was not influenced by diet CP concentration, but SID of CP and all indispensable and dispensable AA increased (linear, $P < 0.05$) when dietary CP was reduced. In experiment 2, the six protein containing diets from experiment 1 were used but Cr_2O_3 was replaced by corn and no nitrogen-free diet was used in this experiment. Twenty-four growing pigs (initial BW: 29.9 ± 2.4 kg) were allocated to 6 calorimeter chambers with 4 pigs per chamber. The 6 chambers were allotted to the six diets

using a 6 × 6 Latin square design with 6 periods of 14 days. Pigs had ad libitum access to diets throughout the experiment except during the final 36 h of each period, when pigs were fasted. Net energy in diets was determined using indirect calorimetry. Results indicated that digestible energy and metabolizable energy was reduced (linear, $P < 0.05$) when dietary CP was reduced, but NE in diets was not influenced by dietary CP. In conclusion, reducing CP of diets for pigs by reducing SBM and increasing corn and synthetic AA does not affect AID of starch, but increases SID of indispensable AA; however, dietary CP does not influence diet NE when fed to group-housed pigs on an ad libitum basis.

Key works: net energy, metabolizable energy, soybean meal, synthetic amino acids

Introduction

Soybean meal (**SBM**) is the major source of amino acids (**AA**) in diets for swine throughout the world. In the U.S., around 18% of all SBM produced is used to feed pigs (Soybean Meal Info Center, 2022). However, due to the emergence of feed-grade synthetic AA and distillers dried grains with solubles, it is estimated that SBM usage by pigs has been reduced over the last 25 years, particularly in countries that do not produce SBM (Van Heugten, 2021; Pope et al., 2023). Also, reducing crude protein (**CP**) in diets, which requires reduction in SBM, has been proposed as a method to reduce the environmental impact of swine production by reducing nitrogen excretion and carbon footprint (Eugenio et al., 2022).

Corn and SBM are assumed to have concentrations of metabolizable energy (**ME**) that are not different, but corn is believed to contain more net energy (**NE**) than SBM (NRC, 2012). Therefore, in theory, corn-SBM diets formulated with synthetic AA contain more NE compared

with corn-SBM diets without synthetic AA because if synthetic AA are used, the inclusion of SBM is reduced and the inclusion of corn is increased. However, recent data indicate that diets based primarily on corn and SBM without synthetic AA contain more NE than diets based on large quantities of synthetic AA, which indicates that NE in SBM may have been underestimated (Cemin et al., 2020). Likewise, recent data from indirect calorimetry experiments (Li et al., 2017; Lee et al., 2021) indicate that the NE in SBM is greater than current book values (Sauvant et al., 2004; NRC, 2012; Rostagno et al., 2024). The NE of SBM calculated from the feed efficiency of growing pigs was also greater than expected if the NE of SBM is close to book values (Ibagon et al., 2024). Thus, results of a number of recent experiments indicate that NE in SBM is likely underestimated, but it is not known by how much and how this likely underestimation impacts the NE of diets with varying concentrations of corn and SBM.

Synthetic AA are expected to be completely digestible (Oliveira et al., 2020) and to be absorbed at a faster rate compared with intact protein (Kodera et al., 2006). Thus, corn-SBM diets with different inclusions of synthetic AA may have variations in digestibility and absorption rates of AA, which may cause AA imbalances at sites of protein synthesis and affect the metabolism of AA in the diet (Trottier, 2006; Selle et al., 2020; Eugenio, 2021). However, it is not known how this may impact NE of diets in which some of the SBM is replaced by synthetic AA. Likewise, if diets contain less SBM and more corn, the concentration of starch will increase, but it is not known if increased dietary starch will affect pre-cecal digestibility of starch. Therefore, two experiments were conducted to test the hypothesis that standardized ileal digestibility (**SID**) of AA and CP, apparent ileal digestibility (**AID**) of starch, and NE in diets based primarily on corn and SBM are not different from that of diets containing less SBM, more corn, and synthetic AA.

Materials and methods

Two experiments were conducted, and the protocol for both experiments were reviewed and approved by The Institutional Animal Care and Use Committee at the University of Illinois before animal work was initiated. Pigs were the offspring of Line 800 boars and Camborough females (Pig Improvement Company, Hendersonville, TN, USA).

Dietary treatment

In experiment 1, seven diets were used. A diet that contained corn, SBM, and no synthetic AA and a diet containing corn, SBM, and synthetic Lys, Met, and Thr were formulated (Table 7.1). These two diets contained 17 and 14% CP, respectively. Four additional diets were formulated to contain 13, 12, 11, or 10% CP, respectively, and these diets contained more corn, less SBM, and more synthetic AA compared with the second diet. Also, a nitrogen free diet was formulated to calculate basal endogenous losses of AA and CP. All 7 diets contained 0.40% chromic oxide as an indigestible marker. All diets, except for the nitrogen free diet, were formulated to meet or exceed the current requirement estimates for standardized ileal digestible AA and all diets met requirements for vitamins and minerals by growing-finishing pigs (NRC, 2012). In experiment 2, six diets were used, and these were identical to the diets used in experiment 1, except that no nitrogen-free diet was used and chromic oxide was replaced by corn.

Animals, housing, and feeding

In experiment 1, seven barrows (average initial body weight: 38.2 ± 1.5 kg) were allotted to a 7 × 7 Latin square design with 7 diets and 7 periods. Thus, there were 7 replicate pigs per diet. Pigs had a T-cannula installed in the distal ileum for collection of digesta (Stein et al., 1998). Pigs were housed in individual pens (1.2 × 1.5 m) in an environmentally controlled room with the ambient temperature maintained between 20 and 24 °C. Pens had smooth sides, and fully slatted

tribar floors, and were equipped with a feeder and a nipple waterer and pigs had free access to water throughout the experiment. Feed allowance was calculated as 3.0 times the maintenance requirement for ME (i.e., 197 kcal ME per kg body weight$^{0.60}$; NRC, 2012) and was adjusted according to the body weight of pigs at the beginning of each period.

In experiment 2, 24 barrows (average initial body weight: 29.3 ± 2.3 kg) were allotted to the six diets in a 6 × 6 Latin square design with 6 indirect calorimetry chambers and 6 consecutive periods. Thus, there were six replicate calorimetry chambers per diet and all diets were provided to each chamber during one period. Four barrows were placed in each chamber. Each chamber was equipped with a stainless steel wet-dry feeder and a nipple waterer was available to ensure free access to water throughout the experiment. Each chamber was also equipped with a fully slatted T-bar floor, stainless steel fecal screens, and urine pans, which allowed for total, but separate, collection of feces and urine. The temperature and relative humidity inside the chambers were maintained at 23 °C and 55%, respectively, and controlled by a temperature and humidity control unit (Model 9241-2220-B1D0000; Parameter Generation & Control, Parameter, Black Mountain, NC, USA). The air velocity was maintained at 1.13 m^3/min using an airflow meter (AccuValve; Accutrol, LLC, Danbury, CT, USA). Diets were fed for 13 days on an ad libitum basis, but in the morning of day 14, feeders were emptied, and pigs were fasted during the following 36 h.

Sample collection

In experiment 1, each period lasted 7 days, where the initial 5 days were considered an adaptation period to the diet and ileal digesta were collected on days 6 and 7 for 9 h each day (from 0700 to 1600 hours) following standard procedures (Stein et al., 1998). A plastic bag was attached to the opened cannula barrel using a plastic cable tie, and digesta flowing into the bag

were collected. Bags were replaced once they were filled with digesta or at least every 30 min and immediately stored at -20 °C to prevent bacterial degradation of AA in the digesta. At the conclusion of the experiment, ileal digesta samples were thawed, mixed, and a subsample was collected, lyophilized, and finely ground in preparation for chemical analysis.

In experiment 2, diets were fed for 12.5 days in which feed intake was not restricted. The initial 6.5 days were considered the adaptation period to the diet starting from midday on day 1, followed by 6 days of total collection of feces and urine. At 0700 h on day 8, the gas analyzers (Classic Line, Sable System Int., North Las Vegas, NV, USA) were turned on to measure O_2 consumption and CO_2 and CH_4 productions and gas measurements ceased at 0700 h on day 14, and these measurements were used to determine total heat production (**THP**). Fecal and urine samples were quantitatively collected from days 8 to 13. At 0700 h on day 14, pigs were deprived of feed for 36 h. This time was considered the fasting period and the initial 24 h of fasting were considered the time that the animals digested and metabolized the remaining feed in the intestinal tract to produce energy. However, the following 12 h of fasting were considered the actual period where the animals mobilized endogenous nutrients to produce energy and the fasting heat production (**FHP**) was measured during this period (De Lange et al., 2006). Fasting heat production was calculated using urine nitrogen and measured O_2 consumption and CO_2 and CH_4 production during this period. Therefore, each period lasted 14 d.

All pigs were weighed prior to being moved into the calorimeter chambers and at the conclusion of each collection period. Chambers were opened every day to add feed to the feeders, and to collect feces and urine during collection periods. Feed spillage on the screens was collected daily during the collection period, and the weight of spilled feed was recorded to determine feed intake. To avoid nitrogen loss from the urine, 50 ml of 3 *N* HCl was added to

each urine pan every day during the collection period. Collected feces were dried immediately after collection in a 65 °C forced air drying oven (Thermo Fisher Scientific Inc.; model Heratherm OMH750, Waltham, MA, USA) until constant weight, and then ground through a 1-mm screen using a hammer mill (model: MM4; Schutte Buffalo, NY, USA). Collected urine was weighed and mixed, and 10% was stored at -20 °C immediately after collection. At the end of the experiment, urine samples were thawed and mixed within chamber and period, and two subsamples were collected and stored at -20 °C. One subsample was thawed and dripped on cotton balls placed in a plastic bag that were then lyophilized (Kim et al., 2009). The other subsample was thawed and used for nitrogen analysis. However, urine collected during the fasting period was only analyzed for nitrogen. Data from the gas analyzers obtained during the period that the chambers were open and until they reached the condition set by the temperature and humidity control unit were disregarded for the final calculation of heat production.

Chemical analysis

In experiment 1, diet and ileal digesta samples were analyzed for dry matter (**DM**; method 930.15; AOAC Int., 2019) and nitrogen using the combustion procedure (method 990.03; AOAC Int., 2019) on a LECO FP628 (LECO Corp., Saint Joseph, MI, USA). Crude protein in diets and ileal digesta samples was calculated as analyzed nitrogen × 6.25. Diet and ileal digesta samples were analyzed for AA (method 982.30 E [a, b, c,]; AOAC Int., 2019) at the Agricultural Experiment Station Chemical Laboratories at the University of Missouri (Columbia, MO, USA) on a Hitachi AA analyzer (Model No. L8800; Hitachi High Technologies America, Inc., Pleasanton, CA, USA) using ninhydrin for postcolumn derivatization and norleucine as the internal standard. Diet and ileal digesta samples were also analyzed for starch using the

glucoamylase procedure (method 979.10; AOAC Int., 2019), and for chromium using Inductive Coupled Plasma Atomic Emission Spectrometric (method 990.08 AOAC Int., 2019).

In experiment 2, diet and fecal samples were analyzed for DM and nitrogen as described for experiment 1. Nitrogen and AA in diets were also analyzed as described for experiment 1, and CP was calculated as nitrogen × 6.25. Diet samples were analyzed for ash (method 942.05; AOAC Int., 2019), and diet, fecal, and lyophilized urine samples were analyzed for gross energy (**GE**) using bomb calorimetry (Model 6400; Parr Instruments, Moline, IL, USA). Urine samples that were not lyophilized were analyzed for nitrogen using the Kjeldahl method (method 984.13; AOAC Int., 2019) on a Kjeltec 8400 (FOSS Inc., Eden Prairie, MN, USA). Acid-hydrolyzed ether extract (**AEE**) in diet and fecal samples was analyzed using acid hydrolysis using 3 *N* HCl (Ankom HCl Hydrolysis System, Ankom Technology, Macedon, NY, USA) followed by fat extraction using petroleum ether (method 2003.06, AOAC Int., 2019; Ankom XT-15 Extractor, Ankom Technology, Macedon, NY, USA). Insoluble dietary fiber (**IDF**) and soluble dietary fiber (**SDF**) were also analyzed in diets and fecal samples on an Ankom Total Dietary Fiber Analyzer (Ankom Technology, Macedon, NY, USA) using method 991.43 (AOAC Int., 2019). Total dietary fiber (**TDF**) was calculated as the sum of insoluble and soluble dietary fiber. Diets were also analyzed for acid detergent fiber (**ADF**; method 12; Ankom 2000 Fiber Analyzer, Ankom Technology, Macedon, NY, USA).

The same batches of corn and SBM were used in experiments 1 and 2. Samples were analyzed for DM, GE, AEE, IDF, SDF, ash, ADF, AA, starch, and nitrogen following methods as described for experiment 2. Crude protein and TDF for corn and SBM were also calculated as previously described. Soybean meal was also analyzed for trypsin inhibitors (method Ba 12-75; AOCS, 2006).

Calculations

In experiment 1, AID of CP, AA, and starch was calculated using analyzed CP, AA, starch, and Cr in diets and ileal digesta (Stein et al., 2007). The basal endogenous losses of CP and AA were calculated from pigs fed the N-free diet, and SID of CP and AA was calculated by correcting the AID for the basal endogenous losses of CP and AA (Stein et al., 2007).

In experiment 2, the apparent total tract digestibility (**ATTD**) of GE, DM, TDF, and AEE were calculated for each diet (Adeola, 2001), and DE and ME in each diet were calculated as well (NRC, 2012). Nitrogen intake, nitrogen excretion, ATTD of nitrogen, retention of nitrogen, and biological value were also calculated (Pedersen et al., 2007).

For calculation of NE in diets, concentrations of O_2, CO_2, and CH_4 were averaged separately for the fed period and for the last 12 h of the fasting period. The respiratory quotient (**RQ**) was calculated as the ratio between CO_2 production (L/d) and O_2 consumption (L/d; Richardson, 1929). Total heat production (**THP**) was calculated from gas exchanges during the fed period using the following equation (Brouwer, 1965):

$THP_{kcal} = [(3.866 \times O_2) + (1.200 \times CO_2) – (0.518 \times CH_4) – (1.431 \times \text{urine nitrogen})]$,

where O_2, CO_2, and CH_4 were expressed as L, and urine nitrogen was expressed as g. The fasting heat production (**FHP**) measured during the fasting period was calculated as described for THP. Heat increment was calculated by subtracting FHP from THP and NE in each diet was calculated using the following equation (modified from NRC, 2012):

$$NE_{kcal/kg} = \frac{ME - (THP - FHP)}{\text{feed intake}},$$

where ME is in kcal/kg, THP and FHP are in kcal, and feed intake is in kg and refers to feed intake during the collection period. The RQ was calculated as the ratio of the volume of CO_2 produced to the volume of O_2 consumed. Net energy in diets was also calculated (Table 2) using

the published NE values for yellow dent corn and dehulled, solvent-extracted SBM (NRC, 2012). In addition, NE in each diet was calculated using equation 1–7 (NRC, 2012).

Statistical analysis

Data from experiments 1 and 2 were analyzed using the MIXED procedure in SAS (SAS Institute Inc., Cary, NC, USA). Homogeneity of the variances among treatments was confirmed using the UNIVARIATE procedure. The MIXED procedure in SAS was used to generate studentized residuals and outliers were defined as means with residuals greater than 3 or less than -3. However, no outliers were detected in either of the two experiments. For experiment 1, the statistical model included diet as the fixed effect, and period and animal as random effects. The pig was the experimental unit. For experiment 2, the statistical model included diet as fixed effect and chamber and period as random effects. The calorimeter chamber was the experimental unit. For both experiments, least square means were calculated, and contrast coefficients were generated from analyzed dietary CP using the Interactive Matrix Language procedure of SAS. These coefficients were used to determine linear and quadratic effects of reducing dietary CP from 17 to 10%. Statistical significance and tendency were considered at $P < 0.05$ and $0.05 \leq P < 0.10$, respectively.

Results

Pigs remained healthy during both experiments and no feed refusals were observed. Analyzed concentrations of nutrients in diets (Table 7.2 and 7.3) were in agreement with calculated values.

Experiment 1

Results indicated that there was no effect of reducing dietary CP from 17 to 10% on AID of CP, total dispensable AA, total AA, or starch (Table 7.4). The AID of Lys, Met, Thr, Trp, Glu, and

total indispensable AA increased (linear, $P < 0.05$) as dietary CP was reduced, and the AID of His, Leu, and Val tended to increase (linear, $P < 0.10$) as dietary CP was reduced. However, the AID of Arg decreased (linear, $P < 0.05$) as dietary CP was reduced.

Reducing dietary CP in diets increased the SID of CP and the SID of each indispensable and dispensable AA (linear, $P < 0.05$; Table 7.5). Likewise, when determining the SID of total indispensable or total dispensable AA, an increasing effect (linear, $P < 0.05$) was observed when dietary CP was reduced.

Experiment 2

Results indicated that reducing dietary CP tended to increase (linear, $0.05 < P < 0.10$) feed intake and ATTD of DM (Table 7.6). Reducing dietary CP reduced (linear, $P < 0.05$) ATTD of TDF, DE, and ME in diets. However, reducing dietary CP did not affect the ATTD of AEE, ATTD of GE, daily fecal GE excretion, daily urine GE excretion, daily THP, daily FHP, daily retained energy, RQ during fasted or fed state, or NE in diets.

Reducing dietary CP decreased (linear, $P < 0.05$) daily nitrogen intake, daily nitrogen excreted in feces or in urine, daily absorbed nitrogen, and daily retained nitrogen (Table 7.7). Likewise, reducing dietary CP decreased (quadratic, $P < 0.05$) the ATTD of nitrogen. However, reducing dietary CP increased (linear, $P < 0.05$) nitrogen retention as percent of intake and biological value of CP.

Discussion

The analyzed concentration of nutrients in corn and SBM were in agreement with previous data (NRC, 2012). The analyzed values for CP and total AA in diets from both experiments were in close agreement with formulated values, which indicates that diets were

mixed correctly. The analyzed starch in diets increased as dietary CP was reduced and this was because corn inclusion increased, which increased the supply of starch to the diets. In general, the analyzed concentration of all indispensable AA in diets decreased as dietary CP was reduced and this was because more synthetic AA were included in the diets as SBM was reduced, and it was assumed that synthetic AA have absorptions close to 100% compared with the digestibility of AA in SBM that is around 90%. Therefore, slightly less concentrations of indispensable AA were needed to meet the standardized ileal digestible indispensable AA requirements. However, concentration of Met increased as dietary CP was reduced and this was because as SBM was reduced, Cys concentration was also reduced, and therefore, more synthetic Met was added to diets to furnish both Met and Cys in the diets.

Experiment 1

The observed AID of starch in all diets was in agreement with previous data (Lin et al., 1987; Bach Knudsen et al., 2006; McGhee and Stein, 2020; Lee et al., 2023). From the analyzed nutrients in ingredients, it was observed that most of the starch in the ingredients was supplied by corn, therefore the digestibility of starch in the diets is mainly influenced by the digestibility of starch of corn. The lack of effects of diet on AID of starch indicates that the changes in TDF in the diets as CP changed did not impact starch digestibility. Starch is absorbed mainly in the small intestine of the pig, therefore, determining ileal digestibility of starch yields a more accurate digestibility estimate compared with total tract digestibility (Stein and Bohlke, 2007).

The AID or SID values observed for CP and AA in the 17% CP diet agreed with values for AID or SID in corn and SBM (NRC, 2012) and were in agreement with reported AID of AA in corn-SBM diets with no synthetic AA fed to growing pigs (Urriola and Stein, 2010). The SID of AA in SBM is greater than in corn (NRC, 2012), and even though less SBM and more corn

was included as dietary CP was reduced, the SID of AA increased which is, likely due to the high absorption of synthetic AA. This observation indirectly confirms that the SID of synthetic AA is greater than that of the AA in SBM and that the absorption of synthetic AA may be close to 100%, as indicated by others (Chung and Baker, 1992; Trottier, 2006; Selle et al., 2020). The concentration of TDF in diets was reduced as CP was reduced due to the reduction of SBM. It has been indicated that fiber has a negative effect on digestibility of CP or AA (Gutierrez et al., 2013) and the increased SID of AA that was observed as CP was reduced may be partly due to the reduction in TDF. An increase in SID of AA was also reported when synthetic AA were added to a diet based on corn, further indicating that the increased supplementation with synthetic AA as diet CP was reduced contributed to the increased SID of AA (Oliveira et al., 2020).

Experiment 2

The observed feed intake and the daily fecal and urine GE excretion were in agreement with data from group-housed pigs fed corn-SBM diets (Lee et al., 2024). Likewise, the observed ATTD of DM, AEE, TDF, and GE were in agreement with previous data (Urriola and Stein, 2010; Rojas and Stein, 2013; Navarro et al., 2018; Abelilla and Stein, 2019; Liu et al., 2019; Rodriguez et al., 2020; Lee et al., 2024). Soybean meal and corn have similar ATTD of DM, which is around 90% (Li et al., 2017; Navarro et al., 2018). Therefore, no effects in ATTD of DM was expected by replacing SBM with corn and, which was confirmed in the present experiment. The lack of effects in ATTD of AEE indicates that there is no difference in how AEE in corn or SBM are digested by growing pigs or that the differences may be masked by the added soybean oil to the diets.

Soybean meal contains more TDF and IDF, and slightly more SDF compared with corn (NRC, 2012; Navarro et al., 2018; Abelilla and Stein, 2019; Rodriguez et al., 2020). These differences in TDF were also observed in the analyzed composition of corn and SBM in the present experiment. The fact that the ATTD of TDF decreased as SBM was replaced by corn indicates a greater digestibility of TDF in SBM compared with corn. Furthermore, because most of the TDF in SBM is composed of IDF, the fermentation of IDF in SBM may be greater than the fermentation of IDF from corn. The differences in ATTD of TDF or IDF caused by varying composition of ingredients in diets were previously reported (Abelilla and Stein, 2019). The decrease in ATTD of TDF as dietary CP was reduced may suggest a decrease in ATTD of GE because less energy from fermentation of fiber was synthesized (Abelilla and Stein, 2019). However, the lack of effects observed in ATTD of GE among diets may indicate that the differences in ATTD of TDF may not be big enough to be reflected in differences in ATTD of GE. Therefore, it appears that corn and SBM have ATTD of GE that is not different.

The values for THP, FHP, RQ in fed and fasted state were in agreement with data for a corn-SBM diet that were determined in the same facility (Munoz, 2020; Leet et al, 2024). However, THP and FHP were greater compared with data obtained with individually housed pigs although the RQ in fed or fasted state were observed in this experiment were close to reported data (Noblet et al., 1994; Li et al., 2017; Kim et al., 2018; Lyu et al., 2023). Nonetheless, it appears that THP or FHP were consistent among diets indicating that there is no influence of dietary CP on THP or FHP. The observed greater THP in the present experiment compared with previous data is likely due to the ad libitum feeding and that pigs were group-housed in the present experiment, whereas restricted feeding and individually housed pigs were used in previous experiments. Therefore, it is likely that the estimations for THP and FHP, and

subsequent NE from the present experiment are more accurate representations of a commercial system compared with the settings used in previous indirect calorimetry experiments.

The observed DE and ME were generally in agreement with previous data (Noblet et al., 1994; Rojas and Stein, 2013; Munoz, 2020; Lee et al., 2024). The reduction of DE in diets as SBM was replaced by corn indicates that the DE in SBM is greater than in corn. This observation has also been reported by others (Rojas and Stein, 2013; Sotak-Peper et al., 2015), and the decrease in DE in diets with reduced dietary CP has also been reported (Le Bellego et al., 2001; Cristobal et al., 2024a). Likewise, the observed decrease in ME as SBM was replaced by corn indicates that SBM may contain more ME than corn. However, this observation differs from previous data that indicated no difference in ME between corn and SBM (Sotak-Peper et al., 2015; Li et al., 2017; Cristobal et al., 2024b) or from data that indicated more ME in corn compared with SBM (NRC, 2012). However, all those experiments determined ME in individually housed pigs, and determining ME in group-housed pigs may influence the estimations.

The observed NE were in agreement with a previous experiment using group-housed pigs (Lee et al., 2024), but was slightly greater than determined in experiments with individually housed pigs (Noblet et al., 1994; Li et al., 2017). The NE obtained in the present experiment were also greater than the NE calculated from the analyzed composition of diets using published equations (NRC, 2012). According to NRC (2012), corn contains more NE compared with SBM, and if SBM inclusion is reduced while corn inclusion is increased, it was expected that NE of the diet would increase. However, no effects on NE were observed as dietary CP was reduced in diets indicating that the NE in SBM is equal or close to that in corn and this is supported by previous data that indicated no differences in NE between corn and SBM or greater NE in SBM

than previously thought (Li et al., 2017; Cemin et al., 2020; Lee et al., 2021; Ibagon et al., 2024). Results from experiment 1 indicate that the lack of effects on NE among diets is not caused by differences in AID of starch and certainly not caused by a decrease in SID of indispensable AA. Therefore, the lack of effects of dietary CP on NE in diets and the fact that the determined NE in the diets is greater than calculated NE, indicates that NE in SBM has been underestimated.

The underestimation of NE in SBM is likely due to an underestimation of the nitrogen retention coefficient. The equations published by NRC (2012) are based on published equations by Noblet et al. (1994) and these equations were developed from experiments conducted prior to that time. Therefore, it is likely that nitrogen retention may have changed over the years. Early studies reported nitrogen retention between 40 and 50% of consumed nitrogen (McConnell et al., 1971; Carr et al., 1977; Campbell and Dunkin, 1983), and later experiments reported values between 50 and 60% (Kerr and Easter, 1995; Lenis et al., 1999; Le Bellego et al., 2001; Otto et al., 2003; Pedersen et al., 2007; Patrás et al., 2012). Modern pigs, especially those with improved genetic traits, have nitrogen retention greater than 60% in diets containing corn and SBM (Rojas and Stein, 2013; Li et al., 2017; Cristobal et al., 2024b; Corassa et al., 2024; Ochoa et al., 2024). This improvement in nitrogen retention is likely due to enhanced efficiency in converting dietary protein into body protein, which may be attributed to selective breeding for lean deposition. Increased nitrogen retention indicates that modern pigs are better at utilizing AA for protein synthesis, reducing the need for deamination and urea cycle activity. As deamination is energy-consuming, this reduced activity may contribute to more NE in protein ingredients.

The ATTD of N, absorbed and retained nitrogen calculated as g per day or nitrogen retention calculated as percent of intake were in agreement with previous data (Rojas and Stein, 2013; Li et al., 2017; Cristobal et al., 2024b; Lee et al., 2024). Results indicate that there is a

relationship between reducing dietary CP and changes in nitrogen balance in growing pigs. As expected, nitrogen intake decreased as dietary CP was reduced, with pigs fed diets with 10% CP consuming significantly less nitrogen than those fed 17% CP, which is consistent with previous data (Kerr and Easter, 1995). The nitrogen excreted in urine followed a similar trend, and decreased with lower CP diets. This reduction in nitrogen excretion is likely due to the improved efficiency of nitrogen utilization in pigs fed lower protein diets, as previously indicated by others (Wang et al., 2018). In contrast, the reduction in nitrogen excreted in feces was much less than the reduction in urine nitrogen indicating that the changes in nitrogen excretion were mainly influenced by the urinary losses rather than fecal losses, which was expected because fecal losses of nitrogen are only influenced by nitrogen intake and ATTD of nitrogen. Although the ATTD of nitrogen was reduced as diet CP was reduced due to the lower ATTD of nitrogen in corn than in SBM, the effect of reduced intake was greater than the effect of reduced ATTD of nitrogen, which is the reason for the reduced fecal excretion of nitrogen as diet CP was reduced.

The absorbed nitrogen and retained nitrogen by pigs also decreased with lower CP diets, primarily due to the lower nitrogen intake, which is in agreement with recent data (Cristobal et al., 2024b). However, the nitrogen retention as a percentage of intake increased as the dietary CP decreased, which is in agreement with previous research (Kerr and Easter, 1995). This increase in nitrogen retention, measured as a percentage of intake, indicates that pigs on lower CP diets are more efficient at utilizing available nitrogen. This could be due to a reduction in the amount of excess AA that need to be deaminated and excreted. As fewer AA are deaminated, more nitrogen is retained for protein synthesis, resulting in higher retention efficiency.

Additionally, the biological value of CP increased as the CP level decreased, which may be attributed to the more balanced amino acid profile in low-CP diets supplemented with

synthetic AA. These diets likely provided a better match between the requirements and the amino acids available for protein synthesis. This observation aligns with the hypothesis that low-CP diets supplemented with synthetic AA offer a more efficient means of meeting AA needs, reducing waste and improving nitrogen retention, which has been indicated previously (Eugenio et al., 2022). Nevertheless, the reduced nitrogen retention calculated as g per day, indicates that overall less protein synthesis may occur when SBM is reduced and more synthetic AA are added to diets for growing pigs fed ad libitum. It is, therefore, likely that pigs fed diets with reduced CP have reduced protein deposition and, therefore, reduced lean meat percentage as has been reported previously (Smith et al., 1999; Li et al., 2016; Wang et al., 2017).

Conclusions

The hypothesis that reducing dietary CP by reducing dietary SBM and increasing corn and synthetic AA would not affect AID of starch was confirmed. However, the hypothesis that reducing dietary CP and supplementing with synthetic AA would not affect SID of CP and AA was rejected as a linear increase was observed for SID of CP and for indispensable and dispensable AA. The hypothesis that NE is changed as corn and synthetic AA are increased and SBM is reduced is accepted as the NE in diets did not increase or decrease with decreasing dietary CP. Overall, these results indicate that there is no advantage of reducing diet SBM concentrations, but the observation that the NE in SBM is likely greater than current book values may imply a need for reevaluation of published nutrient tables or correction of equations to determine NE to adjust for current nitrogen retention by growing pigs. These results support flexibility in SBM inclusion in grower pig diets, allowing nutritionists to adjust formulations

based on the market availability of SBM and synthetic AA. Because NE remains unaffected by varying SBM levels, diets can be economically optimized without compromising energy supply.

Tables

Table 7.1. Ingredient and nutrient composition of experimental diets, experiment 1[1]

Item[2]	Dietary crude protein						
	17%	14%	13%	12%	11%	10%	N-free[2]
Ground corn	69.28	76.68	78.94	81.19	83.35	85.42	-
Soybean meal	26.97	19.10	16.65	14.20	11.76	9.34	-
Soybean oil	1.00	1.00	1.00	1.00	1.00	1.00	4.00
Dicalcium phosphate	0.73	0.86	0.90	0.94	0.98	1.03	1.75
Ground limestone	0.73	0.70	0.70	0.69	0.68	0.68	0.30
Cornstarch	-	-	-	-	-	-	68.15
Sugar	-	-	-	-	-	-	20.00
Magnesium oxide	-	-	-	-	-	-	0.10
Potassium carbonate	-	-	-	-	-	-	0.40
L-Lys-HCl, 78% Lys	-	0.22	0.30	0.37	0.45	0.53	-
DL-Met, 98% Met	-	0.08	0.10	0.13	0.15	0.17	-
L-Thr, 98% Thr	-	0.07	0.10	0.13	0.17	0.20	-
L-Trp, 98% Trp	-	-	0.01	0.03	0.04	0.05	-
L-Val, 98% Val	-	-	-	0.03	0.07	0.12	-
L-Ile, 98% Ile	-	-	-	-	0.02	0.06	-
L-Phe, 98% Phe	-	-	-	-	0.01	0.06	-
L-His, 98% His	-	-	-	-	0.02	0.04	-
Solka floc[3]	-	-	-	-	-	-	4.00
Sodium chloride	0.40	0.40	0.40	0.40	0.40	0.40	0.40

Table 7.1 (cont.)

Chromic oxide	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Vitamin-mineral premix[4]	0.50	0.50	0.50	0.50	0.50	0.50	0.50

[1]Diets in experiment 2 were identical to those used in experiment 1 with the exception that chromic oxide was replaced by corn and no N-free diet was used.

[2]Nitrogen-free diet

[3]Fiber Sales and Development Corp., Urbana, OH, USA.

[4]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 D_3 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 B_{12}, 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.

Table 7.2. Analyzed nutrients composition of diets, as-fed basis, experiment 1

Item	Dietary crude protein						
	N-free[1]	17%	14%	13%	12%	11%	10%
Dry matter, %	92.15	87.19	86.91	86.71	86.80	86.88	87.51
Crude protein, %	0.61	18.66	13.77	13.31	13.10	11.36	10.65
Starch, %	56.90	45.00	45.60	47.70	52.50	51.80	55.20
Indispensable amino acids, %							
Arg	0.01	1.24	0.85	0.78	0.80	0.62	0.58
His	0.01	0.50	0.36	0.34	0.35	0.31	0.31
Ile	0.01	0.88	0.61	0.56	0.57	0.47	0.47
Leu	0.02	1.62	1.23	1.16	1.16	1.02	0.98
Lys	0.02	1.07	0.91	0.89	0.88	0.88	0.86
Met	0.01	0.27	0.25	0.33	0.30	0.30	0.32
Phe	0.01	0.98	0.69	0.64	0.65	0.54	0.55
Thr	0.01	0.72	0.55	0.64	0.61	0.51	0.58
Trp	0.02	0.18	0.14	0.14	0.14	0.14	0.13
Val	0.01	0.92	0.66	0.61	0.64	0.60	0.57
Total	0.13	8.38	6.25	6.09	6.10	5.39	5.35
Dispensable amino acids, %							
Ala	0.02	0.93	0.72	0.68	0.63	0.61	0.59
Asp	0.02	1.98	1.34	1.23	1.20	0.97	0.89
Cys	0.00	0.30	0.21	0.22	0.20	0.18	0.17
Glu	0.03	3.40	2.44	2.25	2.22	1.89	1.78

Table 7.2 (cont.)

Gly	0.01	0.80	0.57	0.53	0.54	0.44	0.42
Pro	0.04	1.08	0.84	0.80	0.80	0.70	0.60
Ser	0.02	0.83	0.61	0.56	0.57	0.47	0.45
Tyr	0.01	0.64	0.46	0.43	0.44	0.35	0.33
Total	0.15	9.96	7.19	6.70	6.60	5.61	5.23
Total amino acids	0.28	18.34	13.44	12.79	12.70	11.00	10.58

[1] Nitrogen-free diet.

Table 7.3. Analyzed nutrient composition of ingredients and diets, experiment 2

Item[2]	Corn	SBM[1]	Dietary crude protein					
			17%	14%	13%	12%	11%	10%
Dry matter, %	86.09	88.71	87.01	87.09	86.94	86.81	86.89	86.91
Gross energy, kcal/kg	3,821	4,209	3,845	3,801	3,800	3,788	3,785	3,786
Metabolizable energy, kcal/kg[2]	3,395	3,294	3,340	3,332	3,328	3,323	3,316	3,307
Net energy, kcal/kg[2]	2,672	2,087	2,500	2,534	2,543	2,552	2,559	2,564
Net energy, kcal/kg[3]	2,686	2,081	2,470	2,481	2,497	2,524	2,525	2,558
Crude protein, %	6.45	46.80	17.97	14.31	13.11	11.89	11.56	10.57
Acid-hydrolyzed ether extract, %	2.53	1.07	2.55	2.60	2.80	2.77	2.51	2.56
Starch, %	64.50	2.30	45.00	45.60	47.70	52.50	51.80	55.20
Ash	1.32	6.22	4.27	4.21	4.13	4.08	4.06	4.01
Total dietary fiber	10.70	17.10	11.80	10.50	10.60	10.00	10.20	9.90
Soluble dietary fiber	ND[4]	2.30	ND[4]	ND[4]	ND[4]	0.90	0.30	1.40
Insoluble dietary fiber	10.70	14.80	11.80	10.50	10.60	9.10	9.90	8.50
Acid detergent fiber	2.88	5.28	4.00	3.82	3.73	3.67	3.61	3.16
Indispensable amino acids, %								
Arg	0.32	3.33	1.14	0.90	0.79	0.69	0.67	0.55
His	0.19	1.23	0.47	0.38	0.35	0.31	0.31	0.29
Ile	0.25	2.31	0.77	0.60	0.55	0.48	0.48	0.49
Leu	0.76	3.65	1.48	1.26	1.16	1.05	1.05	0.96
Lys	0.24	2.95	1.01	0.96	0.92	0.87	0.87	0.88
Met	0.13	0.64	0.24	0.27	0.28	0.27	0.26	0.31

Table 7.3 (cont.)

Phe	0.32	2.41	0.88	0.70	0.64	0.57	0.57	0.54
Thr	0.24	1.81	0.68	0.60	0.56	0.57	0.57	0.61
Trp	0.05	0.63	0.20	0.17	0.17	0.16	0.14	0.14
Val	0.32	2.34	0.87	0.70	0.64	0.60	0.63	0.61
Total	2.82	21.30	7.74	6.54	6.06	5.57	5.54	5.38
Dispensable amino acids, %								
Ala	0.48	2.03	0.87	0.76	0.69	0.64	0.64	0.58
Asp	0.45	5.33	1.78	1.38	1.23	1.08	1.03	0.86
Cys	0.14	0.64	0.27	0.23	0.21	0.18	0.19	0.18
Glu	1.20	8.51	3.17	2.57	2.32	2.05	2.01	1.75
Gly	0.27	1.96	0.73	0.60	0.53	0.48	0.47	0.40
Pro	0.56	2.37	0.98	0.84	0.78	0.72	0.66	0.60
Ser	0.30	1.97	0.78	0.65	0.56	0.51	0.50	0.43
Tyr	0.20	1.69	0.59	0.47	0.44	0.38	0.37	0.33
Total	3.60	24.50	9.17	7.50	6.76	6.04	5.87	5.13
Total amino acids	6.42	45.80	16.91	14.04	12.82	11.61	11.41	10.51

[1]Trypsin inhibitor units per mg: 3.42.

[2]Calculated using published metabolizable energy or net energy values for yellow dent corn and dehulled, solvent-extracted SBM (NRC, 2012).

[3]Calculated with published equation (equation 1–7; NRC, 2012) to predict net energy in diets from analyzed nutrient composition and the determined metabolizable energy from experiment 2.

[4]Not detectable.

Table 7.4. Apparent ileal digestbility (AID) of crude protein, starch, and amino acids (AA)[1], experiment 1

Item	Dietary crude protein							Contrast *P*-value	
	17%	14%	13%	12%	11%	10%	SEM	Linear[2]	Quadratic[2]
Crude protein	76.84	77.07	75.97	79.57	76.89	78.33	1.32	0.242	0.671
Starch	94.14	95.48	95.55	95.20	95.30	94.86	0.53	0.319	0.419
Indispensable AA									
Arg	87.04	85.45	84.13	86.61	83.63	84.72	0.93	0.006	0.410
His	81.22	81.70	79.75	82.25	82.23	83.18	0.82	0.088	0.106
Ile	81.14	80.12	78.45	81.53	78.94	80.53	0.84	0.413	0.223
Leu	81.36	82.31	81.32	83.61	82.30	82.88	0.79	0.085	0.920
Lys	75.40	77.40	76.61	81.41	80.40	81.13	0.96	<0.001	0.463
Met	84.51	86.95	89.57	88.99	90.03	90.74	0.57	<0.001	0.282
Phe	81.39	81.35	80.08	82.70	80.57	82.87	0.77	0.292	0.136
Thr	71.14	72.50	76.58	78.33	75.65	79.89	1.00	<0.001	0.645
Trp	78.25	81.49	81.17	82.33	84.41	84.85	0.91	<0.001	0.632
Val	79.50	78.91	77.04	80.68	80.93	81.05	0.85	0.068	0.274

Table 7.4 (cont.)

Total	80.35	80.61	80.03	82.69	81.37	82.61	0.76	0.014	0.267
Dispensable AA									
Ala	75.46	75.19	74.10	78.12	74.87	75.30	1.24	0.854	0.920
Asp	76.09	76.21	74.98	78.47	75.44	76.04	0.93	0.873	0.728
Cys	69.51	70.30	70.65	71.50	71.48	71.40	1.24	0.121	0.876
Glu	81.10	83.20	82.73	84.58	83.12	83.87	0.86	0.004	0.195
Gly	61.19	59.34	56.62	62.38	56.16	59.52	3.35	0.432	0.597
Pro	54.20	47.71	41.76	58.33	41.98	48.74	11.61	0.569	0.674
Ser	76.79	77.15	75.95	78.57	75.81	77.31	0.99	0.836	0.961
Tyr	80.40	80.28	78.88	81.75	78.42	79.15	0.76	0.171	0.498
Total	74.31	73.98	72.26	76.85	72.37	73.98	2.00	0.832	0.974
Total AA	76.84	77.07	75.97	79.57	76.89	78.33	1.31	0.242	0.671

[1]Each least squares means represent 7 observations.

[2]Linear or quadratic effects of reducing dietary protein from 17% to 10%.

Table 7.5. Standardized ileal digestbility (SID) of crude protein and amino acids (AA)[1,2], experiment 1

Item	Dietary crude protein							Contrast *P*-value	
	17%	14%	13%	12%	11%	10%	SEM	Linear[3]	Quadratic[3]
Crude protein	85.45	88.74	88.22	91.61	91.01	92.95	1.31	<0.001	0.713
Indispensable AA									
Arg	92.79	93.85	93.28	95.51	95.13	97.02	0.93	<0.001	0.078
His	85.17	87.19	85.55	87.88	88.58	89.54	0.82	0.004	0.249
Ile	84.66	85.21	83.98	86.95	85.52	87.11	0.84	0.041	0.279
Leu	84.70	86.72	85.99	88.15	87.61	88.41	0.79	0.003	0.926
Lys	82.01	85.18	84.64	89.00	88.25	89.34	0.96	<0.001	0.806
Met	87.48	90.16	92.00	91.84	92.70	93.25	0.57	<0.001	0.188
Phe	85.02	86.51	85.64	88.08	87.15	89.22	0.77	0.002	0.243
Thr	78.68	82.37	85.05	87.21	86.27	89.23	1.00	<0.001	0.868
Trp	84.10	89.02	88.68	89.83	91.91	92.93	0.91	<0.001	0.873
Val	83.62	84.65	83.25	86.58	87.24	87.70	0.85	<0.001	0.092
Total	85.04	86.91	86.50	89.05	88.63	89.94	0.76	<0.001	0.452

Table 7.5 (cont.)

Dispensable AA									
Ala	82.70	84.55	84.00	87.86	85.89	86.71	1.24	0.008	0.836
Asp	79.98	81.96	81.22	84.61	83.36	84.67	0.93	<0.001	0.743
Cys	76.02	79.62	79.53	81.26	82.33	82.89	1.24	<0.001	0.873
Glu	83.82	87.00	86.85	88.59	88.01	89.07	0.86	<0.001	0.274
Gly	84.44	92.01	91.69	96.75	98.38	103.76	3.35	<0.001	0.339
Pro	115.13	126.12	123.96	139.39	135.86	146.85	11.61	0.005	0.576
Ser	83.03	85.66	85.20	87.65	86.83	88.82	0.99	<0.001	0.766
Tyr	84.55	86.05	85.05	87.76	85.99	87.17	0.76	0.013	0.845
Total	86.15	90.39	89.85	94.12	93.35	96.20	2.00	<0.001	0.658
Total AA	85.45	88.74	88.22	91.61	91.01	92.95	1.31	<0.001	0.713

[1]Each least squares means represent 7 observations.

[2]Values for SID were calculated by correcting values for apparent ileal digestibility for basal ileal endogenous losses. Basal ileal endogenous losses were determined (g/kg of dry matter intake) as crude protein, 10.54; Arg, 0.39; His, 0.13; Ile, 0.28; Leu, 0.40; Lys, 0.29; Met, 0.07; Phe, 0.26; Thr, 0.44; Trp, 0.08; Val, 0.31; Ala, 0.43; Asp, 0.58; Cys, 0.16; Glu, 0.76; Gly, 0.97; Pro, 2.68; Ser, 0.37; Tyr, 0.20; and total AA, 6.10.

[3]Linear or quadratic effects of reducing dietary protein from 17% to 10%.

Table 7.6. Effects of reducing crude protein on apparent total tract digestibility (ATTD) of dry matter (DM), acid-hydrolyzed ether-extract (AEE), total dietary fiber (TDF), and gross energy (GE), and concentrations of digestible energy (DE), metabolizable energy (ME), and net energy (NE) in diets and total heat production (THP) and fasting heat production (FHP), pig basis[1], experiment 2

Item	Dietary crude protein							Contrast *P*-value	
	17%	14%	13%	12%	11%	10%	SEM	Linear[2]	Quadratic[2]
Feed intake, kg/day	2.75	2.70	2.73	2.82	2.95	2.89	0.19	0.084	0.241
Fecal GE output, kcal/day	1,274	1,248	1,275	1,276	1,352	1,313	78	0.144	0.277
ATTD of DM, %	88.93	89.14	89.09	89.43	89.24	89.54	0.27	0.066	0.751
ATTD of AEE, %	52.76	47.90	52.38	52.23	51.16	49.35	1.54	0.303	0.746
ATTD of TDF, %	60.95	60.53	56.30	57.03	57.54	55.89	1.47	0.009	0.933
ATTD of GE, %	87.89	87.79	87.76	87.97	87.82	87.93	0.32	0.906	0.698
Urine GE output, kcal/day	213	182	181	187	174	173	29	0.575	0.714
THP, kcal/$BW^{0.6}$/day	384	375	378	376	390	377	20	0.621	0.704
FHP, kcal/ $BW^{0.6}$/day	223	220	235	218	238	221	17	0.878	0.481
Retained energy, kcal/$BW^{0.6}$/day	411	399	406	432	478	433	43	0.100	0.405
Respiratory quotient, fasted state	0.66	0.64	0.63	0.64	0.64	0.67	0.03	0.112	0.217

Table 7.6 (cont.)

Respiratory quotient, fed state	0.99	1.03	1.01	1.05	1.05	1.04	0.04	0.199	0.444
Energy in diets, kcal/kg									
GE	3,846	3,802	3,800	3,788	3,785	3,787	-	-	-
DE	3,380	3,337	3,335	3,333	3,324	3,330	12	0.001	0.075
ME	3,306	3,272	3,269	3,261	3,266	3,271	11	0.008	0.057
NE	2,603	2,606	2,663	2,610	2,665	2,634	53	0.333	0.853

[1]Each least squares means represent 6 observations.

[2]Linear or quadratic effects of reducing dietary protein from 17% to 10%.

Table 7.7. Effects of reducing dietary crude protein on nitrogen balance by group housed growing pigs (FHP), one-pig basis[1], experiment 2

Item	Dietary crude protein							Contrast *P*-value	
	17%	14%	13%	12%	11%	10%	SEM	Linear[2]	Quadratic[2]
Nitrogen intake, g/day	72.40	60.75	52.78	49.90	47.44	40.53	3.67	0.001	0.930
Nitrogen excreted in feces, g/day	9.78	9.39	9.09	9.09	9.18	8.53	0.49	0.005	0.730
Nitrogen excreted in urine, g/day	18.76	11.88	9.57	8.22	6.08	6.37	2.40	0.001	0.079
Absorbed nitrogen, g/day	62.62	51.36	43.69	40.81	38.26	32.05	3.33	0.001	0.370
Retained nitrogen, g/day	43.86	39.48	34.12	32.59	32.18	25.80	2.04	0.001	0.230
ATTD[3] of nitrogen, %	86.30	84.40	82.75	81.50	80.49	78.77	0.76	0.001	0.011
Nitrogen retention, % of intake	61.29	65.57	65.45	66.01	67.76	64.58	3.00	0.031	0.106
Biological value of crude protein, %[4]	71.10	77.85	79.14	81.14	84.32	82.10	4.01	0.001	0.281

[1]Each least squares mean represents 6 observations.

[2]Linear or quadratic effects of reducing dietary protein from 17% to 10%.

[3]ATTD = apparent total tract digestibility.

[4]Calculated according to Rojas and Stein, 2013.

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CHAPTER 8: Conclusions

High-oil soybean expellers (**SBE-HO**) have a nutritional value that is generally not different from conventional soybean expellers (**SBE-CV**), although SBM-HO have slightly greater concentrations of some digestible AA despite minor reductions in the standardized ileal digestibility (**SID**) of some indispensable AA. Whereas SBE-HO did not have greater SID of AA or metabolizable energy (**ME**) compared with SBE-CV, it did have greater concentrations of certain limiting AA, which may allow for lower inclusion rates in animal diets without affecting performance.

Inclusion of synthetic AA in low-protein diets maintain growth performance and nitrogen retention as a percentage of intake, whereas daily nitrogen retention expressed as grams per day was reduced. Results demonstrated that reducing dietary crude protein supplementing with synthetic AA did not negatively impact growth or nutrient deposition in pigs. However, this reduction in CP resulted in a decrease in digestible energy (**DE**) of the diet and reduced daily nitrogen retention, although no significant effect on net energy (**NE**) was observed. Results also indicated that reducing dietary protein through the inclusion of synthetic AA increased the SID of CP and AA of the diet. In contrast, starch digestibility remained unaffected by the reduction in protein concentration. These observations demonstrate that reducing SBM and supplementing with synthetic AA does not compromise starch digestibility or overall AA availability for pigs. However, results indicated that inclusion of synthetic AA and more corn instead of SBM in the diets allowed for a reduction in dietary protein, but NE was not increased, which differs from previous assumptions that indicated a greater NE in low-protein diets due to less energy expenditure for deamination of excess AA from the diet. The implications of these observations

are that SBM likely contain NE that is close to that of corn and the actual NE in SBM, therefore, is likely greater than current book values.

Intestinal health markers, such as cytokine levels and abundance of genes for AA transporters were not changed by dietary protein reduction. This observation, along with the consistent nutrient deposition data, supports the conclusion that reduced protein diets, when balanced with synthetic AA, do not negatively impact immune response or gut health.

In conclusion, results from the experiments included in this dissertation support the hypothesis that SBM produced from genetically improved soybeans has a nutritional value comparable to that of SBM from conventional soybean sources and suggest its potential to enhance the overall nutritional value of SBM. These findings support flexibility in SBM inclusion in growing pig diets, allowing nutritionists to adjust formulations based on the market availability of SBM and synthetic AA. Dietary protein may be reduced by partially replacing SBM with corn and synthetic AA without negatively affecting growth performance or nutrient digestibility; however, daily nitrogen retention may be reduced, and there is no evidence that low-protein diets provide more NE than diets with greater protein concentrations. This observation suggests that the NE of SBM is close to, or potentially greater than, that of corn. Further research to improve NE prediction equations for low-protein diets and to expand the use of such diets in poultry may enhance our understanding of the practical applications of intact protein and synthetic AA in animal nutrition.