Use of Soybean Meal in Diets for Growing and Reproducing Swine

by

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Dissertation

Submitted in partial fulfillment of the requirements
for the degree of Doctor of Philosophy in Animal Sciences
in the Graduate College of the
University of Illinois Urbana-Champaign, 2026

Urbana, Illinois

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Abstract

Eight experiments were conducted to test the hypothesis that soybean meal (**SBM**) in diets for gestating and lactating sows and growing-finishing pigs cannot be reduced without negatively impacting growth performance, nitrogen balance, or changing blood immune parameters, even if diets are fortified with crystalline amino acids (**AA**) to meet requirements, and to test the hypothesis that replacing SBM with alternative ingredients will increase the environmental impact by increasing nitrogen excretion or greenhouse gas production. In experiment one, the objective was to test the hypothesis that the energy value of SBM is greater than the current NRC (2012) value. A regression of gain to feed for pigs fed diets without SBM against increasing levels of soybean oil (**SBO**) was used to create an equation to predict the response in gain to feed (**G:F**) of adding SBO to the diets. Results demonstrated that G:F increased (linear, $P < 0.001$) by increasing SBO in diets, and the G:F of pigs fed the diet containing 12% SBM corresponded to a diet containing 4.70% SBO, which is equivalent to 2,955 kcal NE per kg. It was concluded that NE of SBM in diets for pigs is greater than previously thought. In experiment two, the objective was to test the hypothesis that nitrogen retention, measured as a percentage of ingested nitrogen, is greater than 50% regardless of the dietary protein level and the BW of pigs. Results indicated that nitrogen retention relative to intake ranged from 60.9 to 71.9% and increased ($P < 0.001$) as dietary protein was reduced, but nitrogen retention measured as g/day was reduced ($P < 0.05$) as dietary protein was reduced. It was concluded that pigs retain more than 50% of consumed nitrogen, but pigs fed a low-protein diet have reduced nitrogen retention measured as g/day compared with pigs fed a diet with a greater protein concentration.

As a reduction in nitrogen retention was observed in the low-protein diets in experiment two, experiments three and four were conducted to test the hypothesis that reducing the inclusion

of SBM and increasing crystalline AA in corn-SBM diets may negatively impact growth performance, carcass traits, and immune responses of pigs, and that supplementation with soy isoflavones and/or Glu may improve growth performance or immune system. Six experimental treatments were used, including a high-protein diet and a medium-protein diet containing less SBM with Lys, Met, and Thr, and four low-protein diets containing six crystalline AA arranged in a 2 × 2 factorial arrangement with 0 or 0.4% soy isoflavones and 0 or 2.0% Glu. Results of experiment three indicated that carcass backfat tended to increase (linear, $P = 0.075$) in pigs fed the low protein diets. In experiment four, daily nitrogen intake, urinary nitrogen excretion, absorbed nitrogen, and retained nitrogen (g/d) were reduced (linear; $P < 0.001$) as dietary protein decreased. Overall, no effects of adding soy isoflavones or extra nitrogen on pig growth performance were observed, but reducing dietary protein reduced daily nitrogen retention, and carcass was less lean, and backfat was thicker. Experiment five was conducted to test the hypothesis that feeding sows diets based primarily on corn, SBM, and no crystalline AA will result in improved reproductive performance and immunity of sows compared with sows fed diets with less SBM and crystalline AA. Results indicated that nitrogen excretion in feces and urine, absorbed nitrogen, and retained nitrogen (g/d) were greater ($P < 0.05$) in gestating sows fed the high-protein diet compared with sows fed the low-protein diet. Colostrum immunoglobulin G and concentrations of fat, protein, urea nitrogen, lactose, and immunoglobulin G were greater ($P < 0.001$) in milk from sows fed the high-protein diet than in milk from sows fed the low-protein diet. It was therefore, concluded that feeding a low-protein diet to gestating sows decreased daily nitrogen retention, whereas reproductive performance was not affected, but feeding a high-protein diet without crystalline AA resulted in improved milk composition and immune-related characteristics. Experiment six tested the hypothesis that

feeding intact protein from SBM, instead of combinations of SBM with crystalline AA, corn distillers dried grains with solubles (**DDGS**), or high-protein DDGS (**HP-DDGS**), results in greater nitrogen retention and greater digestible energy (**DE**) in the diet without affecting metabolizable energy (**ME**). Results indicated that absorbed nitrogen was greater ($P < 0.05$) for pigs fed 31% SBM compared with pigs fed the other diets, whereas retained nitrogen (g/d) was reduced ($P < 0.05$) in pigs fed the 21% SBM or the HP-DDGS diet with added AA compared with pigs fed the 31% SBM diet or the DDGS diet with added AA. The DE and ME were greater ($P < 0.05$) in SBM and HP-DDGS diets compared with DDGS diets, regardless of addition of AA. It was concluded that feeding SBM increased nitrogen retention and provided greater DE and ME compared with feeding DDGS diets and that replacing SBM with crystalline AA reduces nitrogen retention. For experiments seven and eight, the objective was to test the hypothesis that there are no differences in net energy (**NE**), greenhouse gas emissions, nitrogen balance, and ileal digestibility of AA in pigs fed corn-based diets containing SBM, canola meal (**CM**), or DDGS. Results indicated that the digestibility of AA was greater in the SBM diet than in the CM or DDGS diet. Concentrations of NE were not different between DDGS and SBM diets, and the DDGS diet had greater ($P < 0.05$) NE than the CM diet. Daily O_2 consumption per kg gain was greater ($P < 0.05$), and CO_2 production per kg gain tended to be greater ($P < 0.10$) in pigs fed the CM diet compared with pigs fed the SBM or DDGS diets. However, production of CH_4 did not differ among the three diets. It was concluded that the use of SBM or DDGS increased NE in corn-based diets compared with CM when fed to group-housed pigs. Pigs fed the diet containing SBM had greater absorbed and retained nitrogen (g/d) compared with those fed diets containing CM or DDGS. In conclusion, SBM is a valuable ingredient in diets for sows and growing pigs because it provides digestible AA and more energy than previously reported. Reducing soybean

meal and increasing crystalline AA or alternative ingredients did not fully maintain nitrogen retention, carcass traits, milk composition, or immune-related responses, and did not reduce the environmental impact of production. Therefore, the nutritional value of SBM may be underestimated in current feeding systems.

Acknowledgments

My deepest gratitude is dedicated to Dr. Stein: having you as my advisor was one of the things I never believed I would have. Thank you for accepting me in your lab as a visiting scholar for six months (which later became almost 7 years). I want to thank you for the time you dedicated to me, for all the guidance, the knowledge, and the conversations about pigs, digestibility, and science. Thank you so much for teaching me that success and results come together with hard work. Thanks to you, I became a better scientist, person, and nutritionist. Thanks for believing in me. Without your help and guidance, I would never have finished this dissertation. Thank you also for all the opportunities, beyond allowing me to be your student, to travel and share my work around the world. Your company and guidance made this PhD process fun and enjoyable, and the pain of "there is no PhD without pain" was way less than it should have been.

To my committee members, Dr. Martin Nyachoti, Dr. Bailey Harsh, and Dr. Michael Murphy: together with Dr. Stein, you helped me achieve the success of this process. Thank you for all the time you spent writing countless letters of recommendation for me. As you all know, they were all very successful. For your time reading my dissertation and making comments and corrections, I truly appreciate it. Also, I would like to thank the United Soybean Board for the financial support to conduct this research.

I want to express my gratitude to all past and present Stein team members: the postdocs, graduate students, visiting scholars, and farm staff, for your collaboration. All the experiences, friendships, feedings, fecal and urine collections, and weighing of pigs were worthwhile; thank you so much. But special thanks to Dr. Su A Lee: you taught me all I know as a scientist. From experimental design to writing manuscripts, your guidance shaped the researcher I am today. Thank you for your patience, your cordiality, and your time reviewing my stuff (all of it, again

and again). Thank you for always having your door open, for answering my endless questions. Your dedication and work ethic will always be an inspiration to me. Also special thanks to Diego Rodriguez (former farm manager) and Elizabeth Guevara (lab technician), my family here in Champaign, my co-workers, and my friends: your hard work is also reflected in this dissertation. You helped me a bunch, and that is something I will never forget, and I'm sure you won't either. Thank you for all your support and unconditional love. Familia Lancheros-Stich, you gave me the opportunity to meet my best mate, and that is something I will never forget. The unconditional company and love that Frodito♥ gives me every time he is with me means the world to me.

My friends Natalia Fanelli and Juliana Martins, thank you for the supportive words every day, for your company, the fun, the margaritas, and all the joy. This PhD could not have been more fun without your friendship. A los que creyeron desde el día uno, gracias. Un saludo pa' Cindy Manjarrez: you are always there for me, I love you! And yes, we will do that BBQ with one pound of meat. A ♥.

Finally, and most importantly, my family, my parents and my sisters: mami y papi, muchas gracias por todas las cosas que han hecho por mí para que yo haya llegado hasta acá. Por las llamadas diarias, por el apoyo incondicional a pesar de la distancia, y por todo el amor que me han brindado. Ustedes son mi mayor ejemplo de esfuerzo y dedicación, y este logro también es de ustedes. Hermanas, las amo con todo mi corazón; gracias por estar siempre presentes, por escucharme, y por celebrar conmigo cada paso de este camino. Familia, les agradezco enormemente los esfuerzos que están haciendo para venir a celebrar este gran logro conmigo. Los amo muchísimo.

Table of contents

CHAPTER 1: Introduction

Despite the relatively low oil content of the seed (about 20%; Patil et al., 2018), soybeans are the largest source of edible oil produced in the U.S., accounting for approximately 60% of global oilseed production (USDA, 2024). Soybeans contain approximately 35% crude protein; however, after crushing, most of the fat is removed, resulting in a meal that contains less than 2% crude fat (NRC, 2012). Conventional soybean meal (**SBM**) is the defatted product of soybean flour after oil extraction and is the premium source of protein used in the global swine feed industry. In the 2023/2024 crop-year, around 49 million metric tons of SBM were produced in the United States, and 5.7 million metric tons were used in diets for pigs (ASA, 2024; USDA, 2024). The reason for the widespread use of SBM and its derivatives in the swine feed industry is primarily the balanced amino acid (**AA**) profile and high digestibility of AA (NRC, 2012; Cervantes-Pahm et al., 2014; Casas et al., 2022). Indeed, SBM has a greater concentration of Lys, Trp, Thr, and Ile, compared with other cereal grains (Stein et al., 2008; NRC, 2012). However, SBM is relatively low in Met and Cys, which are present in greater concentrations in protein from cereal grains and cereal grain co-products. Therefore, the AA profile of SBM complements the AA profile of cereal grains for formulating balanced diets. Weanling, growing, and finishing pigs have high requirements for AA to support growth, lean tissue deposition, and immune system (NRC, 2012). Before parturition, reproducing sows require high-quality AA diets to support fetal development and mammary growth. Likewise, during lactation, milk and colostrum production require large quantities of AA to synthesize milk proteins (Tokach et al., 2019; Johannsen et al., 2024). Because of the high requirements for AA by growing and reproducing swine, SBM is the premium protein source used in the swine industry. However, in addition to providing AA, SBM also provides other nutrients and energy in the diets, and both digestible energy (**DE**) and

metabolizable energy (**ME**) in SBM is assumed to be close to the DE and ME in corn (NRC, 2012). In contrast, net energy (**NE**) in SBM is estimated at only around 77% of the NE in corn (Rostagno, 2011; NRC, 2012; Sauvant et al., 2004). This low value for NE is a result of a presumed large negative effect of protein on NE due to a presumed low nitrogen retention by pigs (Noblet et al., 1994). However, data indicate that DE, ME, and NE in SBM may be underestimated by as much as 300 kcal/kg (Baker and Stein, 2009; Sotak-Peper et al., 2015; Cemin et al., 2020; Lee et al., 2024), which at least partly may be a result of modern genotypes of pigs being more efficient in retaining nitrogen in the body. Thus, in addition to the AA contribution, SBM may also offer substantial dietary energy to the diets, but research is required to quantify the amount of NE that pigs can obtain from SBM.

The environmental impact of producing SBM is important because of concerns about P and nitrogen excretion in manure and the emission of greenhouse gases (**GHG**) such as carbon dioxide, methane, from the animals, and nitrous oxide by the stored manure (Opio et al., 2013; FAO, 2017). However, nitrogen and P in manure can be reduced by using accurate formulation of diets and optimizing the nutrient-use efficiency of the ingredients to meet the requirement of animals (de Lange et al., 2012; Shurson et al., 2022). The use of crystalline AA has gradually increased in recent decades because of the competitive prices for most synthetic AA the assumption that as long as the requirements for digestible AA are met, reducing intact protein in the diets will reduce nitrogen excretion and diet cost, but carcass quality and protein deposition of pigs will not be affected (Sutton and Richert, 2004; Wang et al., 2018). However, when pigs were fed diets with reduced concentrations of SBM, a reduction in growth performance, carcass leanness, nitrogen retention, and energy retention were also observed (Kerr and Easter, 1995; Le Bellego and Noblet, 2002; Hinson et al., 2009). Therefore, the assumption that carcass quality

and growth performance are not affected by the reduction of crude protein in the diet may not be entirely true, and research to determine to which degree synthetic AA can substitute SBM without reducing body protein synthesis is required.

Nutrient composition and digestibility of energy and nutrients in SBM and its derivatives fed to growing pigs have been reported (Cervantes-Pahm and Stein, 2008; Wang et al., 2011), and results of recent research quantified the effect of diets containing SBM on the environment and the production of GHG (Trabue and Kerr, 2014; van Zanten et al., 2015). However, there is uncertainty about how using intact protein from SBM or lower protein diets containing SBM, but supplemented with crystalline AA, influences GHG, the energy value of the diets, and the performance of growing and reproducing swine. Therefore, eight experiments were conducted to test the hypothesis that SBM in diets for gestating and lactating sows and growing-finishing pigs cannot be reduced without negatively impacting growth performance or changing blood immune parameters even if diets are fortified with crystalline AA to meet requirements. Likewise, it was also the objective to test the hypothesis that feeding low-protein diets supplemented with crystalline AA to growing-finishing pigs or gestating sows will result in reduced nitrogen balance and protein retention, compared with feeding diets based on SBM and no crystalline AA.

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CHAPTER 2: Soybean meal in the nutrition of pigs. Review of literature

Introduction

Soybeans (*Glycine max* L. Merr.), one of the most cultivated crops in the world, was originally grown in East Asia and was domesticated around 5000 years ago (Hymowitz, 1970; Lee et al., 2011). Soybean is an annual bushy herbaceous legume crop, and it has been adapted to a wide range of geographic and climate regions that have a well-drained and loose soil rich in organic matter (Leff et al., 2004; Edde, 2022). Nitrogen is the most critical limiting element for plant development and crop productivity, after carbon and water (Peoples et al., 1995). However, legumes, including soybeans, are often used in cereal crop rotation since it can reduce the need for mineral nitrogen fertilizer for the subsequent crop because of the ability of legumes to fix atmospheric N_2 in the soil with the *Rhizobia microbes* (Meyer and Badaruddin, 2001; Kakraliya et al., 2018). Effective symbiosis allows soybeans to fix 1.6 million metric tons of nitrogen from the atmosphere each year, or almost 77% of the total amount of nitrogen fixed by legumes (Hartman et al., 2011).

Although soybeans can grow on a wide range of soil types, they mostly prefer neutral to alkaline pH soil (Yang et al., 2001). Soybeans have a reduced tolerance for soil compaction and anaerobic soil conditions. Therefore, good drainage and low temperatures are essential for adequate root oxygen supply (Kuzma et al., 1999; Kunert et al., 2016). In soybean crops, yield depends on the number of seeds produced and their quality. Variations in yields are mainly caused by diseases and biotic and abiotic stresses and can also be related to the environment and genotype of the soybeans used in the crop (Bastidas et al., 2008; Müller et al., 2021). Late

planting of early maturing cultivars of soybeans can shift reproductive growth into a more favorable environment due to the improvement in isolation for germination (Egli and Bruening, 1992). However, drought and warmer temperatures during early summer may result in abiotic stress that can influence the yield of soybeans and the quality of the seeds (Dornbos Jr. et al., 1989; Cox et al., 2008; Hu and Wiatrak, 2012; Dubey et al., 2019). Tolerance to drought and warmer temperatures may be improved by using different growth-promoting rhizobacteria and genetic selection of different cultivars (Dubey et al., 2019). Therefore, the use of new cultivars and rhizobia and mycorrhizae for the cultivation of soybeans in areas with warmer temperatures and low precipitation will increase protein and dry matter (**DM**) yield in short-season conditions (Dashti et al., 1997; Boote, 2011; Schmidt et al., 2015).

Soybean is one of the most important crops worldwide, and in the year 2025/2026 crop, soybean yields accounted for nearly 70% of the global oilseed production. Brazil, is the largest producer of soybeans, followed by the United States and Argentina. In the 2025/2026 crop year, global soybean production was 427 million metric tons, and production is expected to increase to more than 435 million metric tons by 2023/2024 (FAO, 2026; USDA, 2026a). In Europe and the United Kingdom, however, soybean production is only around 2.5 million metric tons (FAO, 2024), and these countries, therefore, import soybean products from South America and the United States. Humans consume soybean protein in the form of soy products such as tofu, miso, soy sauce, and others (Chen et al., 2012; Pimentel et al., 2021). Conventional soybean meal (**SBM**) is the defatted product of soybean flour after oil extraction and is the premium source of protein used in the swine industry worldwide. In the 2025/2026 crop-year, around 55 million metric tons were produced in the United States, and 5.7 million metric tons were used by the swine industry (ASA, 2025; USDA, 2026b).

Chemical composition of soybean meal

Soybeans are legumes with high concentrations of oil and protein in the cotyledons. The protein content is approximately 36%, and soybeans also contain 19% fat (Baker et al., 2010; Medic et al., 2014). However, oil is removed during crushing, and the resulting SBM contains less than 2% fat (NRC, 2012). The DM of SBM is usually 88 – 90%, and the concentration of gross energy is approximately 4,250 kcal/kg on an as-is basis (Baker and Stein, 2009; NRC, 2012; Lagos and Stein, 2017). Dehulled soybean meal is usually referred to as high-protein soybean meal and contains around 47% crude protein (**CP**) and approximately 3% Lys (NRC, 2012). The amount of nitrogen in the seed mainly depends on the genotype of the cultivar, growing conditions, and nitrogen fertilization during germination (Epie et al., 2023). Soybean meal is primarily a source of AA, but there is a significant amount of carbohydrates in the meal. The total dietary fiber content in soybean meal is between 16 and 20%, of which 1 to 2% is soluble dietary fiber (Grieshop et al., 2003; NRC, 2012; Lopez et al., 2020). Fiber in soybean meal is attributed to structural and non-structural carbohydrates (Grieshop et al., 2003). Cellulose, hemicellulose, pectic polysaccharides, and lignin are the most complex structural carbohydrates in soybean meal (Bach Knudsen, 2011; 2014). Oligosaccharides are low molecular weight carbohydrates, including sucrose and galacto-oligosaccharides (e.g., raffinose, stachyose, and verbascose; Johansen et al., 1996). The total concentration of sucrose and galacto-oligosaccharides in soybean meal is 12 to 16% (Cervantes-Pahm and Stein, 2010).

Soybean meal contains slightly less lipids than canola meal, rapeseed meal, and cottonseed meal, but more than sunflower meal, and the acid-hydrolyzed ether extract concentration in soybean meal is around 2% (NRC, 2012; Maison and Stein, 2014; Ibagon et al., 2021). The ash content of SBM is approximately 6%, of which 9 to 10% is P (Rojas and Stein,

2012). Soybean meal contains 0.60 to 0.70% P, which is more than most cereal grains, although almost half of P in SBM is bound to phytic acid (Rojas and Stein, 2012; She et al., 2017). Soybean meal has a greater Ca concentration than cereal grains (approximately 0.30%), which is less than in canola meal (Maison et al., 2015; She et al., 2017).

Digestibility of Nutrients and Energy

The digestibility of nutrients and energy in SBM by growing pigs has been determined and compared with other oilseeds (Cervantes-Pahm and Stein, 2008; Rojas and Stein, 2012; Lopez et al., 2020), but because new varieties of soybeans and new genetics of pigs have been developed, it is important to keep evaluating the nutritional composition and digestibility of nutrients in SBM to avoid an underestimation or overestimation of nutrients or energy by feed manufacturers. The standardized ileal digestibility (**SID**) of CP and most amino acids (**AA**), including Lys, Met, Thr, and Trp, is greater than the SID of AA in proteins from other plant ingredients, including sunflower meal, canola meal, and corn gluten meal (Berrocoso et al., 2015; Lee et al., 2020; Ibagon et al., 2021). In soybean meal, the SID of CP is approximately 87%, and the SID of Lys is approximately 89%, with slight variations depending on the soybean meal variety or cultivar (NRC, 2012; Lagos and Stein, 2017).

The standardized total tract digestibility (**STTD**) of P is greater in SBM than in corn and most plant protein sources when microbial phytase is not included in the diet. Inclusion of microbial phytase in the diet increases the STTD of P in all cereal grains and most oilseeds, and this is true for soybean meal as well; however, the increase in the STTD of P as a result of microbial phytase differs among oilseeds. When microbial phytase is used, the STTD of P does not differ among soybean meal, canola meal, and sunflower seeds (Rodríguez et al., 2013)

The apparent total tract digestibility (**ATTD**) of total dietary fiber is greater in SBM (approximately 70%) than in canola meal, distillers dried grains with solubles, sunflower meal, and corn germ meal, which indicates that the total dietary fiber in SBM is fermentable (Navarro et al., 2018). Among oilseeds, the ATTD of gross energy is greater in SBM (approximately 89%) than in sunflower meal, canola meal, and cotton seed meal. The ATTD of gross energy in SBM is very close to the ATTD of gross energy in cereal grains, such as corn (90%), wheat (87%), or sorghum (86%; Rodríguez et al., 2013; Lopez et al., 2020; Ibagon et al., 2023). The digestible energy (**DE**) and the metabolizable energy (**ME**) in SBM, as determined in growing pigs, are relatively close to the values reported for corn (3,600 and 3,294 kcal/kg as-is basis, respectively; NRC, 2012; Lopez et al., 2020). In contrast, net energy (**NE**) in SBM is estimated at only around 77% of the NE in corn (Rostagno, 2011; NRC, 2012; Sauvant et al., 2004). This low value for NE is a result of a presumed large negative effect of protein on NE due to a presumed low nitrogen retention by pigs. However, data indicate that DE, ME, and NE in SBM may be underestimated by as much as 300 kcal/kg (Baker and Stein, 2009; Sotak-Peper et al., 2015; Cemin et al., 2020; Lee et al., 2024), which at least partly may be a result of modern genotypes of pigs being more efficient in retaining nitrogen in the body. Thus, in addition to the AA contribution, SBM may also offer substantial dietary energy to the diets, but research is required to quantify the amount of NE that pigs can obtain from SBM.

Performance of pigs fed diets containing soybean meal

The relatively high concentration of anti-nutritional factors such as trypsin inhibitors, antigens, α-galactosides and lectins in raw soybeans has resulted in conservative upper limit recommendations for the inclusion of full-fat soybeans in diets for pigs to prevent reduction in feed intake and growth performance (Palacios et al., 2004). Therefore, to avoid this problem,

soybean products need to be heated to inactivate those anti-nutritional factors. For example, the inclusion of soybean meal and its derivates in diets for pigs less than 7 kg should not be greater than 15%, and pigs from 7 to 15 kg should be allowed no more than 20 to 24% of soybean meal in the diets (National Swine Nutrition Guide, 2010; Navarro et al., 2017). When pigs are heavier than 20 kg, their diet is typically based on cereal grains and soybean meal, with no inclusion of any specialty protein source such as fermented soybean meal, soy protein concentrate, or enzyme-treated soybean meal (National Swine Nutrition Guide, 2010; Navarro et al., 2017). In diets for growing and finishing pigs, SBM can be used as the only protein ingredient and the inclusion can be up to 30% to ensure the requirement for all indispensable AA is met (NRC, 2012). Because this recommendation stipulates meeting the AA requirements for growing and finishing pigs, but not the energy requirements, and because the NE of SBM appears to be underestimated (Sotak-Peper et al., 2015; Cemin et al., 2020), it is possible that pigs can be fed greater amounts of SBM than is currently recommended without affecting growth parameter or the health status of the animal.

New cultivars and varieties of soybeans contain less anti-nutritional factors than older varieties of soybeans (Clarke and Wiseman, 2000; Vollmann et al., 2003; Pesic et al., 2007), and elimination of most of the remaining anti-nutritional factors can effectively be controlled through feed processing and manufacturing of the beans (Goebel and Stein, 2011). Glycinin and β-conglycinin, two proteins in soybeans, are considered the primary antigenic proteins responsible for SBM hypersensitivity in nursery pigs (Li et al., 1990). However, after being exposed to soy proteins, nursery pigs that exhibited hypersensitivity developed tolerance within 7 to 10 days (Heppell et al., 1989).

Weanling pigs

The nutritional quality and content of the diet for weanling pigs have a critical role in the development of the gastrointestinal tract, as well as in the reduction in the incidence of post-weaning diarrhea. Therefore, utilization of SBM in the diets for weanling pigs may be limited due to the content of antinutritional factors and allergenic proteins in soybeans, which can reduce growth performance and nutrient utilization (Qin et al., 1996). However, after first exposure, pigs begin to develop tolerance to soy proteins after 7 to 10 days (Barrat et al., 1978). Different strategies have been used in the swine industry to ameliorate the negative effects of transitioning from a liquid (milk) to a solid diet in the post-waning stage of the pigs. The level of protein in diets is often reduced to reduce post-weaning diarrhea and other health problems (Chiba, 2001; Nyachoti et al., 2006). Diets for weanling and nursery pigs generally contain no less than 20% of CP to support maximum rates and lean tissue gain (NRC, 2012). Feeding low-protein diets supplemented with AA may reduce the adverse effects of soybean hypersensitivity by slowly increasing levels of soybean meal in the diet. However, feeding low-protein diets often results in reduced growth performance of pigs. Therefore, it is important to make sure the requirement for all indispensable AA is met if dietary protein is reduced (Limbach et al., 2021).

A reduction in average daily gain (**ADG**) and gain-to-feed ratio (**G:F**) when 10 kg pigs were fed diets based on sorghum and SBM, and contained 14.5% CP, supplemented with Lys, Met, Thr, and Trp, compared with diets containing 20.5% CP was observed (Zamora et al., 2011). However, Figueroa et al. (2012) did not observed any difference in ADG or G:F when feeding pigs a low-protein diet (17%) supplemented with Lys, Met, Thr, and Trp compared with a high-protein diet (20.5%). Zamora et al. (2011) reduced the protein in the diet by 6%, whereas Figueroa et al. (2012) reduced it by 3%, and therefore, it was concluded that reducing CP to 4%

below NRC (1998) requirements and supplementing with Lys, Met, Thr, and Trp, growth performance during the postweaning period will not be affected (Htoo et al., 2007).

However, a decrease in growth performance when decreasing CP from 23% to 19 or 17%, regardless of the supplementation of Lys, Met, Thr, and Trp was observed (Nyachoti et al., 2006). The cause of these discrepancies in results is not clear. Likewise, Limbach et al. (2021) observed a reduction in ADG and G:F in weanling pigs fed diets containing 16% CP, compared with those fed 22% CP. However, it is possible there was an imbalance or deficiency of other nutrients in the low-protein diets or that the nitrogen in the low-protein diets is insufficient to support synthesis of all needed dispensable AA (Nyachoti et al., 2006; Jones et al., 2014). Lys, Met, Thr, and Trp are the first limiting AA in corn-SBM diets, which is the reason it is necessary to supplement these AA when dietary protein is reduced in diets for weanling pigs (Kerr et al., 2003). Postweaning diarrhea was reduced when dietary protein was reduced from 20 to 17 % when the low-protein diet was supplemented with Lys, Met, Thr, Trp, Ile, and Val (Heo et al., 2009). Reducing protein in nursery pigs may reduce postweaning diarrhea, but can compromise growth. However, if the diet is supplemented with Ile or Val, or both, it may not compromise growth performance (Lordelo et al., 2008). When dietary protein is reduced from 20% to 14%, with the addition of His, Ile, Leu, and Phe as well as Lys, Met, Thr, and Trp, nitrogen retention in weanling pigs was reduced, suggesting that protein deposition can be compromised by feeding low-protein diets (Gloaguen et al., 2014). Therefore, it is worth considering if the biological value of the protein coming from the crystalline AA or from SBM need to be reconsidered.

Growing and finishing pigs

As is the case for weanling pigs, the level of SBM in the diet influences diet concentration of CP. Thus, reducing the concentration of soybean meal in diets will reduce CP,

and to compensate for the reduced contribution of AA from SBM, synthetic AA are usually added. Indeed, pigs fed a high-protein diet (19% CP) with SBM as the only source of protein had greater ADG and feed efficiency compared with pigs fed a diet containing 15% CP without AA supplementation, but no differences in growth performance were observed between the high-protein diet and a low-protein diet supplemented with Lys, Trp, and Thr. (Kerr et al., 1995; 2003a). However, when growing-finishing pigs were fed diets containing 16% CP or 12% CP supplemented with Lys, Trp, and Thr, feed consumption was not different, but ADG was reduced (Kerr et al., 2003b). On the other hand, Madrid et al. (2013) demonstrated that by formulating diets using the ideal protein concept, CP can be decreased from 16 to 14% when the low-protein diet is supplemented with crystalline AA. According to Qiu et al. (2018), when supplementing the diet with all dispensable AA except Leu, CP can be decreased 18% to 14% without compromising growth performance. However, while keeping the proper ratio of all dispensable AA under the NRC (2012) guidelines, decreasing CP in diets from 18 to 15% resulted in a reduction in ADG and G:F in growing pigs (Li et al., 2018). Additionally, the reduction of CP from 17 or 18% to 13%, with supplementation of all dispensable AA, has a negative impact on growth performance in growing pigs (Roux et al., 2011; Che et al., 2017). Variation in CP levels used in the control diets may explain the inconsistencies between experiments (Che et al., 2017). Control diets containing 16% or 20% CP differ in their content of intact proteins, peptides, and indispensable AA. As a result, reducing CP levels further has different effects on growth performance of animals.

For the finishing phases, less protein is used due to the reduced requirements for indispensable AA. However, feed intake increases, and nutrient efficiency has an important impact on the cost of production (van Milgen et al., 2015). When CP was reduced from 14 to

12% in diets for finishing pigs, with supplementation of all dispensable AA, no changes in ADG and G:F were observed (Qin et al., 2015). However, when reducing CP from 16% to 13% with diets balanced for all dispensable AA, ADG and G:F was decreased (Li et al., 2018). Reduction of CP from 13 to 9% in late-finishing pigs will negatively impact G:F and ADG, even if the low-protein diet is supplemented and balanced for all dispensable AA, including Val and Ile (Soto et al., 2019). The negative impact of reducing intact protein from SBM in diets may be related to the reduction in the biological compounds of soybeans such as isoflavones and saponins (Soto et al., 2019). Also, a negative effect on growth performance can be related to the underestimation of NE in SBM (Cemin et al., 2020; Stein et al., 2024). Therefore, growth performance may be negatively impacted by reducing SBM in diets for growing-finishing pigs.

Reproductive performance of sows

Reproductive performance of sows is influenced by the nutritional composition of the diet during both gestation and lactation, with particular importance of protein and AA supply to support fetal development, milk production, and subsequent reproductive cycles (Muller et al., 2025). Soybean meal is the primary source of protein and AA in diets for gestating and lactating sows, due to its highly digestible and balanced AA profile (NRC, 2012). However, reducing protein by decreasing SBM inclusion and supplementing with crystalline AA has been explored as a strategy to reduce nitrogen excretion and improve environmental sustainability.

During gestation, adequate protein intake is essential to support placental development and fetal growth (Herring et al., 2018). However, it is possible that excess dietary protein may increase nitrogen excretion without improving reproductive outcomes. Results of recent experiments demonstrated that reducing CP in gestation diets, while meeting requirements for standardized ileal digestible AA, does not negatively affect litter size or pig birth weight (Huber

et al., 2015). Similarly, reducing SBM inclusion in gestation diets did not impair reproductive performance when AA requirements were met (Munoz Alfonso et al., 2024). However, Jan et al. (2014) demonstrate that when protein is increased in gestation diets from 11.4 to 17.13%, the ADG of the litter increased, but no effects of increasing protein in gestation diets on composition of colostrum or milk during lactation were observed.

In lactating sows, the demand for AA and energy increases substantially due to milk production (Strathe et al., 2017a). Insufficient protein intake during lactation will result in mobilization of body protein reserves, leading to excessive body weight loss and reduced nutrient availability for milk synthesis (Strathe et al., 2017b). Consequently, litter growth may be compromised if the dietary supply of protein is not sufficient to support both milk production and maintenance of maternal body tissues.

In addition to supporting protein synthesis, SBM contains bioactive compounds such as isoflavones, which have been associated with antioxidant and immunomodulatory effects (Smith and Dilger, 2018). These compounds may contribute to improved sow health and potentially influence reproductive performance, particularly during the high metabolic demands of lactation, and may also positively influence growth performance of the offspring (Li et al., 2022). Supplementation of sow diets with isoflavones during late gestation and lactation has been demonstrated to increase ADFI, reduce the weaning-to-estrus interval, and improve offspring growth performance, likely as a consequence of improved antioxidant status in the sow (Hu et al., 2015; Li et al., 2021).

Conclusions

In conclusion, SBM is a highly valuable ingredient in diets for pigs at all production stages because it provides digestible AA, energy, and bioactive compounds. Its greater nutrient digestibility supports growth and reproductive performance more effectively than when protein is replaced with crystalline AA. Although reducing dietary crude protein by decreasing SBM and supplementing crystalline AA may reduce nitrogen excretion and sometimes maintain performance, these output do not always provide optimal nitrogen retention measure as grams per day, needed for protein deposition. In addition, the energy value of SBM be underestimated in current feed book values, which emphasizes its importance in pig diets. Therefore, soybean meal should be considered not only as a source of AA, but also as a multifunctional ingredient that supports nutrient utilization, productive performance, and the antioxidant function in pigs.

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CHAPTER 3: The soybean oil equivalency of soybean meal indicates a high energy value of soybean when fed to growing pigs[1]

Abstract

An experiment was conducted to test the hypothesis that the energy in soybean meal (**SBM**) fed to pigs determined using a fat equivalency procedure is greater than current book values for net energy (**NE**). A total of 120 growing pigs were allotted to five dietary treatments. A diet based on corn, soy protein concentrate (**SPC**), and synthetic cellulose and three diets containing 2%, 4%, or 6% soybean oil (**SBO**) were formulated. A fifth diet that contained corn, SPC, and 12% SBM, but no SBO, was also formulated. Pigs were fed experimental diets for four weeks and daily gain, daily fed intake, and gain-to-feed ratio (**G:F**) were calculated. Regression of G:F for pigs fed diets without SBM against the increasing levels of SBO was used to create an equation to predict the response in G:F of adding SBO to the diets. Results demonstrated that G:F increased (linear, $P < 0.001$) by increasing SBO in the diets and the G:F of pigs fed the diet containing 12% SBM corresponded to a diet containing 4.70% SBO, which is equivalent to 2955 kcal NE per kg. In conclusion, the NE of SBM in diets for pigs is greater than previously thought.

Key Words: net energy, pigs, soybean meal, soybean oil.

[1]Material from: Ibagon et al. The soybean oil equivalency of soybean meal indicates a high energy value of soybean meal when fed to growing pigs. Canadian Journal of Animal Sciences. Published 2025, publisher: Canadian Science Publishing. The copyright owner has provided permission to reprint.

Introduction

The most expensive component in swine diets is energy, and lipids are often included in diets for pigs due to their high energy content (Patience et al. 2015). Increasing the concentration of fat in diets improves dietary energy, growth performance, and gain-to-feed ratio (**G:F**) of pigs, but at the same time, dietary fat increases diet costs (Shurson et al. 2015).Therefore, accuracy in determining the energy provided by each ingredient is necessary to evaluate the tradeoff between growth performance and diet costs. Soybean meal (**SBM**) is the primary source of amino acids (**AA**) in diets for pigs in most pig-producing countries in the world, and the net energy (**NE**) of SBM has been reported as 2,087 kcal/kg (NRC 2012). However, results of recent research indicated that the NE of SBM is greater than this value (Liet al. 2017; Cemin et al. 2020). Improvements in feed efficiency with increasing levels of SBM in the diet have also been reported (Moran et al. 2017), which indicates that mod-ern genotypes of pigs have a greater ability to utilize energy in SBM than previously thought and that NRC (2012) may underestimate the NE of SBM. Previously, the NE of SBM was determined using indirect calorimetry (Li et al. 2017) or estimated from G:F in pigs used in growth assays (Cemin et al.2020). However, because the NE of soybean oil (**SBO**) is well defined, it is also possible to calculate the energy value of a feed ingredient in terms of SBO equivalency using G:F as the response variable, but to our knowledge, this approach has not been used to estimate the energy value of SBM. Therefore, the objective of this experiment was to test the hypothesis that the energy value of SBM is greater than the current NRC (2012) value and that the usable energy in SBM can be calculated from a regression equation that predicts the G:F response of pigs fed diets with different levels of dietary SBO.

Materials and Methods

The protocol for the experiment was approved by the Institutional Animal Care and Use Committee at the University of Illinois, Urbana-Champaign, USA. Pigs used in the experiment were the offspring of Line 800 boars mated to Camborough females (Pig Improvement Company, Hendersonville, TN, USA).

Pigs and housing

A total of 120 growing pigs (initial body weight:24.76 ± 3.07 kg) were used in a 4-week experiment. Pigs were randomly allotted to five dietary treatments with two pigs per pen (one barrow and one gilt) for a total of 12replicate pens per treatment. Pens (0.9 m × 1.8 m) had fully slatted concrete floors, a feeder, and a cup waterer. Feed and water were available at all times, and all diets were feeding a meal form. Experimental diets were formulated based on corn, soy protein concentrate (SPC; Stutzman's Feed &Supply, Arthur, IL, USA), and synthetic cellulose (Solka Floc, J.Rettenmaier, MI, USA; Tables 3.1 and 3.2). Diets were formulated to meet current estimates for nutrient requirements for 25–50 kg pigs (NRC 2012). The basal diet contained corn, SPC, and 12% synthetic cellulose, and this diet contained no SBO, but synthetic Lys, Met, Thr, Trp, and Val were included in the diet to meet the requirement for digestible indispensable AA. Three additional diets were formulated by adding 2.0%, 4.0%, or 6.0% SBO to the basal diet. A fifth diet containing corn, SPC, and 12% SBM, but only 0.89% synthetic cellulose and no SBO or synthetic AA, was also formulated. Individual pig weights were recorded at the beginning of the experiment and at the conclusion of the experiment. Feed addition was recorded daily, and the weight of feed left in the feeder was recorded at the conclusion of the experiment. At the end of the 28-day experiment, pig weight and feed allowance data were summarized and

used to calculate total feed consumption, average daily gain (ADG), average daily feed intake (ADFI), and G:F for each pen and treatment group.

Chemical analysis

All diet and ingredient samples were ground using a 500G stainless steel swing-type mill grinder (Model RRH-500, Zhejiang Winki Plastic Industry Co., Ltd., Zhejiang, China) prior to chemical analyses. Diet and ingredient samples were analyzed for dry matter (method 927.05; AOAC Int. 2019) and ash (method 942.05; AOAC Int. 2019). Gross energy was analyzed using an isoperibol bomb calorimeter (Model 6400, Parr Instruments, Moline, IL, USA). Benzoic acid was used for standard calibration. The concentration of nitrogen was analyzed by combustion (method 990.03; AOAC Int. 2019) using a LECO FP628 analyzer (LECO Corp., Saint Joseph, MI, USA) with subsequent calculation of crude protein as nitrogen × 6.25. All diets and ingredients were analyzed for AA on a Hitachi Amino Acid Analyzer, Model No. L8800 (Hitachi High Technologies America, Inc.; Pleasanton, CA, USA) using ninhydrin for post-column 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). Ingredients and diets were also analyzed for acid hydrolyzed ether extract using the acid hydrolysis filter bag technique (Ankom HCl Hydrolysis System; Ankom Technology, Macedon, NY, USA) followed by crude fat extraction using petroleum ether (method 2003.06, AOAC Int. 2019) in an AnkomXT15 Extractor (Ankom Technology).

Calculations and statistical analysis

Data were analyzed using the MIXED Procedure (SAS Inst. Inc., Cary, NC, USA) with the pen as the experimental unit. Model assumptions of the residuals were confirmed using the MIXED procedure and the Brown-Forsythe test of the GLM procedure of SAS. Outliers were detected using the ROBUSTREG procedure and were removed before final statistical analyses. Two outliers were removed from the diet containing no SBO and no SBM. Linear and quadratic effects of increasing dietary SBO were determined using orthogonal polynomial CONTRAST statements. Statistical significance and tendencies were considered at $P < 0.05$ and $0.05 \leq P < 0.10$, respectively. A single degree of freedom contrast was used to compare the effect of the diet containing 12% SBM and no SBO with the diet containing no SBO and no SBM. A regression equation to estimate the improvement in G:F that was observed for pigs fed the diet containing SBM against the levels of dietary oil (i.e., 0, 2, 4, or 6%) was developed using the REG procedure in SAS, with SBO as the independent variable. Using the developed equation, the SBO level that corresponded to the G:F of pigs fed the diet containing SBM was calculated. By assuming an NE of 7,545 kcal/kg in SBO (NRC, 2012), the corresponding NE in SBM was calculated.

Results

The final body weight of pigs increased (quadratic, $P < 0.05$) as SBO increased in diets containing no SBM (Table 3.3). However, the weight of pigs was greater ($P < 0.01$) in pigs fed the diets containing 12% SBM compared with the weight of pigs fed the diet without SBO oil and no SBM. Increasing SBO in the diets increased (quadratic, $P < 0.001$) ADG, and pigs fed the diet containing SBM had a greater ($P < 0.001$) ADG than pigs fed the diet with no SBO or

SBM. Average daily feed intake decreased (quadratic, $P < 0.001$) as dietary SBO increased, but G:F increased (linear, $P < 0.001$) as SBO increased in diets containing no SBM. However, addition of 12% SBM to the diet without SBO increased ($P < 0.001$) G:F of pigs.

A prediction equation for G:F of pigs fed the four diets containing no SBM, and from 0 to 6% SBO was developed (Table 3.4). Results indicated that G:F increased ($P < 0.001$) by 0.013 for each percentage unit increase in SBO inclusion in the diet. From the prediction equation, it was calculated that the G:F of pigs fed the diet with 12% SBM was equivalent to that of pigs fed a diet containing 4.70% SBO (Table 5).

Discussion

Soybean meal is the most commonly used protein source in diets for pigs due to its excellent AA profile and favorable digestibility of AA (Cervantes-Pahm and Stein 2008). However, besides providing AA, SBM also provides other nutrients and energy to the diet. Although it is not a nutrient, energy is necessary for all biological processes in pigs. Because most of the variable costs in swine production are related to diet cost (Patience et al. 2015), and because energy is the costliest component of diets, accurate estimation of feed energy values may reduce production costs (Noblet et al. 1994; Kil et al. 2013). The NE in SBM is around 77% of the NE in corn according to current book values, which corresponds to 2,319 kcal/kg DM (Sauvant et al. 2004; Rostagno et al. 2011; NRC 2012). However, Sotak-Peper et al. (2015) calculated the NE of 22 sources of SBM from determined values for digestible energy and concluded that the average NE in SBM was around 2,467 kcal/kg DM. Likewise, Li et al. (2017) conducted an experiment using indirect calorimetry and determined that the NE of SBM was 2,710 kcal/kg DM. Results from an experiment conducted under commercial conditions that used

caloric efficiency to estimate the energy in SBM indicated that the energy value of SBM ranges between 105% and 125% of the NE in corn, which correspond to NE values of 3,171 to 3,752 kcal/kg DM, respectively (Cemin et al. 2020).Thus, results of all recently conducted experiments indicate that current book values may significantly underestimate the NE of SBM. To address this uncertainty, the current research aimed to determine the energy value of SBM using G:F as the response variable. Because the NE in SBO is well-defined and has been measured by indirect calorimetry and prediction equations (NRC 2012; Li et al. 2018), and growth assays to test the energetic values of ingredients have been conducted (Boyd et al. 2010; Cemin et al. 2020), we calculated the energy value of SBM as the SBO equivalency using G:F as the response variable. To our knowledge, estimation of the SBO equivalency of SBM using G:F as the response criteria has not been reported before, but it is recognized that this approach does not take possible changes in body composition into account. Changes in body composition may influence feed efficiency (Campbell and Taverner 1988), and values obtained from this procedure are not always equivalent to NE values determined using indirect calorimetry. The analyzed AA composition of ingredients and diets were within the expected values and consistent with calculated values (NRC 2012). Likewise, by removing the synthetic AA from the SBM containing diet, it was possible to formulate all diets with equivalent concentrations of the limiting indispensable AA. The basal diet was formulated to contain 12% synthetic cellulose and increasing concentrations of SBO were included at the expense of synthetic cellulose. Dietary fiber is resistant to digestion by the enzymes of pigs, and fermentation of non-digested nutrients in the hindgut can be a source of energy for the pigs. However, the source of synthetic cellulose used in the current experiment is completely unfermentable and contributes no energy to the diet (Cervantes-Pahm et al.2014). Dietary fiber can contribute to the loss of endogenous lipids, which

can decrease the apparent digestibility of lipids(de Lange 2000; Urriola et al. 2013). However, cellulose con-sists of sugars arranged in a crystalline, straight-chain structure, which limits its accessibility to microbial degradation and reduces its interaction with dietary fats, minimizing its effect on fat digestibility (Ndou et al. 2019). Additionally, increasing levels of purified cellulose do not significantly affect lipid digestibility, suggesting that the simplified chemical structure of purified fiber is less likely to interfere with lipid digestion (Kil et al. 2010). The calculated metabolizable energy was 2,926, 3,,098, 3269, and 3,441 kcal/kg in the diets containing 0%, 2%, 4%, and 6% SBO, respectively (NRC 2012).The linear increase in G:F of pigs fed the diets with increasing concentrations of SBO is likely a result of the increased energy density of the diets containing SBO. This effect was expected and has been demonstrated in previous research (Kilet al. 2011; Adeola et al. 2013; Espinosa et al. 2021). Therefore, by including four levels of SBO in the diets, it was possible to establish a regression equation that could be used to calculate the response in G:F of SBM (Espinosa et al. 2021). The observation that addition of SBM to the diet with no SBO increased ADG of pigs is in agreement with data indicating that partially or fully replacing crystalline AA with SBM may improve growth performance of pigs (Holen et al. 2022). In the current experiment, concentrations of indispensable AA met or exceeded requirements for 25–50 kg pigs (NRC2012). Diet analysis indicated that the concentration of the first limiting AA in the SBM diet was not greater than in the diets without SBM because the synthetic AA were removed from the SBM diet, but all diets met requirements. Therefore, the increase in G:F of pigs fed the diet containing 12% SBM and no SBO compared with the diet containing no SBM, and no SBO was not due to an increase in limiting AA in the diets. It may be speculated that the fiber in the diet with no SBM would increase passage rate. This could theoretically reduce digestibility of energy and nutrients, which could be a reason for the

difference between the two diets, but because we did not measure passage rate, we cannot confirm this hypothesis. However, the inclusion rate of high-fiber ingredients in diets for growing pigs did not impact the energy value of the ingredients (Navarro et al. 2018) and it is, therefore, unlikely that differences in the concentration of fiber among diets impacted energy digestibility. The observation that G:F of pigs fed the diet containing 12% SBM corresponded to an inclusion of 4.70% SBO indicates that NE under the condition of this experiment was close to 2,955 kcal per kg, assuming that the NE of SBO reported by the NRC (2012) is accurate. This value is much greater than current book values (Sauvant et al. 2004; Rostagno et al. 2011; NRC 2012), but a greater NE in SBM than current book values has been reported numerous times in recent years, indicating that the energy value of SBM is underestimated in current feed tables (Sotak-Peper et al. 2015; Li et al. 2017; Cemin et al.2020; Lee et al. 2021). However, as mentioned above, energy estimations of SBM using changes in G:F may overestimate NE because possible changes in body composition of pigs are not taken into account, which may explain why the value obtained in the current experiment may be different from NE values obtained from pigs placed in calorimeter chambers. The current experiment was conducted with only one source of SBM containing 46% protein. Soybean meals vary in composition due to differences in residual oil, dehulling, and processing methods, which may affect nutrient composition and energy digestibility. Although only minor variability among different sources of SBM in digestible energy and metabolizable energy is observed (Sotak-Peper et al. 2015), it cannot be ruled out that a different response would be obtained if a different source of SBM was used. Nevertheless, because results from this and other recently conducted experiments demonstrated a greater energy value in SBM than book values, and because no experiments observed NE in SBM to be less than book values, there is strong evidence that the NE in SBM is

currently being underestimated. This conclusion is supported by results from recently published experiments in which it was observed that the NE of corn-SBM diets were greater than those calculated from ingredient NE values (NRC2012; Kim et al. 2020; Lyu et al. 2023; Ibagon et al. 2024; Lee et al. 2024; Ochoa et al. 2024). Assuming that the NE of corn is accurate, these results support the hypothesis that SBM contributes more NE to the diets than previously estimated.

Conclusion

Results of this experiment indicate that the overall G:F of pigs increased as SBO increased in diets containing no SBM. From the prediction equation, it was calculated that the G:F of pigs fed the diet containing 12% SBM corresponded to that of a diet containing 4.70% SBO. Assuming there is 7,545 kcal NE per kg in SBO, this corresponds to an NE value of 355 kcal/kg from 12% SBM, which corresponds to 2955 kcal NE per kg SBM.

Tables

Table 3.1. Ingredient composition of experimental diets, as- is basis[1]

Item	No SBM				SBM,
Soybean oil, %:	0	2	4	6	12%
Ingredient, %					
Ground corn	70.03	70.03	70.03	70.03	70.03
Soybean meal, 46% crude protein	-	-	-	-	12.00
Soy protein concentrate	14.38	14.38	14.38	14.38	14.38
Solka floc[2]	12.00	10.00	8.00	6.00	0.89
Soybean oil	-	2.00	4.00	6.00	-
L-Lys·HCl, 78.8%	0.40	0.40	0.40	0.40	-
DL-Met, 99%	0.12	0.12	0.12	0.12	-
L-Thr, 99%	0.13	0.13	0.13	0.13	-
L-Trp, 99%	0.03	0.03	0.03	0.03	-
L-Val, 99%	0.02	0.02	0.02	0.02	-
Dicalcium phosphate	1.25	1.25	1.25	1.25	1.00
Ground limestone	0.74	0.74	0.74	0.74	0.80
Sodium chloride	0.40	0.40	0.40	0.40	0.40
Vitamin-mineral premix[3]	0.50	0.50	0.50	0.50	0.50
Total	100	100	100	100	100
Analyzed nutrients					
Dry matter, %	88.79	89.17	89.11	89.02	87.66
Ash, %	3.03	3.07	3.04	3.17	4.33

Table 3.1. (Cont.)

Gross energy, kcal/kg	3,848	4,014	4,142	4,224	3,914
Crude protein[4], %	15.36	15.99	15.80	16.01	20.44
Acid hydrolyzed ether extract, %	2.78	4.58	6.63	8.12	2.47
Indispensable amino acids, %					
Arg	0.87	0.93	0.97	0.92	1.33
His	0.39	0.42	0.43	0.41	0.56
Ile	0.65	0.70	0.72	0.67	0.94
Leu	1.34	1.44	1.44	1.42	1.83
Lys	1.13	1.15	1.12	1.09	1.16
Met	0.30	0.32	0.33	0.32	0.30
Phe	0.72	0.78	0.79	0.76	1.04
Thr	0.64	0.64	0.67	0.65	0.74
Trp	0.19	0.20	0.21	0.19	0.21
Val	0.75	0.80	0.81	0.76	1.00
Dispensable amino acids, %					
Ala	0.77	0.81	0.82	0.82	1.06
Asp	1.42	1.47	1.57	1.45	2.08
Cys	0.24	0.24	0.26	0.24	0.32
Glu	2.63	2.80	2.88	2.76	3.75
Gly	0.59	0.61	0.64	0.60	0.86
Pro	0.96	1.01	1.01	1.01	1.30
Ser	0.60	0.63	0.68	0.65	0.89

Table 3.1. (Cont.)

Tyr	0.43	0.46	0.48	0.48	0.67
Total amino acids, %	14.62	15.41	15.83	15.2	20.04

[1]SBO, soybean oil; SBM, soybean meal

[2]J. Rettenmaier USA LP., Schoolcraft, MI, USA.

[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 hydroiodide; 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.

[4]Crude protein was calculated by multiplying analyzed nitrogen by 6.25.

Table 3.2 Analyzed nutrient composition of ingredients, as-is basis[1]

Item	Corn	Soybean meal	Soy protein concentrate
Dry matter, %	87.46	89.66	91.88
Ash, %	1.26	6.41	5.01
Gross energy, kcal/kg	3,784	4,101	4,629
Crude protein, %	7.48	45.76	64.74
Acid hydrolyzed ether extract, %	3.37	1.05	2.99
Indispensable amino acids, %			
Arg	0.22	3.35	4.55
His	0.14	1.08	1.40
Ile	0.19	2.15	3.09
Leu	0.84	3.32	5.36
Lys	0.20	3.16	3.79
Met	0.15	0.76	1.01
Phe	0.29	1.41	4.38
Thr	0.25	2.06	2.32
Trp	0.03	0.56	0.78
Val	0.30	2.43	2.84
Dispensable amino acids, %			
Ala	0.70	1.66	2.92
Asp	0.44	5.11	7.38
Cys	0.11	0.10	0.62

Table 3.2. (Cont.)

Glu	1.28	8.44	11.82
Gly	0.21	2.09	2.85
Pro	0.56	2.43	3.48
Ser	0.28	2.06	3.03
Tyr	0.25	1.79	1.96
Total amino acids, %	6.43	43.95	63.56

[1]SBO, soybean oil; SBM, soybean meal

Table 3.3. Growth performance of pigs fed diets containing 0, 2, 4, or 6 % soybean oil (SBO) and 12% of soybean meal (SBM)[1,2]

Item	No soybean meal				Soybean meal, 12%	Pooled SEM	Contrast *P*-value		
							No SBM		-SBO vs.
SBO %	0	2	4	6	0		Linear	Quadratic	-SBO + SBM
day 1 to 28									
Initial body weight	24.69	24.78	24.67	24.64	24.78	.	.	.	.
ADG[3], kg	0.84	0.80	0.76	0.88	0.94	0.05	0.198	< 0.001	< 0.001
ADFI[3], kg	1.79	1.62	1.48	1.62	1.80	0.14	0.001	< 0.001	0.872
G:F[3]	0.47	0.50	0.51	0.55	0.53	0.02	< 0.001	0.795	0.001
Final BW, kg	48.14	47.04	45.85	49.39	51.18	3.84	0.451	0.003	0.007

[1]SBO, soybean oil; SBM, soybean meal

[2]Least square means represents 12 observations, except for the diet containing no SBO and no SBM (n = 10).

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

Table 3.4. Regression coefficients used for estimating gain-to-feed ratio (G:F) response of including soybean oil (SBO) in diets[1, 2]

		Standard error		*P*-value		Statistical parameter		
Dependent variable	Prediction equation	Intercept	Slope	Intercept	Slope	R^2	RMSE	*P*-value
G:F	0.468 + 0.013 (SBO, %)	0.009	0.002	< 0.001	< 0.001	0.406	0.034	< 0.001

[1]Data were subjected to linear regression analysis with the % inclusion of SBO as the independent variable and G:F as the dependent variable. The regression coefficients indicate the change in G:F for each % point change of SBO included in the diet.

[2]RMSE, root means square of error; R^2 = coefficient of determination.

Table 3.5. Soybean oil (SBO) equivalence (%) of soybean meal (SBM) in the diet containing 12% SBM[1,2]

Item	12% SBM
	0 % SBO
G:F	0.528
SBO equivalence, %	4.70

[1]SBO, soybean oil; SBM, soybean meal

[2]Equivalence was calculated from the prediction equation [0.468 + 0.013 (SBO, %); Table 4] used to estimate the corresponding SBO inclusion for a given gain-to-feed ratio in SBO.

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CHAPTER 4: Nitrogen retention in weanling and finishing pigs fed corn-soybean meal-based diets is greater than 60%, regardless of diet composition

Abstract

The objective was to test the hypothesis that nitrogen retention by weanling and finishing pigs, measured as a percentage of nitrogen intake, is greater than 50%, regardless of the dietary protein level and the body weight (**BW**) of pigs. Six diets containing corn and soybean meal (**SBM**) were formulated. Diets were fed to pigs in two phases (i.e., weanling and finishing). Within each phase, a high-protein, medium-protein, and a low-protein diet were formulated. Twenty-four weanling pigs and 24 finishing pigs were allotted to the three dietary treatments. Pigs were housed individually in metabolism crates, and feces and urine were collected quantitatively for five days. Nitrogen retention relative to intake ranged from 60.9 to 71.9% and increased ($P < 0.001$) as dietary protein was reduced, but nitrogen retention measured as g/day was reduced ($P < 0.05$) as dietary protein was reduced. In conclusion, the hypothesis that pigs retain more than 50% of consumed nitrogen was accepted, but pigs fed low-protein diet have reduced nitrogen retention measured as g/day compared with pigs fed diets with greater protein concentrations.

Key Words: nitrogen balance, nitrogen retention, soybean meal

Introduction

Soybean meal (**SBM**) is the primary plant-protein source in diets for pigs and provides both amino acids (**AA**) and energy to the diets. Current estimates for net energy in SBM (Sauvant et al., 2004; NRC, 2012) are less than for cereal grains, based on the assumption that there is more nitrogen to be deaminated if ingredients are high in protein because deamination and excretion of nitrogen via the urea cycle are energy-requiring processes, and therefore, reduce energy efficiency. It has been suggested that pigs retain only 45 to 50% of absorbed nitrogen (Noblet et al., 2004), which corresponds to 40 to 45% of ingested nitrogen. Modern genotypes of pigs, however, have improved the capacity for protein synthesis and may retain more nitrogen than older genotypes, which would result in less AA deamination and, therefore, less energy loss to deaminate AA and excrete nitrogen. Indeed, results of recent research indicate that pigs fed corn-SBM based diets retain more than 60% of ingested nitrogen (Corassa et al., 2024; Ochoa et al., 2024; Cristobal et al., 2025), indicating that protein retention by modern genotypes of pigs is more efficient than by older genotypes. It is likely that as breeding companies have selected for leaner pigs (Fix et al., 2010; Gozalo-Marcilla et al., 2021), they have also selected genotypes that are more efficient in converting dietary protein into body protein. It is, however, not known if the greater nitrogen retention that has been recently reported is experienced by all pigs regardless of body weight (**BW**) and if it is true for all types of diets regardless of the dietary level of protein. Therefore, the objective of this experiment was to test the hypothesis that nitrogen retention, measured as a percentage of ingested nitrogen, is greater than 50% regardless of the dietary protein level and the BW of pigs.

Materials and Methods

The protocol for the experiment was reviewed and approved by the Institutional Animal Care and Use Committee at the University of Illinois.

Experimental diets, animals, and housing

Diets were fed to pigs in two phases (weanling and finishing pigs), and there were three dietary treatments within each phase (Tables 4.1, 4.2, and 4.3). Therefore, there were a total of six diets in the experiment. Within each phase, a high-protein diet was formulated based on corn and SBM (and whey powder for weanling pigs), and this diet contained no crystalline AA, with the exception that 0.036% $_{DL}$-methionine was included in the high-protein diet fed to weanling pigs. A medium-protein diet contained less SBM and more corn than the high-protein diet, and this diet was formulated by allowing crystalline Lys, Met, and Thr to enter the formulation to meet required levels of indispensable AA (NRC, 2012). A low-protein diet that contained less SBM and more corn than the other two diets was also formulated, and five crystalline AA (i.e., Lys, Met, Thr, Trp, and Val) were used in the formulation of this diet to maintain required levels of digestible AA. All diets were formulated to meet or exceed the estimated requirements for standardized ileal digestible AA, vitamins, and minerals for weanling or finishing pigs (Table 3; NRC, 2012). All diets were fed in meal form. The same batch of corn and SBM was used in all diets, and the same batch of whey powder was used in the three diets fed to weanling pigs. Twenty-four weanling pigs (initial BW: 11.37 ± 0.62 kg) and 24 finishing pigs (initial BW: 76.22 ± 3.10 kg) were allotted to the three dietary treatments using a randomized complete block design within each phase, for a total of eight replicate pigs per diet in each phase. Initial BW was the blocking factor. All pigs were castrated male pigs that were the offspring of Line 800 males mated to Camborough females (Pig Improvement Company, Hendersonville, TN, USA). Pigs

were weaned at approximately three weeks of age and weanling pigs were allotted to the experiment three weeks post-weaning whereas finishing pigs were used 15 weeks post-weaning. Pigs were housed individually in metabolism crates (0.67 × 0.84 m for weanling pigs and 0.85 × 152.4 m for finishing pigs). Crates were equipped with a self-feeder, a nipple waterer, a fully slatted floor, a screen floor, and urine trays that allowed for the total, but separate, collection of urine and fecal materials from each pig. Throughout the experiment, pigs had free access to water. The daily feed allowance was calculated as 3.2 times the maintenance requirement for metabolizable energy [i.e., 197 kcal metabolizable energy (ME) per kg $BW^{0.60}$; NRC, 2012]. Feed was provided daily in two equal meals that were provided at 0700 h and 1600 h.

Sample Collection

Pigs were fed experimental diets for 13 days, with feed disappearance recorded daily. The initial five days were considered the adaptation period to the diet, and urine and fecal samples were collected from the feed provided during the following five days according to the marker-to-marker method (Adeola, 2001). The start marker (i.e., indigo carmine) was included in the morning meal on day six, and fecal collections were initiated when the marker appeared in the feces. Fecal collections ceased when the stop marker (i.e., ferric oxide), which was included in the morning meal on day 11, appeared in the feces. Feeding of experimental diets continued until day 13 to allow sufficient time for marker passage to the feces. Urine was collected in urine buckets containing 50 mL of 6 *N* HCl as a preservative and placed under the metabolism crates. Urine buckets were emptied daily, the weight of the collected urine was recorded, and 20% was stored at −20 °C. Orts were collected daily prior to feeding the morning meal, pooled for the duration of the collection period, dried in a 50 °C forced-air drying oven, and weighed at the

conclusion of the experiment to determine feed intake. Fecal samples, orts, and urine were stored at −20 °C immediately after collection.

Chemical analysis

At the conclusion of the experiment, fecal samples were dried at 65 °C in a forced-air oven (Thermo Fisher Scientific Inc.; model Heratherm OMH750, Waltham, MA, USA) and then finely ground using a 500 G stainless steel mill grinder (RRH, Zhejiang, China) prior to chemical analysis. Urine samples were thawed, mixed, subsampled, and stored at −20 °C until analysis for nitrogen. A second urine sample was lyophilized and used to quantify gross energy (**GE**) in urine (Kim et al., 2009). All diets, ingredients, and fecal samples were analyzed for dry matter (**DM**; method 930.15; AOAC Int., 2019), and diets and ingredients were also analyzed for ash (method 942.05; AOAC Int., 2019). The concentration of nitrogen in diets and ingredients was analyzed using the Kjeldahl method (method 984.13; AOAC Int., 2019) on a Kjeltec 8400 (FOSS Inc., Eden Prairie, MN, USA) with subsequent calculation of crude protein using a conversion factor of 6.25. These samples were also analyzed for AA [method 982.30 E (a, b, c); AOAC Int., 2019] on a Hitachi Amino Acid Analyzer, Model No. L8800 (Hitachi High Technologies America, Inc.; Pleasanton, CA, USA). Diet, ingredient, fecal, and lyophilized urine samples were analyzed for GE using bomb calorimetry (Model 6400; Parr Instruments, Moline, IL, USA) with benzoic acid being used as the standard for calibration. Fecal and urine samples that were not lyophilized were analyzed for nitrogen as described for diets.

Calculations and statistical analysis

The apparent total tract digestibility (**ATTD**) of nitrogen in each experimental diet and nitrogen retention for each pig were calculated as described by Pedersen et al. (2007):

ATTD (%) = [nitrogen intake (g/day) ÷ nitrogen in feces (g/day)]/ nitrogen intake (g/day) × 100

Nitrogen retention (g/day) = nitrogen intake (g/day) ÷ nitrogen in feces (g/day) ÷ nitrogen in urine (g/day)

The ATTD of DM and GE were calculated as ATTD of nitrogen and digestible energy (DE) and ME were also calculated for each diet (NRC, 2012). The biological value of nitrogen in diets was calculated using the following equation (Rojas and Stein, 2013):

$$\text{Biological value, \%} = (\text{retained nitrogen} \div \text{absorbed nitrogen}) \times 100,$$

where retained nitrogen and absorbed nitrogen are expressed in g/day. Retained nitrogen was calculated by subtracting fecal and urine nitrogen outputs from nitrogen intake, and absorbed nitrogen was calculated by subtracting fecal nitrogen output from nitrogen intake.

Data were analyzed using the MIXED procedure of SAS, and pig was the experimental unit for all analyses. Model assumptions on the residuals were confirmed using the MIXED procedure and the Brown-Forsythe test of the GLM procedure of SAS (SAS Inst. Inc., Cary, NC, USA). The MIXED procedure in SAS was used to generate studentized residuals, and outliers were defined as data points having studentized residuals greater than 3 or less than −3. One outlier was removed from the low-protein diet in the weanling phase, and one from the high-protein diet in the finishing phase, but all other data were included in the analysis. The statistical model included diet, phase, and the interaction between diet and phase as fixed effects, and block (i.e., weanling or finishing pigs) within phase as a random effect. For all treatments, means were calculated and separated using the LSMEANS statements, and if the model was significant, means were separated using the PDIFF option with Tukey's adjustment. Statistical significance and tendencies were considered at $P < 0.05$ and $0.05 \leq P < 0.10$, respectively.

Results

The analyzed composition of corn, SBM, and whey powder was close to expected values (Table 4.4). Pigs were monitored daily for health status and showed no clinical signs of disease or distress during the experiment; no feed refusal was observed, and all pigs completed the experiment. There were no interactions between dietary protein and phase for feed intake, GE intake, dry feces output, GE in feces, nitrogen excretion in feces, urine output, GE in urine, the ATTD of DM, the ATTD of GE, the ATTD of nitrogen, retained nitrogen (g/day and % of intake), biological value or ME to GE and ME to DE ratios (Table 4.5). Nitrogen intake, nitrogen excretion in urine, absorbed nitrogen, DE, and ME decreased as dietary nitrogen was reduced, but more so for finishing pigs than for weanling pigs (interaction, $P < 0.05$). Excretion of GE in urine, retained nitrogen (g/day), and ATTD of nitrogen were reduced ($P < 0.01$) as dietary protein was reduced, whereas nitrogen retention (% of intake), biological value, and the ME:DE ratio increased ($P < 0.001$) as dietary protein was reduced. All measured parameters except nitrogen retention (% of intake) and biological value were greater ($P < 0.001$) in finishing pigs than in weanling pigs. Nitrogen retention (% of intake) tended ($P < 0.10$) to be reduced, and biological value was reduced ($P < 0.01$) in finishing pigs compared with weanling pigs.

Discussion

The analyzed AA profile and nutrient composition of the ingredients used in the experiment were as expected and in agreement with reported values (NRC, 2012). Diets for both weanling and finishing pigs were formulated to meet requirements for all indispensable AA (NRC, 2012), but because of the differences in inclusion levels of SBM, the indispensable AA to Lys ratios were not constant among diets. The diets with the greatest concentrations of SBM

were formulated to meet requirements for standardized ileal digestible Lys (and for weanling pigs also Met), but other indispensable AA were supplied in excess of the requirements. The medium-protein diets were formulated to meet requirements for standardized ileal digestible Lys, Met, Thr, and Trp or Val, but other indispensable AA were in excess of the requirement. Likewise, the low-protein diets were formulated to meet requirements for standardized ileal digestible Lys, Met, Thr, Trp, Val, and His or Ile, but other indispensable AA were included in excess of the requirement. The protein reduction in diets for all phases was achieved by reducing the amount of SBM, increasing corn, and adding crystalline AA to the diets to meet requirements for indispensable AA.

The reason diets with different protein concentrations were used in both phases was that nitrogen retention is influenced by diet protein concentration (Kerr and Easter, 1995; Cristobal et al., 2025a). Therefore, to be able to address the hypothesis that pigs have nitrogen retention that is greater than 50%, it was necessary to use diets that were expected to result in different nitrogen retention values.

The ATTD of DM in the low-protein diets was expected to be greater than in the high-protein diets due to less total dietary fiber in corn compared with SBM (NRC, 2012), but a tendency for a reduction in the ATTD of DM was observed when crude protein and SBM were reduced in the diets, which is in agreement with results of other recent experiments in which high and low-protein diets were used (Gómez et al., 2002; Cristobal et al., 2025a). This observation is likely a result of the impact of dietary protein on microbial fermentation because low protein diets provide fewer substrates for fermentation, which limits the microbial activity in the hindgut and thus results in reduced total tract digestibility of nutrients (Zhou et al., 2022). The observation that the ATTD of nitrogen was reduced as SBM was reduced and replaced with corn was

expected, because the ATTD of crude protein in corn and SBM is approximately 80.70% and 89.15%, respectively, in growing pigs (Dong et al., 2020). Therefore, because protein also contributes to DM, the reduction in ATTD of nitrogen may also have contributed to the reduced ATTD of DM, which is in agreement with previous data (Zervas and Zijlstra, 2002; Fabian et al., 2004; Cristobal et al., 2025a).

The reduction in DE that was observed as SBM in the diets was reduced confirms that DE in SBM is greater than in corn, as has also been previously reported (Le Bellego et al., 2001; Sotak-Peper et al., 2015; Cristobal et al., 2025a). The interaction for ME that was observed indicates that for weanling pigs, the ME of SBM is likely close to that of corn because no change in ME was observed when corn increased and SBM was reduced. However, the reduction in ME that was observed when SBM was replaced by corn in diets for finishing pigs indicates that SBM has a greater ME than corn when fed to finishing pigs, which is likely because of the greater nitrogen retention in finishing pigs. These observations are also in agreement with previous data (Li et al., 2017; Sotak-Peper et al., 2015). The average values for DE and ME in all diets are in agreement with data for corn-SBM diets fed to growing pigs (Lee et al., 2024; Cristobal et al., 2025a; Ibagon et al., 2025a).

Lysine is the first limiting AA in most swine diets containing corn and SBM although Met may become first limiting in diets for weanling pigs due to the low concentration of sulfur AA in milk proteins. Therefore, if crystalline Lys is not used, SBM is included in diets to meet the requirement for Lys (Liao et al., 2015; Remus et al., 2019), which also increases the dietary concentrations of other AA and results in an excess of AA other than Lys. However, the excess AA that are not used for protein synthesis are deaminated, which results in nitrogen excretion in the urine (van Milgen and Dourmad, 2015). Therefore, low-protein diets that contain less

protein-bound AA, but more crystalline AA, have been used to reduce urine nitrogen excretion (Figueroa et al., 2002; Wang et al., 2018). A reduction of 5% units in dietary protein for both phases in the current experiment decreased nitrogen excretion in urine by 3.5 and 8.7 g per day in the weanling and finishing phases, respectively. This reduction is in agreement with data from experiments where nitrogen excretion in urine decreased when low-protein diets were used (Canh et al., 1998; Wang et al., 2018; Cristobal et al., 2025a). The observation that despite the decrease in nitrogen intake observed in the low- and medium-protein diets compared with the high-protein diets, fecal nitrogen excretion was not different among diets, is likely a consequence of the reduced ATTD of nitrogen for the low protein diets compared with the diets with greater protein. It is, however, also possible that pigs fed the diets with greater protein had greater absorption of ammonia from the hindgut (Zervas and Zijlstra, 2002; Yen et al., 2004). Reducing dietary crude protein usually results in reduced fecal nitrogen excretion (Kerr and Easter, 1995; Le Bellego et al., 2001; Figueroa et al., 2002; Otto et al., 2003). However, increasing inclusion of fiber in diets increases fecal nitrogen excretion due to greater microbial nitrogen incorporation and endogenous nitrogen losses (Zervas and Zijlstra, 2002), and because fiber concentration was less in the low protein diets than in the high protein diets, this effect may have contributed to the reduced nitrogen excretion in feces from pigs fed low-protein diets.

Because all diets were formulated to meet the requirements for standardized ileal digestible indispensable AA, which was supposed to result in an equal supply of available AA to support protein synthesis, nitrogen retention was expected to not be different among diets within each phase. However, the observation that nitrogen retention, measured as grams per day, decreased in pigs fed low-protein diets compared with pigs fed high-protein or medium-protein diets may be a result of differences in absorption rates between crystalline AA and protein-bound

AA, resulting in an imbalance in AA supply at the sites of protein synthesis (Yen et al., 2004; Zhang et al., 2022). According to the adaptive regulation mechanisms, AA transporter activity decreases when blood concentrations of AA are high (Kilberg et al., 1985). As a result, absorption of AA from protein-bound AA is also reduced by reduced activity of transporters in the small intestine, which may negatively impact protein synthesis after absorption (Morales et al., 2015; Wang et al., 2021). Therefore, the reduction in nitrogen retention, measured in grams per day, by pigs fed the low-protein diets is likely a result of differences among diets in availability of AA in the cells synthesizing protein (Canh et al., 1998; Portejoie et al., 2004; Yen et al., 2004). However, because neither AA deamination nor protein synthesis were measured in this experiment, this hypothesis was not verified in the present work and it is acknowledged that conclusions about the effectiveness of crystalline AA for protein synthesis cannot be made from data generated in this experiment. Nevertheless, the implication of the reduction in nitrogen retention is that pigs fed a low-protein diet supplemented with crystalline AA may have reduced protein synthesis, reduced carcass leanness, and increased back fat deposition (Tuitoek et al., 1997; Kerr and Easter, 1995; Deng et al., 2009). In the present experiment, diets were formulated to meet minimum requirements for standardized ileal digestible AA. However, because the digestibility of crystalline AA is believed to be 100% (Oliveira et al., 2020), concentrations of total AA is expected to be reduced if crystalline AA are used (Cristobal et al., 2025b). The traditional AA analysis will also sometimes miss some of the crystalline AA in the analysis, which may further reduce the analyzed total AA in diets containing crystalline AA. However, for protein synthesis to be maximized it is critical that all AA meet the minimum requirements by the pigs because if one or more AA are fed below the requirement, nitrogen retention will be compromised.

Nitrogen utilization is usually calculated as the amount of nitrogen retained in the body divided by the nitrogen intake from the diet and refers to the nitrogen retained in the body for protein synthesis. The observation that pigs fed the low and medium-protein diets had increased nitrogen retention as a percentage of nitrogen intake and as a percentage of absorbed nitrogen indicates that the utilization of dietary AA is more efficient when using low-protein diets due to the reduction of excess AA that high-protein diets provide. The standardized ileal digestibility of crystalline AA is close to 100% (Chung and Baker, 1992; Oliveira et al., 2020), which indicates that the crystalline AA are absorbed more efficiently than protein-bound AA. The high digestibility of crystalline AA will reduce fecal nitrogen excretion, and the improved AA balance in low-protein diets is expected to result in a greater percentage of dietary nitrogen being used for protein synthesis and deposition as was also demonstrated in the present experiment. This observation is in agreement with data from pigs fed diets with a reduction of protein from 16% to 12% (Kerr and Easter, 1995).

It has been suggested that nitrogen retention in most growth stages of pigs is less than 45% of nitrogen intake (Noblet et al., 2004). Indeed, data from 1960 to 1990 reported values of nitrogen retention from balanced diets in the range of 30 to 40% of nitrogen intake. For example, Cromwell et al. (1965) reported nitrogen retention of 32% of nitrogen intake, similar to the average values reported between 1960 and 1990 for balanced diets with a maximum of three crystalline AA (McConnell et al., 1971; Stewart et al.,1983; Noblet et al., 1987). However, data from 1990 to 2010 indicated that nitrogen retention as a percentage of intake increased to 50 to 60% (Kerr and Easter, 1995; Noblet et al., 2001; Htoo et al., 2007). Data from experiments conducted in the last 10 to 15 years in which modern genotypes of pigs were used indicated that nitrogen retention now is between 60 and 70% of nitrogen intake (Rojas and Stein, 2013;

Corassa et al., 2024; Ochoa et al., 2024; Cristobal et al., 2025a; Ibagon et al., 2025a; 2025b). Therefore, the observation that nitrogen retention as a percentage of intake in the current experiment averaged 67% for all pigs, regardless of growth phase, indicates that advances in pig genetics have resulted in greater efficiency in utilizing dietary nitrogen for muscle protein synthesis (Stein et al., 2024). It is, however, acknowledged that this conclusion is based on corn and soybean meal diets being provided, but if pigs are fed diets with co-products that are high in fiber and possibly unbalanced in AA, nitrogen balance may be less than the values obtained with corn-soybean meal diets as has been demonstrated (Corassa et al., 2024; Ochoa et al., 2024; Ibagon et al., 2025b).

The ability to formulate diets using standardized ileal digestible AA has increased the precision in diet formulation and resulted in a more balanced AA supply, which also results in greater efficiency of retention of nitrogen by the pigs. Therefore, modern pig genotypes fed well balanced diets will deaminate less AA, resulting in reduced nitrogen excretion compared with older genotypes. Consequently, the hypothesis that modern genotypes of pigs can retain more than 50% of nitrogen intake was confirmed.

The net energy of feed ingredients may be estimated using prediction equations based on digestible energy values adjusted for nutrient composition and utilization efficiencies (Noblet et al., 1994). Protein is believed to be a less efficient energy source than carbohydrates and fats because of the energy expenditure for urea synthesis, protein turnover, and nitrogen excretion that requires ATP and produces heat (van Milgen et al., 2001; Bender, 2012). Therefore, a diet containing high levels of protein is believed to reduce net energy (Le Bellego et al., 2001). Consequently, high-protein ingredients generally have lower calculated net energy compared with high-starch ingredients, which has resulted in an estimation of the net energy in SBM of

approximately 78% of the net energy in corn (NRC, 2012). However, as demonstrated in the current experiment, nitrogen retention in modern genotypes of pigs is close to 70% of nitrogen intake, indicating that a greater proportion of dietary protein is utilized for tissue protein synthesis, and less nitrogen is excreted after AA deamination. As a result, the efficiency of protein utilization has been improved as pig genotypes have become leaner, which possibly increases the net energy of high-protein ingredients. If this hypothesis is correct, it may at least partly explain why net energy in SBM appears to be greater than previously calculated (Li et al., 2017; Lee et al., 2022).

Conclusion

Results indicate that modern genotypes of pigs retain between 60% and 72% of dietary nitrogen regardless of growth phase if fed a corn-SBM diet, but retention rate is greater if crude protein is reduced and crystalline AA are used. However, results also indicated that regardless of BW of pigs, daily nitrogen retention is greater if diets with only intact protein are provided compared with diets containing less protein and crystalline AA.

Tables

Table 4.1. Ingredient composition of experimental diets, as-fed basis

Item, %	Weanling pigs			Finishing pigs		
Phase[†]:						
Dietary protein:	High	Medium	Low	High	Medium	Low
Ground corn	41.894	53.480	57.287	73.359	80.781	85.434
Soybean meal, 46% crude protein	43.600	31.260	27.100	23.850	16.030	11.000
Whey powder	10.000	10.000	10.000	-	-	-
Soybean oil	2.000	2.000	2.000	0.500	0.500	0.500
L-Lys·HCl	-	0.362	0.485	-	0.229	0.378
DL-Met	0.036	0.140	0.175	-	-	0.044
L-Thr	-	0.094	0.149	-	0.047	0.113
L-Trp	-	-	0.010	-	-	0.027
L-Val	-	-	0.066	-	-	0.012
Monocalcium phosphate	0.509	0.676	0.732	0.511	0.616	0.684
Limestone	1.061	1.088	1.096	0.880	0.897	0.908
Sodium chloride	0.400	0.400	0.400	0.400	0.400	0.400
Vitamin-mineral premix[1]	0.500	0.500	0.500	0.500	0.500	0.500

[*]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-tocopherol 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-

Table 4.1. (Cont.)

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 dihydroiodide; 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.

†Initial weights were 11.37 and 76.22 kg for weanling and finishing pigs, respectively.

Table 4.2. Analyzed nutrient composition of experimental diets, as fed basis

Item Phase:	Weanling pigs			Finishing pigs		
Dietary protein:	High	Medium	Low	High	Medium	Low
Dry matter, %	88.49	88.23	88.42	87.16	86.85	86.94
Ash, %	5.58	4.99	4.71	3.62	3.30	4.03
Crude protein, %	24.39	20.97	19.24	16.48	13.41	11.91
Gross energy, kcal/kg	4,068	4,013	4,036	3,902	3,765	3,780
Indispensable amino acids, %						
Arg	1.56	1.29	1.12	0.97	0.73	0.60
His	0.64	0.53	0.48	0.44	0.35	0.30
Ile	1.16	0.96	0.84	0.73	0.56	0.47
Leu	2.02	1.74	1.60	1.50	1.29	1.16
Lys	1.49	1.54	1.46	0.87	0.87	0.81
Met	0.42	0.48	0.43	0.27	0.22	0.24
Phe	1.23	1.03	0.91	0.82	0.65	0.56
Thr	0.96	0.86	0.85	0.60	0.50	0.49
Trp	0.31	0.23	0.23	0.17	0.13	0.12
Val	1.26	1.05	0.99	0.84	0.66	0.59
Dispensable amino acids, %						
Ala	1.16	0.99	0.93	0.88	0.77	0.69
Asp	2.62	2.15	1.87	1.55	1.16	0.93
Cys	0.39	0.32	0.30	0.29	0.22	0.19

Table 4.2. (Cont.)

Glu	4.48	3.74	3.36	3.00	2.44	2.07
Gly	1.01	0.82	0.74	0.66	0.52	0.43
Pro	1.35	1.17	1.07	1.04	0.90	0.81
Ser	1.04	0.89	0.80	0.65	0.54	0.46
Tyr	0.78	0.69	0.60	0.50	0.41	0.36
Total amino acids, %	23.88	20.44	18.56	15.77	12.92	11.28

Table 4.3. Calculated concentration of standardized ileal digestible indispensable amino acids, metabolizable energy, calcium and P in experimental diets, as fed basis

Item	Phase:	Weanling pigs*			Finishing pigs†		
	Dietary protein:	High	Medium	Low	High	Medium	Low
Arg, %		1.49	1.15	1.04	0.95	0.74	0.60
His, %		0.59	0.48	0.44	0.40	0.33	0.28
Ile, %		1.06	0.84	0.76	0.66	0.52	0.43
Leu, %		1.88	1.59	1.49	1.39	1.21	1.09
Lys, %		1.35	1.33	1.32	0.78	0.77	0.76
Met, %		0.38	0.42	0.44	0.24	0.21	0.23
Phe, %		1.13	1.02	0.98	0.75	0.61	0.52
Thr, %		0.84	0.67	0.61	0.53	0.47	0.46
Trp, %		0.28	0.22	0.21	0.15	0.13	0.13
Val, %		1.14	0.91	0.85	0.75	0.61	0.53
Arg, %		1.49	1.15	1.04	0.95	0.74	0.60
Metabolizable energy, kcal/kg		3,371	3,358	3,351	3,319	3,313	3,306

Table 4.3. (Cont.)

Total Ca, %	0.72	0.72	0.72	0.52	0.52	0.52
Standardized total tract digestible P, %	0.35	0.35	0.35	0.24	0.24	0.24

*Requirements (NRC, 2012) for standardized ileal digestible amino acids and minerals (%) for weanling pigs are as follows: Arg, 0.56; His, 0.42; Ile, 0.63; Leu, 1.23; Lys, 1.23; Met, 0.36; Phe, 0.72; Thr, 0.73; Trp, 0.20, Val, 0.78; Ca, 0.70; standardized total tract digestible P: 0.33.

† Requirements (NRC, 2012) for standardized ileal digestible amino acids and minerals (%) for finishing pigs are as follows: Arg, 0.33; His, 0.25; Ile, 0.39; Leu, 0.74; Lys, 0.73; Met, 0.21; Phe, 0.44; Thr, 0.43; Trp, 0.13, Val, 0.48; Ca, 0.52; standardized total tract digestible P: 0.24.

Table 4.4. Analyzed nutrient composition of ingredients, as fed basis

Item	Corn	Soybean meal	Whey powder
Dry matter, %	86.08	88.74	90.39
Ash, %	1.06	5.98	7.88
Crude protein, %	7.92	46.17	11.97
Gross energy, kcal/kg	3,756	4,266	3,593
Indispensable amino acids, %			
Arg	0.34	3.25	0.26
His	0.22	1.24	0.21
Ile	0.29	2.27	0.71
Leu	0.97	3.59	1.15
Lys	0.26	3.01	0.96
Met	0.16	0.67	0.19
Phe	0.38	2.39	0.36
Thr	0.27	1.82	0.74
Trp	0.05	0.61	0.20
Val	0.39	2.44	0.65
Dispensable amino acids, %			
Ala	0.59	2.04	0.56
Asp	0.51	5.31	1.18
Cys	0.16	0.67	0.28
Glu	1.49	8.57	1.98
Gly	0.29	1.99	0.23

Table 4.4. (Cont.)

Pro	0.68	2.34	0.64
Ser	0.33	1.96	0.47
Tyr	0.24	1.53	0.26
Total amino acids, %	7.62	45.70	11.03

Table 4.5. Effects of dietary phase and dietary protein on 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[*]

	Weanling pigs			Finishing pigs			SEM	*P*-value		
Dietary protein:	High[‡]	Med	Low	High	Med	Low		Dietary protein	Phase	Interaction
Intake										
Feed, kg/d	0.88	0.89	0.91	2.53	2.62	2.63	0.09	0.601	< 0.001	0.793
Gross energy, Mcal/day	3.55	3.54	3.65	9.87	9.88	9.97	0.36	0.910	< 0.001	0.999
Nitrogen, g/d	32.05[c]	28.14[c]	25.78[c]	66.43[a]	56.66[b]	50.99[b]	2.36	< 0.001	< 0.001	0.036
Fecal excretion										
Dry feces output, g/d	81.38	79.41	85.43	195.12	208.32	227.95	18.02	0.208	< 0.001	0.392
Gross energy, kcal/day	369.19	363.59	392.93	898.56	960.23	1008.47	76.49	0.350	< 0.001	0.610
Nitrogen, g/d	4.04	3.78	4.02	6.73	6.82	7.19	0.71	0.768	< 0.001	0.839
Urine excretion										
Urine output, kg/d	3.75	3.52	3.73	10.73	7.44	8.28	1.60	0.176	< 0.001	0.248
Gross energy, kcal/day	127.20	83.55	67.97	274.95	180.24	188.26	23.56	0.005	< 0.001	0.553
Nitrogen, g/d	7.07[bc]	3.92[c]	3.66[c]	18.37[a]	10.18[b]	9.65[b]	1.24	< 0.001	< 0.001	0.002

Table 4.5. (Cont.)

ATTD of dry matter, %	89.16	89.46	88.73	91.23	90.81	90.05	0.43	0.130	< 0.001	0.616
ATTD of gross energy, %	88.28	88.38	87.67	90.64	89.86	89.49	0.45	0.170	< 0.001	0.624
Nitrogen balance										
Absorbed nitrogen, g/d	27.8[c]	24.17[c]	21.55[c]	59.5[a]	49.64[b]	43.6[b]	2.07	< 0.001	< 0.001	0.010
Retained nitrogen, g/d	20.13	19.64	17.23	40.52	38.86	33.34	2.33	0.008	< 0.001	0.398
ATTD of nitrogen, %	86.41	85.31	82.46	89.70	87.41	85.23	0.73	< 0.001	< 0.001	0.715
Nitrogen retention, % of intake	63.70	71.91	68.87	60.88	69.11	66.23	1.87	< 0.001	0.078	0.999
Biological value, %	73.79	84.29	83.50	67.87	79.06	77.71	2.11	< 0.001	0.002	0.985
Energy in diets, kcal/kg										
Digestible energy	3591[a]	3547[a]	3538[a]	3537[a]	3383[b]	3383[b]	17.82	< 0.001	< 0.001	0.005
Metabolizable energy	3417[a]	3432[a]	3447[a]	3420[a]	3309[b]	3307[b]	19.40	0.034	< 0.001	0.001
Energy efficiency, %										
ME to GE	88.28	88.38	87.67	90.64	89.86	89.49	0.45	0.170	< 0.001	0.624
ME to DE	95.18	96.76	97.43	96.70	97.80	97.76	0.40	< 0.001	0.005	0.339

[*]Least square means represent eight observations for all treatments, except for the low-protein diet of weanling pigs (n = 7).

Table 4.5. (Cont.)

[†]Initial weights were 11.37 and 76.22 kg for weanling and finishing pigs, respectively.

[‡]High = Corn-SBM diet with no crystalline AA; Medium = Less SBM, and more corn with Lys, Met, and Thr; Low = Less SBM than medium protein diet, and more corn with Lys, Met, Thr, Trp, and Val.

[a-c]Within a row, means without a common superscript differ ($P < 0.05$).

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CHAPTER 5: Reducing dietary protein results in less nitrogen retention and carcass leanness, but extra nitrogen or soy isoflavones did not change growth performance or immune responses of growing pigs

Abstract

Two experiments were conducted to test the hypothesis that reducing the inclusion of soybean meal (**SBM**) and increasing synthetic amino acids (**AA**) in corn-SBM diets may impact nitrogen balance, growth performance, carcass traits, and immune responses of pigs, and that supplementation with soy isoflavones and/or Glu may improve growth performance or immune function. Six experimental treatments were used. A high-protein diet containing no synthetic AA and a medium-protein diet containing less SBM and synthetic Lys, Met, and Thr, were formulated. Four low-protein diets containing less SBM and synthetic Lys, Met, Ile, Thr, Trp, and Val, that were arranged in a 2 × 2 factorial with 0 or 0.4% soy isoflavones and 0 or 2.0% Glu were also formulated. In experiment 1, a total of 240 pigs (initial weight: 9.65 ± 0.87 kg) were allotted to the six diets in a randomized complete block design with eight replicate pens per treatment. Pigs were fed a 5-phase feeding program (11 to 125 kg), and growth performance, carcass characteristics, and blood parameters were determined. One pig per pen was euthanized at the conclusion of phase one to determine abundance of genes associated with intestinal barrier function. In experiment 2, 60 pigs (initial weight: 17.8 ± 0.9 kg) were allotted to the six phase one diets used in experiment 1, in a randomized complete block design and housed in metabolism crates for urine and fecal collection. Results of experiment 1 indicated that average daily gain, average daily feed intake, and gain to feed ratio for the entire experimental period

were not affected by diet. Hot carcass weight was greater ($P < 0.05$) for pigs fed the medium-protein diet compared with pigs fed the low-protein diet containing soy isoflavones and Glu. Carcass backfat tended to increase (linear, $P = 0.075$) in pigs fed the low protein diets. Plasma urea nitrogen decreased (linear, $P < 0.001$) as protein was reduced. In experiment 2, daily nitrogen intake, urinary nitrogen excretion, absorbed nitrogen, and retained nitrogen (g/d) were reduced (linear; $P < 0.001$) as dietary protein decreased, but the digestibility of nitrogen was not affected. In conclusion, overall growth performance, immune responses, and abundance of gut-protective proteins were not affected by protein level, but carcass lean was reduced, and backfat tended to be thicker in pigs fed a diet containing less protein than in pigs fed the high-protein diet. Additionally, no effects of adding soy isoflavones or extra nitrogen on pig growth performance were observed, but reducing dietary protein reduced daily nitrogen retention.
Keywords: amino acids, carcass composition, growth performance, nitrogen balance, soybean meal

Abbreviations: AA, amino acids; ADFI, average daily feed intake; ADG, average daily gain; ATTD, apparent total tract digestibility; BW, body weight; cDNA, deoxyribonucleic acid; G:F, gain to feed ratio; IFN-γ, interferon-gamma; IL, Interleukin; MUC-2, mucin 2; PUN, plasma urea nitrogen; RNA, ribonucleic acid; SBM, soybean meal; TNF-α, tumor necrosis factor alpha; ZO-1, Zona occludins.

Introduction

Soybean meal (**SBM**) is the major source of amino acids (**AA**) and energy in diets for swine throughout the world (NRC, 2012). However, to decrease nitrogen excretion, formulators have

tried to reduce dietary protein by decreasing SBM inclusion and increasing synthetic AA usage in diets (Cappelaere et al., 2021). Nonetheless, lowering dietary protein does not always result in the same growth performance as obtained by feeding high-protein diets, even when the requirement for all digestible indispensable AA is met (Tuitoek et al., 1997; Wang et al., 2019). Dispensable AA are needed for protein synthesis as well as metabolic processes such as antioxidative responses and development of the immune system (Wu, 2009). Therefore, reducing dietary protein may compromise the availability of non-essential AA, resulting in a need for extra nitrogen to support de novo synthesis of those AA (Gloaguen et al., 2014; Wang et al., 2018). Additional sources of nitrogen, such as free AA (e.g., Glu) may provide extra dietary nitrogen to maintain growth performance of pigs fed low-protein diets and also contribute to intestinal homeostasis (Stoll et al., 1999; Hou and Wu, 2018).

Decreasing SBM inclusion may also reduce the intake of soybean-derived bioactive compounds, such as soy isoflavones, which are absorbed in the small intestine and act as immunomodulators that enhance immune function, resulting in better growth performance of the pigs (Walsh et al., 2009; Smith and Dilger, 2018). Indeed, Guay et al. (2006) and Kerr et al. (1995) indicated that reducing SBM in the diet may not only affect nitrogen metabolism but also reduce the supply of bioactive compounds that are needed to support immunity and intestinal health of pigs. However, no data are available to confirm this hypothesis. Therefore, two experiments were conducted to test the hypothesis that negative effects on growth performance, carcass composition, nitrogen balance, and immunity of reducing dietary protein by decreasing SBM and increasing synthetic AA in diets may be overcome by adding additional nitrogen from Glu and/or soy isoflavones to diets for growing-finishing pigs.

Materials and Methods

Two experiments were conducted, and the protocols 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 used in both experiments were the offspring of Line 800 boars mated to Camborough females (Pig Improvement Company, Hendersonville, TN, USA).

Experimental diets, animals, and housing

The same batches of corn and SBM were used to produce all six diets in both experiments (Table 5.1). In experiment 1, pigs from 9 to 125 kg were fed diets using a five-phase feeding program. A high-protein diet with no synthetic AA and a medium-protein diet with inclusion of synthetic Lys, Met, and Thr were formulated within each phase. Four low-protein diets containing less SBM and six synthetic AA (i.e., Lys, Met, Ile, Thr, Trp, and Val) were also prepared using a 2 × 2 factorial arrangement with two levels of isoflavones (i.e., 0 or 0.4% isoflavones) and two levels of extra nitrogen (i.e., 0 or 2% $_{L}$-Glu). Diets were fed in five phases, with phase one diets being provided for 21 days (nursery phase; 9 to 20 kg), phase two for 28 days (early grower phase; 20 to 45 kg), phase three for 28 days (late grower phase; 45 to 70 kg), phase four for 28 days (finisher phase; 75 to 100 kg), and phase five for 28 d (late finisher phase; 100 to 125 kg). Therefore, a total of 30 diets were formulated for the five phases (Tables 5.2, 5.3, and 5.4). All diets were formulated to meet or exceed the estimated requirements for standardized ileal digestible AA, vitamins, and minerals for 10 to 25, 25 to 50, 50 to 75, 75 to 100, and 100 to 135 kg pigs, respectively (NRC, 2012).

In experiment 1, 240 pigs [initial body weight (**BW**) 9.65 ± 0.87 kg] were allotted to one of six dietary treatments using a randomized complete block design with two blocks of 24 pens in each block. Therefore, a total of 48 pens were used, with five pigs per pen (three females and

two barrows) and eight replicate pens per treatment. The weaning group was the blocking factor. In phase one, pigs were housed in two different environmentally controlled barns in pens with fully slatted plastic floors, equipped with a feeder and a nipple drinker. In phases two to five, pigs were housed in an environmentally controlled grower facility with partially slatted concrete floors, a nipple waterer, and a stainless-steel feeder. Pens measured 1.83 × 2.59 m, which resulted in a floor space of 1.18 m^2 for each pig. Feed was provided on an *ad libitum* basis and water was available at all times. All diets were fed in mash form for all five phases.

In experiment 2, sixty growing pigs with an initial BW of 17.75 ± 0.91 kg were allotted to a randomized complete block design with two blocks of 30 pigs and with five pigs per diet in each block for a total of 10 replicate pigs per diet. The two blocks contained pigs from two weaning groups that were weaned 14 days apart. The weaning group was the blocking factor. Pigs were housed individually in metabolism crates (0.71 × 0.84 m) that were equipped with a self-feeder, a nipple waterer, a fully slatted floor, and a screen floor to allow for total collection of fecal materials. The phase one diets from experiment 1 were also fed to pigs in experiment 2. The daily feed allowance was calculated as 3.2 times the estimated maintenance requirement for metabolizable energy (i.e., 197 kcal/kg × $BW^{0.60}$; NRC, 2012), and was provided each day in two equal daily meals at 0700 and 1600 hours. Addition of feed to each pig was recorded daily. Water was available at all times throughout the experiment.

Sample and data collection

A sample of all diets was collected at the time of diet mixing and used for chemical analysis. In experiment one, individual pig weights were recorded at the beginning of the experiment and at the conclusion of each phase. Feed in the feeders was checked daily. Feed allowance was recorded daily, and the weight of feed left in the feeder was recorded at the end of

each phase to calculate pen feed disappearance. If a pig was removed from a pen during the experiment, the weight of all pigs in the pen and of the feed in the feeder at the time of removal were recorded, which allowed for calculation of individual feed intake of pigs within the pen (Lindemann and Kim, 2007). Data collected for pig weights and feed allowance were summarized and used to calculate average daily gain (**ADG**), average daily feed intake (**ADFI**), and gain-to-feed ratio (**G:F**) for each pen and treatment group. Data were summarized for each phase and for the entire experiment.

At the conclusion of phase one, the pig in each pen with BW closest to the pen average was identified (four barrows and four gilts per treatment), and two blood samples were collected from the jugular vein of each selected pig via vena puncture. The first blood sample was collected in heparinized vacutainers and centrifuged (Model Sorvall ST8, Thermo Fisher Scientific, Waltham, MA, USA) at room temperature at 4000 × g for 13 min to recover the plasma, which was stored at −20 °C until analysis for plasma urea nitrogen (**PUN**), albumin, and total plasma protein using a Beckman Coulter Clinical Chemistry AU analyzer (Beckman Coulter, Inc., Brea, CA, USA). The second blood sample was collected in vacutainers with ethylenediaminetetraacetic acid and stored on ice immediately after collection. Samples were centrifuged at room temperature at 1000 × g for 30 min to recover the plasma, which was stored at −20 °C until analysis for cytokines: [e.g., interferon-gamma (**IFN-γ**), interleukin- (**IL-**) 1α, IL-1β, IL-1 receptor antagonist, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12, IL-18, and tumor necrosis factor-α (**TNF-α**)]. Cytokines were analyzed using a MILLIPLEX kit (EMD Millipore Corporation, Billercia, MA, USA) in a MagPix instrument with ProcartaPlex- multiplex technology (R&D Systems, Inc., Minneapolis, MN, USA). The pig from which blood was collected was then euthanized via captive bolt penetration. Ileal mucosa samples were collected

for analyzing gene abundance of gut-protective proteins. Samples, approximately 2 to 3 cm long, were collected approximately 80 cm anterior to the ileal-cecal junction. Samples were washed with phosphate-buffered saline, scraped gently, snap-frozen in liquid nitrogen, and stored at −80 °C until analyzed for ribonucleic acid (**RNA**) extraction and quantitative reverse-transcription polymerase chain reaction. 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 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 100ng/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 quantitative reverse-transcription polymerase chain reaction analysis which was performed using 4µL of diluted cDNA and 6µL 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 (Gonzalez et al., 2013) and hypoxanthine-guanine phosphoribosyl transferase (Nygard et al., 2007) were used to normalize the abundance of tested genes. The geometric mean of the internal control genes was determined for target normalization and for the relative abundance of specified genes. Abundance of the following gut-protective protein target genes were measured: occludin, claudin-1, zonula occludens-1 (**ZO-1**), and mucin

2 (**MUC-2**). Because one pig per pen was euthanized at the end of phase one, only four pigs per pen were used in phases 2 to 5. Consequently, growth performance data from pigs in phase one were excluded from the final analysis using the partitioning method (Lee et al., 2016).

At the conclusion of phase 5, one pig per pen was harvested at the Meat Science Laboratory at the University of Illinois. Pigs were held in lairage for 16 h before slaughter. During this time, all pigs were provided ad libitum access to water, but no feed was available during this time. Prior to selecting the pigs for slaughter, pens within treatment were randomly assigned to deliver either a gilt or a barrow for slaughter, in such a way that four gilts and four barrows were selected from each treatment. The pig with the assigned sex that had a body weight closest to the pen average was then identified. Immediately prior to slaughter, pigs were weighed to determine the ending live weight. Head-to-heart electrical stunning was used to immobilize pigs before slaughter by exsanguination. Approximately 45 min postmortem, hot carcass weights were recorded. Carcasses were split down the midline and chilled for 24 h at 4 °C prior to loin quality evaluation on the left side of the carcass.

Loin quality was evaluated on the cut surface of the longissimus thoracis muscle between the 10^{th} and 11^{th} rib following oxygenation of myoglobin for approximately 20 min at 4 °C. Fat depth was measured between the 10^{th} and 11^{th} ribs at three-fourths distance of the longissimus thoracis muscle from the dorsal side of the vertebral column. Instrumental color on the longissimus muscle and fat (L*, a*, and b*; CIE, 1978) was measured using a Minolta CR-400 Chroma meter (Minolta Camera Co., Ltd, Osaka, Japan) with a D65 light source and a 10° observer angle with an aperture size of 8 mm. Ultimate pH of the longissimus thoracis was measured using a handheld pH meter fitted with a Hanna glass electrode calibrated at 4 °C (REED SD-230 Series pH/ORP Datalogger, 0.00 to 14.00 ph/0-199 mV; Hanna FC200B

electrode). Visual color and marbling scores (National Pork Producers Council, 1999) and subjective firmness scores (National Pork Producers Council, 1991) were determined by a single, trained technician. The loin eye area was measured by tracing the surface of the longissimus muscle on a double-matted acetate paper. Tracings were later measured twice using a digitizer tablet (Wacom, Vancouver, WA) and Adobe Photoshop CS6, and the average of the two measurements was recorded (Gaffield et al., 2022). Drip loss was measured according to Boler et al. (2011). Carcass yield (%) was calculated by dividing hot carcass weight by ending live weight and multiplied by 100. Fat-free lean (%) was calculated using the following equation (Lowell et al., 2018):

$$[8.588 + (0.465 \times \text{hot carcass weight}_{lbs}) - (21.896 \times \text{backfat thickness}_{in}) + (3.005 \times \text{loin eye area}_{in^2})]/\text{hot carcass weight}_{lbs}) \times 100.$$

Bellies were measured for length, width, thickness and flop distance (Kyle et al., 2014; Gaffield et al., 2022). Fresh belly thickness was measured at eight individual locations across the belly. To determine thickness, a probe was inserted through the lean side of each belly. Measurements 1 through 4 were taken along the dorsal edge of the belly, beginning at the anterior end, by pressing a sharpened ruler through the belly while it was positioned skin-side down. Measurements 5 through 8 were obtained in the same way along the ventral edge, also starting from the anterior end. Additionally, flop distance was measured by placing the bellies, skin side down, over a bar. The distance was then measured between the inside edges of the bellies. A larger flop distance signals increased belly firmness, while a reduced flop distance signals decreased firmness.

In experiment two, pigs were fed experimental diets for 12 days, the initial five days of the experiment being the adaptation period to the diet, whereas fecal and urine materials were

collected from the feed provided during the following four days according to standard procedures for the marker-to-marker approach (Adeola, 2001). Indigo carmine was used to mark the initiation of feces collection and was included in the morning meal on day 6. Fecal collection ceased when the second marker, ferric oxide, which was included in the morning meal on day 10, appeared in the feces. Urine was collected in urine buckets containing 50 mL 6 *N* HCl as a preservative and placed under the metabolism crates. Orts were collected daily prior to feeding the morning meal, pooled for the duration of the collection period, dried in a 50 °C forced air-drying oven, and weighed at the conclusion of the experiment to determine feed intake from day six to day 10. During the collection period, feces were collected twice daily and stored at −20 ◦C immediately after collection. Collected urine was weighed and mixed, and 20% was stored at −20 °C immediately after collection. At the conclusion of the experiment, fecal samples were dried at 65 °C in a forced air oven (Thermo Fisher Scientific Inc.; model Heratherm OMH750, Waltham, MA, USA) and finely ground using a RRH-500 G stainless steel swing-type mill grinder (Rancho Cucamonga, CA, USA) prior to chemical analysis. Urine samples were thawed, mixed, subsampled, and stored at −20 °C until analysis for nitrogen.

Chemical analyses of diets and ingredients

In experiment 1, within each dietary phase, multiple one-ton batches of diets were mixed. Each batch of diet was subsampled, and at the end of each phase, 1 kg of diet samples from each batch were mixed within diet and phase. A subsample of the mixed samples was then finely ground using a RRH-500G stainless steel swing-type mill grinder, prior to chemical analysis. Diets and ingredients were analyzed for dry matter (method 930.15; AOAC Int., 2019) and ash (method 942.05; AOAC Int., 2019). The concentration of nitrogen was analyzed by combustion (method 990.03; AOAC Int., 2019) using a LECO FP628 analyzer (LECO Corp., Saint Joseph,

MI, USA) with subsequent calculation of crude protein as nitrogen × 6.25. Diets were also analyzed for AA 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]. Soybean meal was analyzed for trypsin inhibitors (method Ba 12-75; AOCS, 2017).

In experiment 2, diets were analyzed for dry matter, ash, gross energy, and AA as described for experiment 1. The concentration of nitrogen in diets, feces, and urine was analyzed using the Kjeldahl method (method 984.13; AOAC Int., 2019) on a KjeltecTM 8400 (FOSS Inc., Eden Prairie, MN, USA). Crude protein was calculated as analyzed nitrogen × 6.25.

Calculation and statistical analysis

For experiment one, data were summarized for each treatment group and ADG, ADFI, and G:F were calculated. In experiment two, the apparent total tract digestibility (**ATTD**) of nitrogen in each experimental diet and nitrogen retention for each pig were calculated as described by Pedersen et al. (2007). The biological value of nitrogen in diets was calculated using the following equation (Rojas and Stein, 2013):

Biological value, % = (retained nitrogen ÷ absorbed nitrogen) × 100,

where retained nitrogen and absorbed nitrogen are expressed in g/d. Retained nitrogen was calculated by subtracting fecal and urine nitrogen outputs from nitrogen intake, and absorbed nitrogen was calculated by subtracting fecal nitrogen output from nitrogen intake.

Data were analyzed using the MIXED procedure of SAS, and for experiment one, pen was the experimental unit for the growth performance analysis, and pig for the carcass traits and blood characteristics analyses, while for experiment two, pig was the experimental unit for all analyses. Model assumptions on the residuals were confirmed using the MIXED procedure and the Brown-Forsythe test of the GLM procedure of SAS (SAS Inst. Inc., Cary, NC, USA). The MIXED procedure in SAS was used to generate externally studentized residuals, and outliers were defined as observations having residuals greater than 3 or less than −3. For blood analyses one outlier was removed from the high-protein, the medium-protein diet, and the low-protein diet containing L-Glu, and two outliers from the low-protein diet containing isoflavones and L-Glu. The statistical model for experiments one and two included diet as the fixed effect and block as the random effect. Least square means were calculated, and if the model was significant, means were separated using the PDIFF option with Tukey's adjustment. Linear effects of reducing dietary protein were determined using orthogonal polynomial CONTRAST statements. Statistical significance and tendencies were considered at $P < 0.05$ and $0.05 \leq P < 0.10$, respectively.

Results

Analyzed nutrient composition in all diets are presented in tables 5.5, 5.6 and 5.7. In both experiments, all pigs consumed their diets throughout the experiment without apparent problems.

Growth performance

Regardless of phase, the average final BW and the ADG were not affected by treatments (Table 5.8). In phase three, ADFI was reduced (linear, $P = 0.039$) as dietary protein was reduced, but ADFI of pigs was not different among dietary treatments in the other phases. Gain to feed

was also not affected by dietary treatments except that G:F of pigs fed the low-protein diet containing L-Glu was greater ($P < 0.05$) in phase one compared with pigs fed the high-protein diet, but no difference was observed among the other diets.

Ending live weight, carcass yield, drip loss, most loin quality traits and backfat color were not affected by dietary treatments (Table 5.9). Hot carcass weight was greater ($P < 0.05$) for pigs fed the medium-protein diet compared with pigs fed the low-protein diet containing soy isoflavones and L-Glu, but no differences were observed among the other diets. Back fat thickness tended to increase (linear, $P = 0.075$) as protein decreased in the diets. Loin eye area was greater ($P < 0.05$) for pigs fed the high- or medium-protein diets compared with pigs fed the low-protein diet containing soy isoflavones and L-Glu. Loin eye area tended to be reduced (linear, $P = 0.058$) as dietary protein decreased. The loin color was lighter (L*; $P < 0.05$) when pigs were fed a low-protein diet compared with the medium-protein diet, but there were no differences among the other diets. The loin lightness (L*) tended to increase (linear, $P = 0.090$) as dietary protein was reduced. The loin of pigs fed the four low-protein diets had more yellow color (b*; $P < 0.05$) compared with pigs fed the medium-protein diet.

Concentrations of PUN in pigs were reduced (linear, $P < 0.001$; Table 5.10) by decreasing dietary protein, but there were no effects of adding isoflavones or L-Glu to the low-protein diets on PUN. Total protein in blood decreased (linear, $P = 0.029$) when protein was reduced in the diets. Serum concentrations of cytokines, TNF-α, and abundance of gut protective genes were not different among treatments.

Nitrogen balance

Pigs remained healthy throughout the experiment, and no feed refusals were observed. Daily feed intake was not affected by dietary treatments (Table 5.11). Daily nitrogen intake,

nitrogen excretions in feces and urine, absorbed nitrogen, and retained nitrogen (g/d) were reduced (linear, $P < 0.001$) by reducing dietary protein, but the ATTD of nitrogen was not affected by dietary protein. Nitrogen retention (% of intake) and biological value increased (linear, $P < 0.001$) as dietary protein was reduced. Fecal excretion was not influenced by the diet.

Discussion

The analyzed values for crude protein and total AA in experimental diets in all phases were in close agreement with formulated values, which indicates correct mixing of diets. Likewise, the analyzed concentrations of nutrients in corn and SBM were consistent with reported data (NRC, 2012).

Growth performance

Reducing crude protein by reducing SBM and increasing synthetic AA in corn-SBM diets is widely used in the swine industry to reduce nitrogen excretion (Wang et al., 2018; Cappelaere et al., 2021). Due to the availability of free AA, swine diets can be formulated to meet individual AA requirements by using synthetic AA instead of intact protein sources such as SBM (Holen et al., 2022). However, reducing dietary protein by reducing SBM in the diets and supplementing with synthetic AA to meet the requirements for indispensable AA does not always yield the same growth performance in pigs (Tuitoek et al., 1997; Soto et al., 2019). Indeed, reduction of dietary protein by more than four percentage units when SBM is replaced by synthetic AA in growing-finishing diets may reduce pig growth performance due to an AA imbalance (Wang et al., 2018).

In addition to supplying indispensable AA to swine diets, SBM also contains bioactive compounds, such as isoflavones, that have been associated with antioxidant and immunomodulatory functions in pigs (Smith and Dilger, 2018). Therefore, health-challenged

pigs fed diets with high inclusion of SBM have less reduction in growth performance compared with pigs fed diets with reduced SBM inclusion (Rochell et al., 2015). This may indicate that the contribution of SBM to pig performance may not always be associated only with indispensable AA because removing non-AA components in SBM from low-protein diets may also limit functions that support normal growth (Boyd et al., 2024).

The observation that G:F ratio was less in phase one for pigs fed the high-protein diet likely reflects the greater inclusion of SBM in the nursery diet. Antinutritional factors in SBM, including antigens and oligosaccharides, can reduce growth performance and increase diarrhea in weanling pigs (Liying et al., 2003; Navarro et al., 2017), which is why diets for weanling pigs typically contain limited amounts of SBM. Monosodium glutamate or L-Glu supplementation has been reported to improve growth performance of young pigs (Rezaei et al., 2013) by enhancing villus height, antioxidant capacity, and growth performance, while reducing diarrhea (Gatel and Guion, 1990; Wu et al., 2009; Rezaei et al., 2013; Lin et al., 2014). Therefore, the observation that pigs fed the low-protein diets supplemented with synthetic AA and 2% L-Glu had greater G:F in phase one may reflect the removal of excess SBM-derived antinutritional factors and a need for additional nitrogen. However, L-Glu did not appear to influence the immune status of pigs during phase one, indicating that the need for extra nitrogen wa sprimarly a need for synthesis of dispensable AA needed for more protein synthesis.

The lack of differences in ADG, ADFI, and G:F among treatments for the overall experiment is consistent with data indicating that low-protein diets fed to growing pigs did not affect growth performance (Kerr et al., 1995; Le Bellego et al., 2001; Cristobal et al., 2025a). Under the conditions of this experiment, the potential influence of soybean bioactive compounds or the effect of L-Glu as an extra source of nitrogen after the phase 1 period on overall growth

performance of pigs also was minimal. This observation is consistent with data indicating that increasing dietary isoflavones above what is naturally present in corn-SBM diets does not improve growth performance, carcass traits, or meat quality (Payne et al., 2001; Boyd et al., 2024). Therefore, the hypothesis that the negative effects of reducing dietary protein on pig growth performance, by decreasing SBM and increasing synthetic AA, may be mitigated by supplying an additional nitrogen sources or soy isoflavones was rejected.

Pigs fed diets with reduced-protein and supplemented with synthetic AA may have reduced carcass leanness and increased back fat deposition (Kerr et al., 1995; Figueroa et al., 2002; Deng et al., 2009; Boyd et al., 2024). The observation that there was a tendency for an increase in fat thickness and a reduction in loin eye area as dietary protein was reduced in the diets, is likely due to the fact that pigs fed diets containing intact protein and synthetic AA may have changed AA metabolism, because free AA, are rapidly absorbed whereas intact proteins release AA at a slow rate (Yen et al., 2004; Zhang et al., 2022). This may result in free AA arriving at the cells before AA from intact protein arrive, leading to greater deamination and usage of the carbon skeletons of deaminated AA to synthesized energy that is deposited as fat (Wang et al., 2018; 2021). Low-protein diets may also supply fewer AA in excess of requirements, thus reducing the energy required for deamination and increasing energy available to the pig, which in turn will further increase fat deposition (Smith et al., 1999; Cristobal et al., 2025a). However, the tendency for a reduced loin eye area indicates that pigs fed the low-protein diets may not have had sufficient AA to maximize protein synthesis. Whether this is due to the deamination of some of the synthetic AA or because of an insufficient supply of dispensable AA remains unclear. Therefore, the increased fat deposition in pigs fed the low-protein diets observed in this experiment may be the combined result of changes in AA metabolism and a

reduced capacity for lean tissue accretion, leading to a limited supply of AA for protein synthesis and a greater lipid deposition.

Blood parameters and abundance of intestinal gut protective proteins

The observed reduction of PUN that was onserved as dietary crude protein was reduced is in agreement with reported data (Limbach et al., 2021; Duarte et al., 2024), and this reduction indicates that excess AA in the high-protein diet were deaminated, resulting in greater urea synthesis. In contrast, reducing dietary protein, while supplementing with synthetic AA likely provided a more balanced supply of AA, decreasing the need for deamination and urea synthesis. Therefore, the reduction in PUN indicates that pigs fed the low-protein diets were more efficient in utilizing dietary AA and reducing nitrogen excretion (Yue and Qiao, 2008; Wang et al., 2018).

Plasma protein and albumin values were in agreement with values obtained by Che et al., (2017) and Cristobal et al. (2025a). However, total plasma protein decreased as dietary protein was reduced even though albumin did not differ among diets. This observation indicates that reducing dietary protein may influence blood protein levels without compromising albumin synthesis. The lack of differences in albumin concentrations may indicate that all diets provided an adequate supply of indispensable AA for protein synthesis, because albumin synthesis is mainly controlled by AA availability and supply (Rothschil et al., 1988). Therefore, the reduction in total plasma protein is more likely a result of decreased nitrogen intake rather than an indication of inadequate AA supply of the diet.

Inflammation and infection stimuli are associated with innate immune cells, which release pro- or anti-inflammatory cytokines (Burger and Dayer, 2002). The observed lack of effect of dietary protein on pro- or anti-inflammatory cytokine levels indicates that under the conditions of this experiment, a reduction in dietary protein from 24 to 17% did not impact the

immune system. Although reduced protein diets have been associated with improved gut function and decreasing post-weaning diarrhea in young pigs (Limbach et al., 2021; Duarte et al., 2024), responses may depend on the presence of intestinal stress or infection rather than the concentration of the protein in the diet. Additionally, pigs were two weeks post-weaning when the current experiment started, and at that time, there is usually less post-weaning diarrhea than during the immediate post weaning period.

The mucosa lining of the gastrointestinal tract represents an assembly of epithelial tissue, immune cells, and microbiota communities (Kissoon-Singh et al., 2013). Tight junctions are essential components of the intestinal epithelium because they regulate the selective permeability of the gut barrier, and tight junction integrity is critical for preserving epithelial function, supporting immune defense, and preventing disease-associated barrier dysfunction (Nusrat et al., 2000). Transmembrane proteins, such as occludin and claudin, as well as cytoplasmic plaque protein ZO-1, and MUC-2 mucin, are among the tight junction proteins that are highly related to gut health and the protective barrier (Mitic and Anderson, 1998).

Cytokines play an important role in the intestinal epithelium by regulating immune activity and maintaining barrier integrity through the tight junction function (Nusrat et al., 2000). When cytokine signaling is altered, epithelial permeability can increase, exposing the animal to pathological conditions. However, the observation that the concentration of cytokines and the abundance of the gut-protective genes were not influenced by dietary treatments indicates that pigs were not under inflammatory or physiological stress, and that the high- or medium protein-diets did not disrupt tight junction function or affect epithelial barrier integrity. This observation is consistent with data from pigs fed diets with different protein levels (Limbach et al., 2021). It is also possible that the reduction of crude protein in the present experiment was not sufficient to

influence the regulation of gut-protective proteins. Greater reductions in dietary protein may be required to observe improvements in the epithelial barrier response (Limbach et al., 2021). Therefore, the hypothesis that addition of soy bioactive compounds or an extra source of nitrogen will improve the immune response of pigs fed low-protein diets was rejected.

Nitrogen balance

The observation that reduction of dietary protein decreased nitrogen excretion in feces and urine is in agreement with results of experiments with pigs fed low-protein diets (Cristobal et al., 2025b; Ibagon et al., 2026). Because all diets were formulated to meet the standardized ileal digestible indispensable AA requirements, it was expected that pigs received an equal supply of digestible AA to support protein synthesis. However, the observation that nitrogen retention measured as grams per day decreased in pigs fed the low-protein diet compared with pigs fed the medium or high-protein diets may be due to the imbalance in the rate of absorption between free AA and protein-bound AA (Zhang et al., 2022). This observation is in agreement with the observation in experiment 1, that pigs fed the low-protein diets tended to have greater back fat thickness and reduced loin eye area. The implication of this observation is that a reduction of SBM in diets may result in less protein synthesis even if overall ADG is not reduced. The fact that this reduction in daily nitrogen retention was not ameliorated by the inclusion of L-Glu in the diets indicates that the reduced nitrogen retention was not caused by a limitation in dispensable AA. However, the reduction in nitrogen retention, measured in grams per day, in pigs fed low-protein diets was in agreement with previous data (Canh et al., 1998; Portejoie et al., 2004; Yen et al., 2004; Cristobal et al., 2025b).

Utilization of dietary AA is more efficient with low-protein than with high-protein diets because less excess AA are provided. Therefore, the observation that pigs fed the high protein

diets had reduced nitrogen retention as a percentage of nitrogen intake as well as reduced biological value compared with pigs fed the low-protein diets was expected and in agreement with reported data (Cristobal et al., 2025b; Ibagon et al., 2026).

Conclusion

Reducing dietary crude protein from 24 to 17% while adding synthetic AA and L-Glu, maintained growth performance, immune and epithelial barrier function in weanling pigs. However, reducing dietary protein reduces nitrogen absorption and retention measured in grams per day, and increases fat deposition. Although PUN decreased as crude protein was reduced, indicating improved nitrogen utilization, neither the additional nitrogen source nor soybean isoflavones improved growth or carcass characteristics under the conditions of this experiment. Likewise, the lack of differences in gut-protective gene expression indicates that the reduction of crude protein in the current experiment was insufficient to improve or modulate responses associated with intestinal barrier function.

Tables

Table 5.1. Analyzed nutrient composition of ingredients as-fed basis[1]

Item,	Corn	Soybean meal
Dry matter, %	87.46	89.66
Ash, %	1.26	6.41
Crude protein, %	6.79	46.12
Acid hydrolyzed ether extract, %	3.25	0.51
Trypsin inhibitors units/mg	-	1.12
Starch, %	69.46	9.79
Insoluble dietary fiber, %	10.10	17.80
Soluble dietary fiber, %	0.70	2.10
Total dietary fiber, %	10.80	19.90
Indispensable amino acids, %		
Arg	0.29	3.25
His	0.19	1.30
Ile	0.23	2.32
Leu	0.65	3.60
Lys	0.25	3.00
Met	0.15	0.65
Phe	0.29	2.43
Thr	0.22	1.79
Trp	0.04	0.64

Table 5.1. (Cont.)

Val	0.31	2.36
Dispensable amino acids, %		
Ala	0.43	2.01
Asp	0.44	5.32
Cys	0.15	0.66
Glu	1.07	8.50
Gly	0.26	1.95
Pro	0.49	2.24
Ser	0.26	1.97
Tyr	0.15	1.57
Total amino acids, %	5.87	45.56

Table 5.2. Ingredient compositions of phase one (9 to 20 kg) experimental diet as-fed basis

Item, %	High-protein	Medium-protein	Low-protein			
			-	Isoflavones	Glu	Isoflavones + Glu
Corn	52.35	64.07	69.50	69.10	67.50	67.10
Soybean meal, 46% protein	43.00	30.50	24.50	24.50	24.50	24.50
Soybean oil	2.00	2.00	2.00	2.00	2.00	2.00
L-Ile, 98% Ile	-	-	0.01	0.01	0.01	0.01
L-Lys·HCl, 78% Lys	-	0.39	0.60	0.60	0.60	0.60
DL-Met, 98% Met	0.03	0.13	0.19	0.19	0.19	0.19
L-Thr, 98% Thr	-	0.11	0.20	0.20	0.20	0.20
L-Trp, 98% Trp	-	-	0.02	0.02	0.02	0.02
L-Val, 98% Val	-	-	0.10	0.10	0.10	0.10
L-Glu, 98% Glu	-	-	-	-	2.00	2.00
Isoflavone product[1]	-	-	-	0.40	-	0.40
Dicalcium phosphate	0.90	1.10	1.20	1.20	1.20	1.20
Limestone	0.82	0.80	0.78	0.78	0.78	0.78
Sodium chloride	0.40	0.40	0.40	0.40	0.40	0.40
Vitamin-mineral premix[2]	0.50	0.50	0.50	0.50	0.50	0.50

[1]Chongqing Honoroad Animal Health Co., Ltd., Hechuan, Chongqing, China. Total gluscosides: 390,000 μg/g.

[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 5.2. (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 5.3. Ingredient compositions of experimental diet phase two and phase three as-fed basis[1]

	Phase 2: Early grower (20 to 45 kg)						Phase 3: Grower (45 to 70 kg)					
Item, %	High-protein	Medium-protein	Low-protein				High-protein	Medium-protein	Low			
			-	Isoflavones	Glu	Isoflavones + Glu			-	Isoflavones	Glu	Isoflavones + Glu
Corn	63.62	73.39	79.16	78.76	77.16	76.76	69.10	76.64	82.61	82.21	80.61	80.21
Soybean meal, 46% protein	32.75	22.40	16.00	16.00	16.00	16.00	27.50	19.50	13.00	13.00	13.00	13.00
Soybean oil	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
L-Ile, 98% Ile	-	-	0.03	0.03	0.03	0.03	-	-	0.02	0.02	0.02	0.02
L-Lys·HCl, 78% Lys	-	0.32	0.53	0.53	0.53	0.53	-	0.25	0.45	0.45	0.45	0.45
DL-Met, 98% Met	-	0.06	0.13	0.13	0.13	0.13	-	0.02	0.09	0.09	0.09	0.09
L-Thr, 98% Thr	-	0.08	0.17	0.17	0.17	0.17	-	0.05	0.14	0.14	0.14	0.14
L-Trp, 98% Trp	-	-	0.04	0.04	0.04	0.04	-	-	0.03	0.03	0.03	0.03
L-Val, 98% Val	-	-	0.09	0.09	0.09	0.09	-	-	0.05	0.05	0.05	0.05
L-Glu, 98% Glu	-	-	-	-	2.00	2.00	-	0.00	-	-	2.00	2.00

Table 5.3. (Cont.)

Isoflavone product[1]	-	-	-	0.40	-	0.40	-	-	-	0.40	-	0.40
Dicalcium phosphate	0.94	1.10	1.20	1.20	1.20	1.20	0.75	0.94	1.00	1.00	1.00	1.00
Limestone	0.79	0.75	0.75	0.75	0.75	0.75	0.75	0.70	0.71	0.71	0.71	0.71
Sodium chloride	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Vitamin-mineral premix[2]	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50

[1]Chongqing Honoroad Animal Health Co., Ltd., Hechuan, Chongqing, China.

[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 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 5.4. Ingredient compositions of experimental diet phase four and phase five as-fed basis

	Phase 4: Early finisher (75 to 100 kg)						Phase 5: Finisher (100 to 135 kg)					
Item, %	High-protein	Medium-protein	Low-protein				High-protein	Medium-protein	Low			
			-	Isoflavones	Glu	Isoflavones + Glu			-	Isoflavones	Glu	Isoflavones + Glu
Corn	74.26	81.85	87.74	87.34	85.74	85.34	79.19	85.89	90.19	87.79	88.19	87.79
Soybean meal, 46% protein	22.50	14.50	8.00	8.00	8.00	8.00	17.75	10.70	6.00	6.00	6.00	6.00
Soybean oil	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
L-Ile, 98% Ile	-	-	0.04	0.04	0.04	0.04	-	-	0.01	0.01	0.01	0.01
L-Lys·HCl, 78% Lys	-	0.25	0.46	0.46	0.46	0.46	-	0.21	0.37	0.37	0.37	0.37
DL-Met, 98% Met	-	0.00	0.07	0.07	0.07	0.07	-	0.00	0.02	0.02	0.02	0.02
L-Thr, 98% Thr	-	0.05	0.15	0.15	0.15	0.15	-	0.05	0.12	0.12	0.12	0.12
L-Trp, 98% Trp	-	-	0.04	0.04	0.04	0.04	-	-	0.03	0.03	0.03	0.03
L-Val, 98% Val	-	-	0.06	0.06	0.06	0.06	-	-	0.02	0.02	0.02	0.02
L-Glu, 98% Glu	-	-	-	-	2.00	2.00	-	-	-	2.00	2.00	2.00

Table 5.4. (Cont.)

Isoflavone product[1]	-	-	-	0.40	-	0.40	-	-	-	0.40	-	0.40
Dicalcium phosphate	0.66	0.75	0.86	0.86	0.86	0.86	0.48	0.60	0.70	0.70	0.70	0.70
Limestone	0.68	0.70	0.68	0.68	0.68	0.68	0.68	0.65	0.64	0.64	0.64	0.64
Sodium chloride	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Vitamin-mineral premix[2]	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50

[1]Chongqing Honoroad Animal Health Co., Ltd., Hechuan, Chongqing, China.

[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 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 5.5. Analyzed nutrient composition of experimental diets for phase one, as-fed basis

	Phase 1: Nursery (10 to 20 kg)					
Item,	High-protein	Medium-protein	Low-protein			
			-	Isoflavones	Glu	Isoflavones + Glu
Dry matter, %	87.17	87.77	87.66	87.65	87.90	87.90
Ash, %	4.47	4.27	4.37	3.83	3.93	3.94
Crude protein, %	24.41	19.87	17.18	17.04	17.96	17.86
Indispensable amino acids, %						
Arg	1.60	1.22	1.07	1.10	1.00	1.02
His	0.67	0.46	0.43	0.44	0.41	0.42
Ile	1.13	0.84	0.76	0.76	0.73	0.74
Leu	1.93	1.55	1.39	1.40	1.36	1.37
Lys	1.43	1.37	1.33	1.32	1.31	1.31
Met	0.35	0.43	0.38	0.42	0.43	0.35
Phe	1.20	0.95	0.82	0.83	0.80	0.81
Thr	0.88	0.73	0.81	0.77	0.81	0.75
Trp	0.33	0.24	0.19	0.20	0.19	0.19
Val	1.22	0.97	0.88	0.90	0.87	0.88
Dispensable amino acids, %						
Ala	1.10	0.95	0.83	0.83	0.79	0.80
Asp	2.61	2.11	1.68	1.70	1.56	1.57
Cys	0.36	0.33	0.26	0.27	0.26	0.25

Table 5.5. (Cont.)

Glu	4.22	3.52	3.02	3.07	4.33	4.42
Gly	1.01	0.83	0.70	0.71	0.66	0.68
Pro	1.32	1.15	0.94	0.95	0.89	0.90
Ser	0.97	0.80	0.71	0.73	0.67	0.66
Tyr	0.83	0.67	0.57	0.58	0.53	0.55
Total amino acids, %	23.16	19.12	16.77	16.98	17.60	17.67

Table 5.6. Analyzed nutrient composition of experimental diets for phase two and phase three, as-fed basis

Item,	Phase 2: Early grower (20 to 45 kg)						Phase 3: Grower (45 to 70 kg)					
	High-protein	Medium-protein	Low-protein -	Isoflavones	Glu	Isoflavones + Glu	High-protein	Medium-protein	Low -	Isoflavones	Glu	Isoflavones + Glu
Dry matter, %	85.01	85.85	85.85	86.04	85.34	86.12	87.54	87.25	87.57	87.30	87.58	87.08
Ash, %	4.43	3.91	3.50	3.53	3.40	3.76	4.37	3.79	3.58	3.60	3.59	3.73
Crude protein, %	20.01	15.55	13.61	13.43	15.21	15.43	18.06	14.93	12.94	13.36	14.67	14.76
Indispensable amino acids, %												
Arg	1.34	0.98	0.75	0.78	0.80	0.87	1.12	0.87	0.67	0.73	0.69	0.68
His	0.55	0.44	0.35	0.35	0.35	0.36	0.53	0.43	0.36	0.37	0.36	0.38
Ile	0.90	0.66	0.54	0.53	0.54	0.53	0.85	0.67	0.53	0.56	0.55	0.58
Leu	1.75	1.43	1.22	1.18	1.18	1.20	1.67	1.44	1.22	1.28	1.23	1.27
Lys	1.14	1.1	1.09	1.11	1.09	1.1	1.01	0.96	0.9	0.92	0.91	1.01
Met	0.32	0.30	0.35	0.34	0.35	0.33	0.30	0.26	0.29	0.30	0.28	0.28
Phe	1.01	0.78	0.65	0.62	0.66	0.67	0.95	0.76	0.62	0.66	0.63	0.65

Table 5.6. (Cont.)

Thr	0.76	0.65	0.62	0.61	0.61	0.64	0.69	0.62	0.55	0.56	0.55	0.56
Trp	0.22	0.16	0.16	0.17	0.15	0.16	0.18	0.14	0.13	0.13	0.14	0.13
Val	1.02	0.74	0.68	0.67	0.68	0.65	0.93	0.75	0.64	0.69	0.66	0.65
Dispensable amino acids, %												
Ala	1.01	0.82	0.68	0.71	0.70	0.72	0.96	0.83	0.73	0.75	0.73	0.75
Asp	2.10	1.50	1.15	1.13	1.28	1.30	1.84	1.43	1.08	1.19	1.11	1.18
Cys	0.32	0.26	0.23	0.23	0.25	0.22	0.31	0.25	0.23	0.25	0.21	0.25
Glu	3.70	2.68	2.25	2.21	3.85	3.80	3.42	2.78	2.25	2.41	3.85	3.70
Gly	0.85	0.64	0.52	0.50	0.56	0.57	0.77	0.62	0.50	0.53	0.51	0.53
Pro	1.13	0.95	0.87	0.82	0.85	0.82	1.13	0.98	0.86	0.88	0.86	0.90
Ser	0.86	0.70	0.65	0.56	0.60	0.58	0.79	0.64	0.52	0.55	0.56	0.57
Tyr	0.68	0.51	0.41	0.41	0.46	0.47	0.53	0.43	0.40	0.39	0.37	0.35
Total amino acids, %	19.66	15.30	13.17	12.93	14.96	14.99	17.98	14.86	12.48	13.15	14.20	14.42

Table 5.7. Analyzed nutrient composition of experimental diets for phase four and phase five, as-fed basis

Item,	Phase 4: Early finisher (75 to 100 kg)						Phase 5: Finisher (100 to 125 kg)					
	High-protein	Medium-protein	Low-protein -	Isoflavones	Glu	Isoflavones + Glu	High-protein	Medium-protein	Low -	Isoflavones	Glu	Isoflavones + Glu
Dry matter, %	87.34	87.14	87.42	87.58	87.48	87.45	86.22	86.79	86.65	86.64	86.97	86.82
Ash, %	3.49	3.66	3.06	2.65	2.39	3.02	3.32	2.48	2.94	2.93	2.86	2.63
Crude protein, %	16.86	13.84	11.55	11.68	12.53	12.74	14.07	11.98	9.82	10.03	11.82	11.59
Indispensable amino acids, %												
Arg	1.00	0.78	0.53	0.54	0.52	0.53	0.84	0.63	0.50	0.53	0.52	0.51
His	0.47	0.38	0.30	0.30	0.30	0.31	0.42	0.33	0.28	0.30	0.29	0.29
Ile	0.75	0.57	0.45	0.46	0.46	0.49	0.63	0.48	0.40	0.41	0.40	0.39
Leu	1.56	1.32	1.07	1.07	1.07	1.08	1.34	1.12	1.03	1.05	1.04	1.00
Lys	0.86	0.84	0.8	0.81	0.79	0.83	0.73	0.68	0.65	0.65	0.64	0.65
Met	0.28	0.22	0.24	0.25	0.25	0.24	0.23	0.20	0.20	0.18	0.19	0.20
Phe	0.86	0.69	0.50	0.51	0.50	0.52	0.71	0.56	0.48	0.50	0.48	0.48

Table 5.7. (Cont.)

Thr	0.62	0.52	0.50	0.48	0.52	0.50	0.51	0.45	0.44	0.43	0.43	0.44
Trp	0.15	0.11	0.10	0.12	0.13	0.12	0.15	0.10	0.09	0.10	0.11	0.09
Val	0.83	0.65	0.53	0.56	0.55	0.56	0.71	0.56	0.47	0.48	0.47	0.46
Dispensable amino acids, %												
Ala	0.90	0.77	0.65	0.65	0.64	0.65	0.79	0.67	0.60	0.63	0.62	0.60
Asp	1.62	1.23	0.83	0.84	0.83	0.85	1.34	1.00	0.79	0.81	0.80	0.80
Cys	0.29	0.23	0.20	0.20	0.19	0.19	0.25	0.20	0.19	0.18	0.19	0.18
Glu	3.12	2.49	1.86	1.88	3.30	3.29	2.60	2.07	1.76	1.86	3.50	3.45
Gly	0.68	0.53	0.40	0.40	0.39	0.41	0.58	0.45	0.37	0.40	0.39	0.38
Pro	1.07	0.91	0.76	0.77	0.75	0.76	0.90	0.77	0.70	0.72	0.72	0.69
Ser	0.80	0.59	0.47	0.47	0.44	0.43	0.58	0.47	0.40	0.43	0.41	0.41
Tyr	0.50	0.39	0.32	0.30	0.29	0.30	0.47	0.36	0.29	0.28	0.30	0.29
Total amino acids	16.36	13.22	10.51	10.61	11.92	12.06	13.78	11.10	9.64	9.94	11.50	11.31

Table 5.8. Growth performance of pigs fed experimental diets, experiment 1[1]

Item	High-protein	Medium-protein	Low-protein				SEM[2]	*P*-value	Linearity[3]
			-	Isoflavones	Glu	Isoflavones + Glu			
Initial body weight, kg	9.67	9.61	9.64	9.68	9.65	9.64	-	-	-
Final body weight, kg									
Phase 1	20.46	20.97	20.69	21.32	21.11	21.11	0.65	0.943	0.730
Phase 2	44.16	44.88	43.13	45.08	44.88	43.86	1.12	0.807	0.644
Phase 3	72.29	73.21	69.59	71.51	71.86	68.84	1.93	0.341	0.330
Phase 4 and 5	138.49	140.07	137.77	135.94	138.21	134.99	2.02	0.536	0.920
Average daily gain, kg/d									
Phase 1	0.51	0.54	0.53	0.55	0.55	0.55	0.02	0.679	0.524
Phase 2	0.82	0.82	0.77	0.82	0.82	0.78	0.02	0.519	0.277
Phase 3	1.04	1.02	0.98	0.98	1.00	0.93	0.05	0.159	0.200
Phases 4 and 5	1.18	1.19	1.22	1.15	1.18	1.17	0.03	0.370	0.299
Overall	0.97	0.98	0.96	0.95	0.97	0.94	0.01	0.477	0.929

Table 5.8. (Cont.)

Average daily feed intake, kg/d									
Phase 1	0.84	0.83	0.83	0.88	0.79	0.86	0.12	0.381	0.729
Phase 2	1.63	1.66	1.57	1.69	1.67	1.59	0.07	0.306	0.413
Phase 3	2.85	2.76	2.59	2.71	2.82	2.66	0.08	0.231	0.039
Phases 4 and 5	3.39	3.41	3.49	3.35	3.42	3.28	0.09	0.436	0.371
Overall	2.49	2.49	2.47	2.47	2.50	2.40	0.05	0.714	0.715
Gain:feed									
Phase 1	0.62[b]	0.66[ab]	0.65[ab]	0.65[ab]	0.70[a]	0.65[ab]	0.10	0.038	0.139
Phase 2	0.50	0.50	0.49	0.49	0.49	0.49	0.02	0.869	0.531
Phase 3	0.37	0.37	0.38	0.36	0.36	0.35	0.02	0.602	0.499
Phases 4 and 5	0.35	0.35	0.35	0.34	0.35	0.36	0.01	0.749	0.924
Overall	0.39	0.39	0.39	0.38	0.39	0.39	0.01	0.708	0.567

[a-b]Within a row, means without a common superscript differ ($P < 0.05$).

[1]Least square means for dietary treatments represent eight observations.

[2]SEM = standard error of the mean.

[3]Linear effects of reducing dietary protein.

Table 5.9. Carcass characteristics and loin quality of pigs fed experimental diets[1]

Item	High-protein	Medium-protein	Low-protein				SEM	*P*-value	Linearity[2]
			-	Isoflavones	Glu	Isoflavones + Glu			
Ending live weight, kg	129.98	139.59	127.01	129.22	136.33	129.33	3.25	0.059	0.913
Hot carcass weight, kg	104.95^{ab}	111.16^{a}	105.12^{ab}	104.10^{ab}	108.61^{ab}	101.18^{b}	1.95	0.009	0.571
Carcass yield, %	80.79	79.83	83.23	80.63	79.78	78.37	1.45	0.312	0.358
Fat thickness, cm	2.29	2.86	2.76	2.76	2.79	2.92	0.21	0.338	0.075
Loin eye area, cm	60.27^{a}	58.05^{a}	53.65^{ab}	54.27^{ab}	54.73^{ab}	48.56^{b}	2.23	0.015	0.058
Drip loss, %	5.35	4.16	6.13	6.22	5.39	4.97	0.80	0.371	0.690
Loin quality traits[3]									
Ultimate pH	5.44	5.55	5.44	5.42	5.45	5.47	0.09	0.153	0.663
Visual color[4]	3.06	3.13	2.81	2.94	2.75	2.88	0.16	0.514	0.359
Visual marbling[5]	2.25	1.81	1.81	2.44	2.50	2.19	0.24	0.194	0.165
Subjective firmness[6]	2.88	3.00	3.00	3.00	3.13	3.13	0.21	0.959	0.646
Lightness, L*	46.79^{ab}	45.53^{b}	49.52^{a}	48.46^{ab}	48.95^{ab}	47.40^{ab}	0.86	0.021	0.090

Table 5.9. (Cont.)

Redness, a*	9.53	8.75	9.30	9.98	9.82	9.73	0.89	0.563	0.609
Yellowness, b*	5.04[ab]	3.71[b]	5.21[a]	5.73[a]	5.73[a]	5.23[a]	0.61	0.001	0.764
Backfat color[3]									
Lightness, L*	73.72	73.8	73.94	74.29	73.31	74.32	0.40	0.499	0.718
Redness, a*	3.46	3.01	3.48	3.13	3.35	3.16	0.26	0.723	0.827
Yellowness, b*	3.71	3.97	3.88	3.53	3.71	3.88	0.22	0.686	0.480
Belly length, cm	70.52	71.56	70.19	72.75	73.10	70.80	1.03	0.064	0.951
Belly width, cm	33.14	32.46	33.58	31.91	32.70	32.07	0.80	0.064	0.951
Belly flop, cm	17.42	17.86	17.98	19.55	17.82	22.98	3.93	0.677	0.837
Belly thickness, cm	4.23	4.31	4.45	4.19	4.42	4.43	0.14	0.751	0.888

[a-b]Within a row, means without a common superscript differ ($P < 0.05$).

[1]Least square means for dietary treatments represents eight observations.

[2]Linear effects of reducing dietary protein.

[3]L*, a*, and b* measure darkness, redness, and yellowness, respectively, where greater values indicate a lighter color, a redder color, or a more yellow color, respectively.

Table 5.9. (Cont.)

[4]National Pork Producers Council color based on the 1999 standards measured in half-point increments where 1 = palest and 6 = darkest.

[5]National Pork Producers Council marbling based on the 1999 standards measured in half-point increments where 1 = least amount of marbling and 6 = greatest amount of marbling.

[6]National Pork Producers Council firmness based on the 1991 scale measured in half-point increments where 1 = softest and 5 = firmest.

Table 5.10. Blood characteristics of pigs fed experimental diets in phase one[1,2]

Item	High-protein	Medium-protein	Low-protein				SEM	*P*-value	Linearity[3]
			-	Isoflavones	Glu	Isoflavones + Glu			
Plasma urea nitrogen, mg/dL	15.38[a]	5.71[b]	2.75[c]	2.88[c]	3.25[c]	3.00[c]	0.50	< 0.001	< 0.001
Total protein, g/dL	5.59	5.25	5.28	5.45	5.25	5.43	0.12	0.184	0.029
Albumin, g/dL	3.35	2.96	3.25	3.38	3.29	3.28	0.11	0.126	0.254
Cytokines									
IFN-γ	1.40	1.45	1.70	2.07	1.41	1.66	0.38	0.721	0.570
IL-1α	0.04	0.03	0.02	0.01	0.04	0.02	0.01	0.649	0.328
IL-1β	0.17	0.20	0.14	0.13	0.17	0.13	0.03	0.423	0.599
IL-1Ra	0.34	0.29	0.19	0.13	0.28	0.17	0.08	0.333	0.196
IL-2	0.18	0.19	0.08	0.06	0.19	0.10	0.07	0.562	0.405
IL-4	0.71	0.67	0.25	0.19	0.60	0.34	0.29	0.675	0.308
IL-6	0.07	0.09	0.05	0.04	0.09	0.06	0.02	0.554	0.566
IL-8	0.03	0.06	0.08	0.02	0.02	0.04	0.03	0.424	0.112

Table 5.10. (Cont.)

IL-10	0.35	0.34	0.20	0.13	0.31	0.26	0.10	0.617	0.379
IL-12	0.92	0.77	0.81	0.75	0.81	0.76	0.10	0.848	0.367
IL-18	0.82	0.75	0.51	0.45	1.00	0.54	0.25	0.432	0.348
TNF-α	0.03	0.04	0.04	0.04	0.03	0.04	0.01	0.712	0.297
Gut-protective proteins									
Occludin 1	0.834	1.077	0.759	0.702	0.827	0.760	0.09	0.102	0.853
Claudin-1	0.853	0.910	0.693	0.916	0.664	0.747	0.11	0.357	0.361
ZO-1[4]	0.920	1.079	0.659	0.787	1.020	1.069	0.11	0.029	0.118
MUC-2	0.779	0.930	0.686	0.701	0.621	0.848	0.12	0.432	0.722

[a-c]Within a row, means without a common superscript differ ($P < 0.05$).

[1]Least square means for dietary treatments represent eight observations except for the high-protein, the medium-protein diet, the low-protein diet containing nitrogen-source, the low protein diet containing isoflavones ($n = 7$) and the low protein diet containing isoflavones and nitrogen-source ($n = 6$).

[2]IFN-γ, interferon-gamma; IL-, interleukin-; IL-1Ra, interleukin-1 receptor antagonist; TNF-α, tumor necrosis factor-α.

[3]Linear effects of reducing dietary protein.

[4]Although the model *P*-value was significant, none of the pairwise comparisons were significant.

Table 5.11. Nitrogen balance and apparent total tract digestibility (ATTD) of nitrogen of growing pigs fed the experimental diets, experiment 2, as fed basis[1]

Item	High-protein	Medium-protein	Low-protein				SEM	*P*-value	Linearity[2]
			-	Isoflavones	Glu	Isoflavones + Glu			
Feed intake, kg/d	1.00	0.99	1.03	1.02	1.02	1.05	-	-	-
Dry feces output, g/d	95.03	94.10	86.83	88.61	92.77	101.76	7.75	0.156	0.216
ATTD of DM, %	89.92	89.89	90.93	90.76	90.34	89.66	0.643	0.204	0.148
Urine output[3], kg/d	6.89	4.93	7.04	7.18	5.32	7.10	2.13	0.033	0.736
Nitrogen intake, g/d	36.68^{a}	29.53^{b}	27.36^{c}	27.05^{c}	29.10^{bc}	29.77^{b}	0.64	< 0.001	< 0.001
Nitrogen excretion in feces, g/d	4.06^{a}	3.48^{ab}	3.14^{b}	3.38^{ab}	3.51^{ab}	3.66^{ab}	0.43	0.055	< 0.001
Absorbed nitrogen, g/d	32.60^{a}	26.06^{b}	24.25^{bc}	23.68^{c}	25.56^{bc}	26.12^{b}	0.54	< 0.001	< 0.001
Nitrogen excretion in urine, g/d	8.39^{a}	4.33^{b}	2.96^{b}	2.85^{b}	3.74^{b}	3.97^{b}	0.48	< 0.001	< 0.001
Retained nitrogen, g/d	24.11^{a}	21.81^{ab}	21.19^{b}	20.89^{b}	21.75^{ab}	22.27^{ab}	0.69	0.030	< 0.001
ATTD of nitrogen, %	88.89	88.24	88.46	87.53	87.94	87.75	1.30	0.784	0.606
Nitrogen retention, % of intake	66.03^{b}	73.56^{a}	77.64^{a}	76.96^{a}	75.00^{a}	74.44^{a}	2.31	< 0.001	< 0.001

Table 5.11. (Cont.)

Biological value[4], %	74.16[b]	83.32[a]	87.76[a]	87.94[a]	85.24[a]	84.87[a]	1.74	< 0.001	< 0.001

[a-c]Within a row, means without a common superscript differ ($P < 0.05$).

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

[2]Linear effects of reducing dietary protein.

[3]Although the model was significant for the urine output (kg/d), the *P*-value of the pairwise multiple comparison was not significant.

[4]The biological value was calculated as retained nitrogen ÷ absorbed nitrogen × 100 (Rojas and Stein, 2013).

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CHAPTER 6: Reducing dietary crude protein for gestating and lactating sows reduces daily nitrogen retention, but reproductive performance is not impacted by diet protein concentration

Abstract

Soybean meal (**SBM**) is a primary protein source in swine diets, but partial replacement with crystalline amino acids (**AA**) is commonly used to reduce dietary protein. Adequate dietary protein and energy are essential to support fetal development, milk production, and litter growth. However, crystalline AA are absorbed more rapidly than AA from intact protein, which may limit protein synthesis due to a lack of AA availability. Therefore, an experiment was conducted to test the hypothesis that feeding sows diets based primarily on corn, SBM, and no crystalline AA will result in improved reproductive performance and immunity of sows compared with sows fed diets with less SBM and more corn and crystalline AA. Results indicate that nitrogen excretion in feces and urine, absorbed nitrogen, and retained nitrogen (g/d) were greater ($P <$ 0.05) in gestating sows fed the high-protein diet compared with sows fed the low-protein diet. Rectal temperature 24 h after farrowing of sows fed the low-protein diet was greater ($P < 0.05$) compared with sows fed the high-protein diet. Number of live-born and total born pigs was not different between treatments, but sows fed the high-protein diet tended to produce fewer ($P <$ 0.10) mummified pigs than sows fed the low-protein diet. Malondialdehyde was greater ($P<$ 0.05) in sows fed the low-protein diet, but serum glutathione peroxidase and white blood cell count were greater ($P< 0.05$) in sows fed the high-protein diet. Colostrum immunoglobulin G and concentrations of fat, protein, urea nitrogen, lactose, and immunoglobulin G were greater (P < 0.001) in milk from sows fed the high-protein diet than in milk from sows fed the low-protein

diet. In conclusion, feeding a low-protein diet to gestating sows decreased daily nitrogen retention. Reproductive performance was not affected, but feeding a high-protein diet without crystalline AA resulted in differences in milk composition and immune-related characteristics compared with feeding a low-protein diet.

Keywords: amino acids, nitrogen balance, reproductive performance, sows, soybean meal

Abbreviations: AA, amino acids; ADFI, average daily feed intake; ADG, average daily gain; ATTD, apparent total tract digestibility; IFN-γ, interferon gamma; IgG, immunoglobulin G; IL, interleukin; MUN, milk urea nitrogen; SBM, soybean meal; SCC, somatic cell count; TNF-α, tumor necrosis factor-α.

Introduction

Soybean meal (**SBM**) is an important protein source that is often used to furnish the majority of amino acids (**AA**) in diets for sows, but reduction in SBM and inclusion of crystalline AA may sometimes reduce diet costs (Pope et al., 2023). Over the past few decades, reproductive efficiency in sows has improved due to genetic selection, resulting in litters that can exceed 20 pigs (Johannsen et al., 2024). This increase in prolificacy has increased the metabolic demands for AA and energy during both gestation and lactation (Boyd et al., 2000; Feyera et al., 2021; Johannsen et al., 2024). Adequate energy intake in gestation supports fetal growth and mammary development (Trottier et al., 2015), but excessive energy intake during gestation may reduce feed intake during lactation, which has a negative effect on milk production (Tokach et al., 2019). However, high protein intake during gestation improves milk production, protein accretion, and

litter and pig weight at weaning (Jang et al., 2014), but it may also result in an increase in fat accretion because greater nutrient supply promotes both protein and lipid deposition in gestating sows (Pettigrew and Yang, 1997).

Although crystalline AA may support growth performance, protein synthesis can only occur when all required AA are simultaneously available in the cell (Che et al., 2017). Crystalline AA may be absorbed more rapidly than AA from intact proteins, which may result in early oxidation of crystalline AA before AA from intact protein arrives in the cell, limiting protein synthesis (Eugenio et al., 2022; Zhang et al., 2022). It is, therefore, unknown, if low-protein diets containing crystalline AA can support the same reproductive performance, litter growth, and immune status of sows as feeding diets that are based on only corn and SBM and no crystalline AA. An experiment was, therefore, conducted to test the hypothesis that feeding sows diets based primarily on corn and SBM, and with no crystalline AA may result in improved reproductive performance and immunity of sows compared with sows fed diets with less SBM and more corn and crystalline AA.

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. The experiment was conducted at the Swine Research Center at the University of Illinois at Urbana-Champaign (IL, USA) from August 2023 to May 2024.

Experimental diets

Two gestation diets and two lactation diets were formulated to meet estimated requirements for AA and other nutrients by gestating and lactating sows (NRC, 2012; Table 6.1).

Within each phase of production, one diet was a high-protein diet in which AA were furnished by corn and SBM, and the other diet was a low-protein diet in which the inclusion of SBM was reduced and crystalline AA were included to meet requirements for gestating or lactating sows. Both gestation diets also contained soybean hulls. The high-protein gestation and lactation diets contained 17.00% and 24.34% SBM, respectively, whereas the low-protein gestation diet contained 7.65% SBM, and the low-protein lactation diet contained 12.60% SBM. All diets were fed as mash diets. Twelve batches of gestation diets and seven batches of lactation diets were mixed during the experiment. Diet samples were collected from each batch, and at the conclusion of the experiment, diet samples were pooled and subsampled for chemical analysis. Ingredient samples were also collected, pooled, and subsampled for analysis.

Animals, housing, and feeding

Gestation housing and feeding

A total of 154 Camborough gilts and sows (Pig Improvement Company, Hendersonville, TN, USA) were bred to terminal line boars (Pig Improvement Company L 800). The initial body weight was 190.04 ± 26.8 kg. Sows and gilts were used in eight blocks of 20 to 28 animals, using a randomized complete block design. The breeding group was the blocking factor. Within each block, animals were allotted to experimental diets on the day of breeding, with parity balanced between treatments, and feeding of gestation diets started on the day of breeding and continued until day 104 of gestation. At this time, sows were moved to the lactation facility, and feeding of the lactation diets was initiated. During gestation, sows were housed individually in gestation stalls (2.1 × 0.6 m). During the gestation period, daily feed allotments were provided at 0600 h. Feed allowance was 1.5 times the maintenance requirement for metabolizable energy for gestating sows (i.e., 100 kcal metabolizable energy/kg body weight$^{0.60}$; NRC, 2012), but feed

allowance was adjusted every other week, to maintain or achieve an ideal sow body condition by visual scoring (approximately 3.0 on a 1 to 5-point scale; Patience and Thacker, 1989).

Nitrogen balance

From the 154 animals that were initially assigned to the experimental diets, 90 sows (parity 2 to 6) were placed in individual metabolism crates (0.91 × 2.08 m) from day 45 to 56 (i.e., mid-gestation). Within each block, the same number of sows from each treatment and with the same parity were selected for placement in metabolism crates, with 12 sows in blocks one through six (i.e., six sows per treatment) and nine sows in blocks seven and eight (i.e., four or five sows per treatment), for a total of 44 and 46 sows for the high-protein and low-protein diets, respectively. Crates were equipped with a self-feeder, a nipple waterer, and a fully slatted floor to allow for total, but separate, collection of urine and fecal materials. The selected 90 sows had an average parity of 3.4 ± 1.3, and an average body weight of 200.4 ± 18.5 kg when moved to the metabolism crates. A screen floor was installed under the slatted floor, and feces were quantitatively collected from the screen floor. A urine tray was installed under the screen floor, and urine was captured in this tray and drained into a urine bucket that was placed under the tray, which allowed for quantitative collection of urine. The initial three days in the metabolism crates were considered the adaptation period to the crates, whereas urine and fecal materials were collected from feed provided during the following five days according to standard procedures using the marker-to-marker approach (Adeola, 2001). Fecal collection was initiated when the first marker (i.e., indigo carmine) appeared in the feces and ceased when the second marker (i.e., ferric oxide) appeared (Adeola, 2001). To avoid nitrogen loss in the urine, 50 mL of 6 *N* HCl was added to the urine bucket daily. Buckets were emptied daily, and the weight of the collected urine was recorded, and 10% was stored at − 20 ◦C until subsampling. Orts were collected daily

prior to feeding the morning meal, pooled for the duration of the collection period, dried in a 65 °C forced-air drying oven (Thermo Fisher Scientific Inc.; model Heratherm OMH750, Waltham, MA, USA), and weighed to determine feed intake during the collection period. Fecal samples from each animal were stored at –20 °C immediately after collection. Urine samples were thawed and mixed within animal and diet at the conclusion of the experiment, and a subsample was stored for nitrogen analysis. Fecal samples from each sow were thawed and mixed, and then dried in a 65 °C forced-air drying oven as described for orts. All dried fecal samples were finely ground using a 500 G stainless steel swing-type mill grinder (RRH, Zhejiang, China), and the ground samples were mixed, and a subsample was collected for chemical analysis.

Lactation housing, feeding, and performance

Sows were moved to the lactation unit on day 104 of gestation and housed individually in farrowing crates. Each farrowing crate (2.1 × 1.5 m) was equipped with a stainless-steel feeder and two nipple waterers. Sows were fed experimental lactation diets starting the day they were moved to the lactation unit. Sows were fed as in gestation from entry to the farrowing unit until farrowing, but diets were provided on an *ad libitum* basis from farrowing until weaning and water was available at all times. According to normal farm procedures, all litters were offered a standard creep diet from day 14 post-farrowing until weaning.

Sow body weights were determined on the day of breeding, when sows were moved in and out of metabolism crates, when sows were moved to the lactation barn, within 24 hours after farrowing, and on the day of weaning. Weaning took place at 20.39 ± 0.71 days post-farrowing. On the day of farrowing and 24 hours later, the rectal temperature of each sow was measured using a digital thermometer while no feeding or nursing activity took place.

The number and body weight of pigs born alive, the number of mummies, stillborn pigs, and total pigs per litter after cross-fostering were recorded, and pigs were weighed again at weaning. For both dietary treatments, cross-fostering was completed within 24 hours of farrowing and pigs were only cross-fostered within treatment group, and each sow had approximately 14 pigs after cross-fostering. Following normal farm procedures, pigs weighing less than 0.9 kg at birth were considered low vitality and euthanized. The weight of pigs that died during lactation, as well as the reason for death (crushed by sow, low vitality/starved, rupture, or euthanized due to congenital deformity), were recorded. Pigs were processed within 24 h of birth. Processing included clipping needle teeth, docking tails, castrating male pigs, administering iron dextran (Uniferon, Pharmacosmos, Watchung, NJ, USA), and centiofur antibiotic (Excede, Zoetis, Parsippany, NJ, USA), and also ear notching for identification.

Blood and milk sample collection

Blood samples were collected from all sows 14 days post-farrowing. Two blood samples were collected from the jugular vein via venipuncture. One blood sample was collected in vacutainers with ethylenediaminetetraacetic acid, and the other blood sample was collected in serum vacutainer tubes containing spray-coated silica as a serum clot activator. Blood samples were stored on ice immediately after collection, and ethylenediaminetetraacetic acid blood samples were delivered to the University of Illinois Veterinary Diagnostic Laboratory (Urbana, IL, USA) for analysis of white blood cells, neutrophil, and lymphocyte cell counts in the whole blood. The blood collected from the serum vacutainer tubes was allowed to clot and then centrifuged at 1,000 × g for 10 min at room temperature. Serum was removed from centrifuged tubes and stored at −80 °C until analysis. Colostrum samples were collected within 24 hours of farrowing, and milk samples were collected on day 14 post-farrowing following administration of 1 mL

oxytocin (Bimeda-MTC Animal Health Inc., Cambridge, ON, Canada) intramuscularly. Approximately 70 mL of colostrum or milk was collected in 50 mL and 25 mL conical sterile polypropylene centrifuge tubes from the first five functional teats on each side of the mammary gland. Colostrum and milk samples were stored at − 20 ºC immediately after collection. Prior to shipping milk for component analysis, all samples were thawed, placed in 60 mL tubes containing a milk preservative, and a subsample of 10 mL was placed in a separate tube for later analysis.

Chemical analyses

Ingredients, diets, and fecal samples were analyzed for dry matter by oven drying at 135 °C for 2 h (method 930.15; AOAC Int., 2019) and for dry ash (method 942.05; AOAC Int., 2019). The concentration of nitrogen in diets and ingredients was analyzed using the Kjeldahl method (method 984.13; AOAC Int., 2019) on a KjeltecTM 8400 (FOSS Inc., Eden Prairie, MN, USA) with subsequent calculation of crude protein using a conversion factor of 6.25. These samples were also analyzed for AA 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 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]. Ingredients were analyzed for phytic acid (Ellis et al., 1977), and total starch was determined using the amyloglucosidase-alpha-amylase procedure corresponding to the enzymatically hydrolyzed starch converted to glucose, followed by analysis of the glucose concentration by spectroscopy

(method 996.11; AOAC Int., 2019), whereas glucose, sucrose, maltose, fructose, stachyose, and raffinose were analyzed using high-performance liquid chromatography (method 977.2, AOAC Int, 2019). Acid hydrolyzed ether extract was also 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). Insoluble dietary fiber and soluble dietary fiber were also analyzed in ingredients according to method 991.43 (AOAC Int., 2019) using the Ankom Dietary Fiber Analyzer (Ankom Technology). Total dietary fiber was calculated as the sum of insoluble dietary fiber and soluble dietary fiber. Soybean meal and soybean hulls were also analyzed for trypsin inhibitors (method Ba 12-75; AOCS, 2017). Fecal and urine samples were analyzed for nitrogen as described for diets. Milk samples were analyzed by Eastern Laboratory Services (Medina, OH, USA) for fat, free fatty acids, protein, milk urea nitrogen (**MUN**), lactose, other solids, total solids, and somatic cell count (**SCC**) using a Milkoscan 7 calibrated for bovine milk (Foss, Hillerød, Denmark), and colostrum samples were analyzed for protein, fat, and total solids. Concentrations of interleukin (**IL**)-1α, IL-1β, IL-1 receptor antagonist, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12, IL-18, interferon-γ (**IFNγ**), and tumor necrosis factor-α (**TNF-α**) in serum samples were measured using a porcine-specific multiplex immunoassay kit (MilliporeSigma, Burlington, MA, USA) and read with a Luminex MagPix instrument (Luminex Corporation, Austin, TX, USA). Malonaldehyde and glutathione peroxidase in serum samples were determined by enzyme-linked immunosorbent assay following the manufacturer's instructions (MyBioSource, Inc., San Diego, CA, USA). Immunoglobulin G (**IgG**) in milk and colostrum was also determined by enzyme-linked immunosorbent assay following the manufacturer's instructions (Bethyl Laboratories, Inc., Montgomery, TX, USA).

Calculations and statistical analysis

At the conclusion of the gestation period, the apparent total tract digestibility (ATTD) of dry matter and nitrogen, retention of nitrogen, and biological value of nitrogen were calculated (NRC, 2012; Rojas and Stein, 2013). Data for body weight gain in gestation, body weight loss in lactation, average daily feed intake (**ADFI**) in gestation and lactation, estimated milk yield (calculated as 4 g milk per g of litter body weight gain; Close and Cole, 2000), and litter performance data were calculated. Litter performance data were calculated for each sow and included number of total pigs born, live born pigs, mummified pigs, and still born pigs; number of pigs after cross-fostering; number of pigs weaned; and pig mortality rates (calculated as the percentage of live born pigs that died before weaning before and after adjusting for cross-fostering). Total live litter birth weight, litter birth weight after cross fostering, litter weight at weaning, and litter average daily gain (**ADG**) were calculated as well. Average pig weights and ADG were also calculated.

Model assumptions on the residuals were confirmed using the MIXED procedure and the Brown-Forsythe test of the GLM procedure of SAS (SAS Inst. Inc., Cary, NC, USA). The MIXED procedure of SAS was used to generate studentized residuals, and outliers were defined as observations having residuals greater than 3 or less than −3. A sow was excluded from statistical analysis if three or more response variables were identified as outliers, but of the 154 sows that farrowed, only one sow from the high-protein diet was identified as an outlier and excluded from statistical analyses. All other sows were included in the final analysis. Except for the number of pigs born alive, the number of mummies, stillborn pigs, and total pigs born per litter, data were considered continuous variables and analyzed using the MIXED procedure in SAS. The number of pigs born alive, mummified pigs, stillborn pigs, and the total pigs born per

litter were considered discrete count variables and analyzed using the GLIMMIX procedure in SAS. Mortality data were considered binomial variables and were also analyzed using the GLIMMIX procedure in SAS. The initial statistical model for the MIXED and the GLIMMIX procedures included the fixed effects of diet, and block and replicate within block as random effects. However, for litter performance response variables, litter weight after cross-fostering was included as a covariate to account for variation in litter weight. The sow was the experimental unit for all sow-related variables, whereas the litter was the experimental unit for litter performance variables. Least square means for somatic cell count and cytokines were reported in the original scale after back-transforming (inverse log) the output from the LSMEANS statement in the MIXED procedure. The LSMEANS statement was used to calculate treatment means for all other variables in the MIXED procedure, along with the inverse link option in the GLIMMIX procedure. Results were considered significant at $P \leq 0.05$ and considered a tendency at $0.05 < P \leq 0.10$.

Results

The chemical compositions of the diets and the ingredients were, in general, in agreement with expected values (Tables 6.1 and 6.2).

Nitrogen balance

Dietary treatment did not affect the initial or final body weight, daily feed intake, weight of feces and urine, or the ATTD of dry matter (Table 6.3). Daily nitrogen intake, nitrogen excretion in feces and urine, absorbed nitrogen, ATTD of nitrogen, and retained nitrogen in grams per day were greater ($P < 0.05$) for sows fed the high-protein diet compared with sows fed the low-

protein diet. However, nitrogen retention as a percentage of intake and biological value were not different between treatments.

Reproductive performance

Differences in body weights of sows between treatment groups were not observed at breeding (Table 6.4), but sows and gilts fed the high-protein diet were heavier ($P < 0.05$) on day 104 compared with sows fed the low-protein diet, and no difference between treatments in the weight of sows at weaning was observed. The ADG tended to be greater ($P < 0.10$) for sows fed the high-protein diet compared with sows fed the low-protein diet during the gestation period, but no difference in ADG was observed during the lactation period. There was no difference between treatments in daily feed intake from breeding to day 104, from day 104 to farrowing or during the lactation period. The temperature of sows fed the low-protein diet was greater ($P < 0.05$) than that of sows fed the high-protein diets at the time of farrowing and 24 hours later.

The total number of pigs born per litter, total number of pigs born alive per litter, number of stillborn pigs per litter, and pigs per litter after cross-fostering were not different between the two treatments (Table 6.5), but sows fed the high-protein diet tended to produce fewer mummified pigs than sows fed the low-protein diet ($P < 0.10$). Live litter birth weight was not affected by diet, but the number of pigs with congenital deformities tended to be greater ($P < 0.10$) for sows fed the high-protein diet than for sows fed the low-protein diet. Litter weight after cross-fostering was greater ($P < 0.05$) for sows fed the high-protein diet than for sows fed the low-protein diet, but the individual pig weight after cross-fostering was less ($P < 0.05$) for sows fed the high-protein diet than for sows fed the low-protein diet. No differences between treatments were observed in litter ADG or individual pig ADG during lactation. Likewise, there was no difference in the number of pigs weaned per litter, in litter weaning weight, or in

individual pig body weight at weaning. Pigs from sows fed the low-protein diet tended to have a greater ($P < 0.10$) survival rate after cross-fostering than pigs from sows fed the high-protein diet. Mortality due to crushing after cross-fostering tended to be greater ($P < 0.10$) for sows fed the high-protein diet than for sows fed the low-protein diet, but there was no effect of dietary treatment for the other causes of mortality after cross-fostering.

Milk composition and immune response

The concentration of fat and total solids in colostrum was not affected by diet (Table 6.6), but the concentration of fat, free fatty acids, protein, MUN, and lactose was greater ($P < 0.05$) in the milk collected on day 14 from sows fed the high-protein diets compared with milk from sows fed the low-protein diets. The IgG in milk and colostrum was also greater ($P < 0.05$) for sows fed the high-protein diet compared with sows fed the low-protein diet.

Malondialdehyde was greater ($P < 0.05$) in serum from sows fed the low-protein diets compared with sows fed the high-protein diets, but white blood cell count and glutathione peroxidase were greater ($P < 0.05$) in serum from sows fed the high-protein diet than those fed the low-protein diet (Table 6.7). Most serum cytokines and white blood cell differential did not differ between treatments on day 14 of lactation, but IL-4 was greater ($P < 0.05$) in sows fed the high-protein diet than in sows fed the low-protein diet. Likewise, sows fed the high-protein diet tended to have greater ($P < 0.10$) concentration of IFN-γ compared with sows fed the low-protein diet, whereas sows fed the low-protein diet tended to have greater ($P < 0.10$) IL-2 in blood compared with sows fed the high-protein diet.

Discussion

The analyzed values for CP and total AA in experimental diets for gestating and lactating sows were in close agreement with formulated values, which indicates correct mixing of diets. Likewise, the analyzed concentrations of nutrients in corn, SBM, and soybean hulls were consistent with reported data (NRC, 2012).

Nitrogen balance

Dietary protein reduction in the low-protein diet was accomplished by reducing SBM and adding more corn and crystalline AA to ensure adequate digestible AA for gestating and lactating sows. The lack of an effect of reducing CP in the diet on feed intake in gestating sows was in agreement with reported data (Theil et al., 2002; Johannsen et al., 2022). The energy value of SBM is close to the energy value of corn (Sotak-Peper et al., 2015; Ibagon et al., 2025), and the observation that there were no differences in ATTD of DM between the high and low-protein diets was, therefore, expected. The ATTD of crude protein in corn is less than the ATTD of crude protein in SBM (Dong et al., 2020), and the reduction in the ATTD of nitrogen that was observed when the SBM was replaced by corn and crystalline AA is, therefore, due to the lower crude protein digestibility in corn. This observation is in agreement with data from growing pigs fed diets where SBM was replaced by corn and crystalline AA (Zervas & Zijlstra, 2002; Cristobal et al., 2025).

Soybean meal is used as the principal source of AA in swine diets in most pig-producing countries (NRC, 2012). When SBM is included in corn-SBM diets to meet the requirements of indispensable AA, dietary concentrations of other AA often exceed the requirement (Liao et al., 2015). The excess AA undergo deamination, resulting in nitrogen losses because of urinary excretion of nitrogen (van Milgen & Dourmad, 2015). Reducing dietary protein by 3 percentage

units in gestation diets in the present experiment decreased urinary nitrogen excretion by approximately 5 g per day, which agrees with data from sows fed low-protein diets (Munoz Alfonso et al., 2024; Wang et al., 2025).

Both gestation diets were formulated to provide sufficient standardized ileal digestible indispensable AA to support fetal development and maternal growth. Therefore, similar nitrogen retention was expected between the diets. However, the observation that nitrogen retention, measured as grams per day, was greater in sows fed the high-protein diet compared with sows fed the low-protein diet supplemented with crystalline AA indicates that crystalline AA were not used with the same efficiency as AA from SBM. This observation is in agreement with reported data (Theil et al., 2002; Johannsen et al., 2024), and may be due to the faster rate of absorption and metabolism of crystalline AA compared with protein-bound AA (Eugenio et al., 2022), resulting in an imbalance in AA supply for the cells at the sites of protein synthesis (Yen et al., 2004). This indicates that a limited inclusion of crystalline AA in low-protein diets is preferred because imbalances in AA supply may reduce nitrogen retention for fetal, mammary, and maternal tissue development (Mallmann et al., 2019). Another possibility is that the reduced crude protein in the diets failed to meet the requirements for dispensable AA in gestating sows, which may have limited protein synthesis and nitrogen retention by sows fed the low-protein diet. However, both diets were formulated to meet or exceed requirements for digestible AA (NRC, 2012), and the analyzed proportion of indispensable AA with total AA in the diets averaged 45.56% and 46.79% corresponding to dispensable AA proportions of 54.44% and 53.21%, respectively. These values are within the range of what has been hypothesized for growing pigs to provide adequate nitrogen for the de novo synthesis of dispensable AA, and a similar proportion would also be expected to be sufficient for sows (Lenis et al., 1999).

Therefore, it is unlikely that there was a lack of nitrogen to synthesize dispensable AA in the low-protein diet. It is, however, also possible that the nitrogen needed to maximize reproductive performance of sows is less than what is needed to maximize nitrogen retention, and if that is the case, the observed difference in nitrogen retention may not have implications for reproductive performance.

Nitrogen retention as a percentage of intake was approximately 46% for both diets, which is greater than values previously reported for gestating sows (Theil et al., 2002;Munoz Alfonso et al., 2024). The implication of this observation is that when formulating diets with a balanced AA profile, nitrogen retention as a percentage of intake is maximized. Formulating diets based on the basis of standardized ileal digestibility of AA also provides balanced diets, resulting in a greater retention of nitrogen, which will reduce AA deamination and, therefore, also reduce nitrogen excretion.

Reproductive performance

During the gestation period, sows are usually restricted in feed intake according to the visual assessment of body condition to avoid problems associated with excessive body weight gain (Carrión-López et al., 2022). The observation that sows fed the low-protein diet had reduced body weight and ADG during the gestation period, even though no differences in feed intake between the two groups of sows were observed, indicates that although diets were balanced in AA and met or exceeded the requirements for digestible indispensable AA, sows fed the high-protein diet had greater whole body protein deposition due to the greater retention of nitrogen in the gestation period, which increased the weight at the end of the gestation period. This observation is in agreement with data from sows fed diets with different levels of crude protein (Mahan, 1998; Jang et al., 2014), which demonstrated that greater dietary protein in the diet can

support greater maternal tissue growth during gestation. It is, however, also possible that the tendency for reduced ADG during gestation was due to reduced backfat deposition, but because backfat was not determined in sows in this experiment, we cannot confirm this hypothesis. The lack of difference between the two treatments for litter size at birth indicates that although sows fed the low-protein diet had lower nitrogen retention, and therefore reduced body weight in late gestation, sows prioritized protein for fetal development. The reduced nitrogen retention in sows fed the low-protein diet, therefore, likely is due to reduced retention of nitrogen in the body of sows.

During lactation, feed is offered to the sow on an ad libitum basis to meet the requirement of sows for milk component synthesis and to limit the mobilization of tissue reserves (Gorr et al., 2024). The lack of differences between treatments in feed intake, body weight at weaning, and ADG of the lactating sows is in agreement with results from other experiments with lactating sows fed diets with different inclusions of protein (Huber et al., 2015; Johannsen et al., 2024), although greater body weight loss of sows during lactation has also been reported for sows fed low-protein diets (Munoz Alfonso et al., 2024). Feeding high-protein diets to sows during gestation and lactation may reduce milk yield by sows fed organic diets (Johannsen et al., 2022), but the calculated milk yield of sows in this experiment was around 11 kg per day and did not differ between treatments, which is in agreement with other experiments where milk yield was between 10.5 and 13.8 kg per day (Renaudeau et al., 2001;Strathe et al., 2020).

In the net energy system, dietary starch and fat are assumed to be used more efficiently than protein because protein catabolism requires ATP for urea synthesis, protein turnover, and nitrogen excretion, which produces heat (van Milgen et al., 2001). Consequently, reducing crude protein in the diet or adding fat is expected to reduce heat production (Noblet & Perez, 1993; Le

Bellego et al., 2001). Although heat production was not measured in the current experiment, the rectal temperature of sows fed the high-protein diet was reduced compared with sows fed the low-protein diet, which does not support the assumption that high-protein diets increase body temperature in sows during farrowing or 24 hours after farrowing. Heat production is positively correlated with energy supply in lactating sows, due to increased feed intake and milk synthesis (Noblet & Etienne, 1987; Renaudeau & Noblet, 2001). Therefore, reduced protein in the diet may have increased the metabolic heat of the sow due to an increase in body protein mobilization for milk protein synthesis, but because heat production was not measured in this experiment, we cannot confirm this hypothesis. It is also possible that the increased temperature is due to an intensified inflammatory response during parturition, but because we did not measure the inflammatory response during parturition, we cannot verify this speculation. It is also noted that sows on both treatments had body temperatures that were within the normal physiological range.

Litter performance

The observation that the number of pigs born, the number of pigs weaned, litter weight at weaning, and litter ADG did not differ between sows fed the two diets is consistent with previous data indicating that nutrients for milk production are derived from both the diet and from body reserves [(Renaudeau et al., 2001; Johannsen et al., 2022, 38, 46], and sows have the ability to maintain milk production even when feed intake is reduced (Pedersen et al., 2016). Milk yield is largely independent of feed intake unless body reserves are depleted, and a decrease in litter growth is more closely associated with energy restriction than with protein restriction (Noblet & Etienne, 1987; Renaudeau & Noblet, 2001). This may be the reason no differences in litter growth were observed between treatments in this experiment, which is also in agreement with data from sows fed low-protein diets (Munoz Alfonso et al., 2024;Huber et al., 2015).

Immunological measurements

Soy bioactive compounds (i.e., isoflavones and saponins) may enhance milk composition and the antioxidant status of the sow (Fangfang et al., 2015). The reduced concentration of IgG in milk on day 14 compared with colostrum is consistent with data that report greater concentration of IgG in colostrum, but a decrease in concentration during lactation (Hurley, 2015). The observation that sows fed the high-protein diet had greater concentrations of IgG, protein, fat, and total solids than sows fed the low-protein diet, is in agreement with data that compared high and low-protein diets or different levels of soy isoflavone inclusion (Hu et al., 2015;Strathe et al., 2020). Likewise, concentrations of lactose, fat, and protein in milk are also in agreement with reported data (Strathe et al., 2020; McGhee and Stein, 2021; Li et al., 2022). The lack of differences in individual pig body weight at weaning between treatments indicates that the improved quality of milk from sows fed the high-protein diet supported adequate growth, even though pigs from these sows had a lower BW after cross-fostering compared with pigs from sows fed the low-protein diet (Berchieri-Ronchi et al., 2011). The implication of this observation is that although sows are capable of mobilizing body reserves to maintain milk quality, inclusion of greater quantities of SBM in the diet provides sufficient nutrients and immune support to compensate for the reduced BW of pigs.

Oxidative stress that animals undergo during gestation may influence the implantation and development of fetuses in the uterus during the early gestation stage (Berchieri-Ronchi et al., 2011; Shahin et al., 2013). Soy bioactive compounds in SBM have also been classified as health-promoting due to their properties as anti-inflammatory agents and antioxidants (White et al., 2024). Malondialdehyde has been used as a marker for lipid peroxidation in sows during lactation (Birben et al., 2012), and enzymatic antioxidants, such as glutathione peroxidase, are

factors that reduce oxidative stress (Arthur, 2001). Lactation is characterized by an increase in metabolic activity associated with milk production, which may increase oxidative metabolism and reactive oxygen species production (Zhao & Kim, 2020).

Although crude protein was reduced by only 3.5%, SBM inclusion in the low-protein diets was reduced by almost 50%, indicating that, in addition to the crude protein concentration, the protein source was also changed. Therefore, differences between the two diets may not only be due to the reduction of crude protein, but also to the reduced supply of soybean protein-associated compounds. During digestion, intact proteins may release different bioactive peptides, whereas crystalline AA provide only the free AA. Consequently, reduced SBM inclusion may have decreased the intake of soy bioactive compounds and peptides with potential antioxidant and anti-inflammatory effects. Therefore, the observed differences in concentrations of glutathione peroxidase and malondialdehyde between treatments during mid-lactation may reflect the reduced inclusion of soy bioactive compounds in the low-protein diet, which may affect the oxidative status of the sows. The implication of this observation is that soy bioactive compounds may improve the antioxidant-related defense of the sow during periods of high metabolic demand, such as lactation and gestation. However, values for malondialdehyde for both groups of sows during mid-lactation were between 2.77 and 2.89 nmol/mL, which are less than values reported for sows kept under thermoneutral conditions and fed diets containing more than 14% protein (Zhao and Kim, 2020; Sun et al., 2022). This indicates that under the conditions of the current experiment, sows were not subjected to extreme oxidative stress. It is, however, acknowledged that there are other biomarkers for antioxidant status that could have been measured (i.e., total antioxidant capacity and superoxide dismutase). Likewise, to provide more robust data for the impact of diet SBM concentration on antioxidant status, and to confirm

the data obtained in this experiment, it may be necessary to collect blood samples throughout gestation, at farrowing, and at several time points in lactation. Having multiple sampling points and analysis of more antioxidant markers would provide an opportunity to elucidate the dynamic impact of diets on antioxidant- and inflammatory status of sows during the entire reproductive period, which could not be provided from the current data where blood was collected only one time during lactation.

When an animal is exposed to different stimuli associated with inflammation and infection, innate immune cells, including white blood cells, release pro- or anti-inflammatory cytokines (Burger & Dayer, 2002). The functional definition of an anti-inflammatory cytokine is the ability to inhibit the synthesis of pro-inflammatory cytokines such as IL-1, IL-2, IL-12, IL-18, IFN-γ, and TNF-α. Therefore, the observation that sows fed the high-protein diet had greater concentrations of anti-inflammatory cytokines indicates that these sows had improved immunity, which may also be a result of the greater intake of bioactive compounds from SBM, which may have improved the immune system of sows. However, due to the design of the experiment it was not possible to separate effects of greater concentrations of protein and possible effects of bioactive compounds and future research is, therefore, needed to specifically address the hypothesis that bioactive compounds in SBM have beneficial effects on reproductive performance of sows. In addition, bioactive compounds were not measured in the diets used in the experiment or the samples collected from sows, which prevents strong conclusion relative to these compounds.

Conclusions

In conclusion, feeding low-protein diets supplemented with crystalline AA to gestating and lactating sows maintained overall reproductive performance, but reduced nitrogen utilization efficiency during gestation compared with sows fed high-protein diets based on only corn and SBM. The greater levels of SBM in high-protein diets also appeared to result in greater antioxidant-related indicators, immune function, and milk quality. These results indicate that maintaining an adequate level of SBM in diets for sows is essential to optimize nitrogen retention, antioxidant-related indicators, and milk composition in sows.

Tables

Table 6.1. Ingredient and nutrient compositions of experimental diets, as-fed basis

Item	Gestation		Lactation	
Dietary protein:	High	Low	High	Low
Ingredient, %				
Corn	68.89	77.61	70.780	81.65
Soybean meal	17.00	7.65	24.34	12.60
Soybean hulls	10.00	10.00	-	-
Soybean oil	1.00	1.00	2.00	2.00
L-Lys·HCl	-	0.29	-	0.37
DL-Met	-	0.06	-	0.02
L-Thr	-	0.12	-	0.12
L-Trp	-	0.03	-	0.04
L-Val	-	-	-	0.15
Dicalcium phosphate	1.42	1.58	1.28	1.47
Calcium carbonate	0.79	0.76	0.70	0.67
Sodium chloride	0.40	0.40	0.40	0.40
Vitamin-mineral premix[1]	0.50	0.50	0.50	0.50
Analyzed nutrients, %				
Dry matter, %	85.34	84.66	84.46	83.76
Ash, %	4.16	4.17	2.11	2.21
Crude protein, %	14.14	11.19	16.14	12.60
Gross energy, kcal/kg	3,901	3,808	3,890	3,832

Table 6.1. (Cont.)

Indispensable amino acids, %				
Arg	0.79	0.55	0.98	0.65
His	0.36	0.28	0.45	0.31
Ile	0.58	0.42	0.72	0.49
Leu	1.28	1.01	1.48	1.16
Lys	0.74	0.73	0.87	0.83
Met	0.22	0.21	0.25	0.22
Phe	0.69	0.51	0.82	0.59
Thr	0.51	0.48	0.60	0.55
Trp	0.12	0.10	0.18	0.13
Val	0.68	0.51	0.80	0.72
Total	5.97	4.80	7.15	5.65
Indispensable amino acids as % of total	44.79	46.33	45.77	47.80
Dispensable amino acids, %				
Ala	0.76	0.62	0.85	0.69
Asp	1.30	0.90	1.55	1.02
Cys	0.24	0.19	0.26	0.22
Glu	2.50	1.85	2.96	2.11
Gly	0.61	0.47	0.66	0.46
Pro	0.88	0.72	0.97	0.79
Ser	0.60	0.47	0.68	0.51

Table 6.1. (Cont.)

Tyr	0.47	0.34	0.54	0.37
Total	7.36	5.56	8.47	6.17
Dispensable amino acids as % of total	55.21	53.67	54.23	52.20
Total amino acids, %	13.33	10.36	15.62	11.82

[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-tocopherol 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 dihydroiodide; 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 6.2. Analyzed composition (as-is basis) of ingredients used in gestation and lactation diets

Item,	Corn	Soybean meal	Soybean hulls
Dry matter, %	87.86	89.66	90.01
Ash, %	1.26	6.41	4.55
Crude protein, %	8.01	47.10	10.99
Acid hydrolyzed ether extract, %	3.68	2.81	1.80
Total dietary fiber, %	12.10	18.30	66.70
Insoluble dietary fiber	11.50	16.30	62.90
Soluble dietary fiber	0.60	2.00	3.80
Phytic acid, %	0.63	1.59	0.16
Starch, %	62.30	0.96	0.96
Glucose, %	0.32	0.05	0.19
Maltose, %	0.52	0.38	0.05
Fructose, %	0.07	0.08	0.14
Sucrose, %	0.52	6.50	0.45
Stachyose, %	0.08	5.44	0.64
Raffinose, %	0.09	1.11	0.16
Trypsin inhibitors units/mg	-	0.04	4.53
Indispensable amino acids, %			
Arg	0.34	3.10	0.59
His	0.21	1.14	0.28
Ile	0.28	2.06	0.51
Leu	0.89	3.36	0.85

Table 6.2. (Cont.)

Lys	0.24	2.83	0.78
Met	0.16	0.65	0.13
Phe	0.38	2.26	0.47
Thr	0.28	1.75	0.47
Trp	0.05	0.60	0.06
Val	0.37	2.19	0.55
Dispensable amino acids, %			
Ala	0.56	1.90	0.52
Asp	0.55	5.00	1.21
Cys	0.17	0.66	0.25
Glu	1.45	8.03	1.42
Gly	0.31	1.84	0.91
Pro	0.63	2.23	0.62
Ser	0.35	1.85	0.59
Tyr	0.22	1.61	0.46
Total amino acids, %	7.44	43.06	10.67

Table 6.3. Effects of dietary protein on apparent total tract digestibility (ATTD) of dry matter and nitrogen balance of gestating sows[1]

Item	Diet		SEM	*P*-value
Dietary protein:	High	Low		
Initial body weight, kg	201.13	200.36	3.733	0.823
Final body weight, kg	209.36	208.30	3.810	0.765
Feed intake, kg/day	2.23	2.22	0.027	0.575
Dry feces output, g/day	0.21	0.21	0.005	0.237
ATTD of dry matter, %	89.93	90.10	0.215	0.515
Urine output, kg/day	14.22	12.86	1.540	0.491
Nitrogen intake, g/day	52.24	39.74	0.557	< 0.001
Nitrogen excretion in feces, g/day	6.77	6.05	0.198	0.001
Absorbed nitrogen, g/day	45.47	33.69	0.501	< 0.001
ATTD of nitrogen, %	87.04	84.79	0.386	< 0.001
Nitrogen excretion in urine, g/day	20.45	15.40	0.666	< 0.001
Retained nitrogen, g/day	24.96	18.28	0.764	< 0.001
Nitrogen retention, % of intake	47.79	45.85	1.422	0.338
Biological value[2], %	54.82	54.15	1.702	0.771

[1]Data are least-square means of 44 observations in the high-protein and 46 in the low-protein diet.

[2]Calculated by dividing retained nitrogen by absorbed nitrogen and multiplying by 100 (Rojas and Stein, 2013).

Table 6.4. Performance of sows fed high or low-protein diets during gestation and lactation[1]

Item	Diet		SEM	*P*-value
Dietary protein:	High	Low		
Parity	1.68	1.66	0.197	0.938
Body weight, kg				
At breeding	191.47	188.18	3.054	0.448
Day 104 gestation	230.06	222.47	2.488	0.033
At 24 hr. after farrowing	213.42	208.05	2.402	0.116
At weaning	204.44	198.83	2.582	0.127
Average daily gain, kg				
Breeding to day 104 of gestation	0.368	0.327	0.016	0.072
Days 1 to 21 of lactation	-0.419	-0.437	0.069	0.836
Average daily feed intake, kg				
Breeding to day 104 of gestation	2.17	2.14	0.021	0.360
Pre-farrowing[2]	2.43	2.46	0.090	0.493
Days 1 to 21 of lactation	5.48	5.59	0.181	0.367
Estimated total milk yield[3], kg	224.78	221.72	3.935	0.539
Estimated daily milk yield, kg	11.05	10.90	0.282	0.534
Temperature at farrowing, ℃	38.12	38.34	0.150	0.046
Temperature 24 hr. after farrowing, ℃	38.39	38.63	0.120	0.017

[1]Least square means for each dependent variable represent 78 and 76 observations for the high-protein and low-protein diet, respectively.

[2]Day 104 of gestation until farrowing.

Table 6.4. (Cont.)

[3]Estimated milk yield was calculated as 4 g milk per 1 g of litter body weight gain (Close and Cole, 200).

Table 6.5. Performance of litters from sows fed diets containing high or low protein during gestation and lactation[1]

Item	Diet		SEM	*P*-value
Dietary protein:	High	Low		
Pigs per litter,				
Total born	16.53	16.07	0.462	0.477
Born alive	15.61	14.83	0.446	0.220
After cross-fostering	13.60	13.00	0.418	0.315
Still born	0.83	0.91	0.119	0.574
Mummified	0.13	0.27	0.051	0.056
Weaned	12.68	12.37	0.405	0.594
Litter weight, kg				
Live at birth	20.53	19.49	0.447	0.103
After cross-fostering	19.00	18.12	0.304	0.041
At weaning	74.01	72.75	1.070	0.378
Litter average daily gain, kg	2.74	2.75	0.044	0.916
Individual pig weight, kg				
After cross-fostering	1.38	1.42	0.012	0.007
At weaning	5.84	5.88	0.074	0.655
Pig average daily gain, kg	0.22	0.22	0.003	0.440
Survival rate[2], %	93.24	95.15	0.738	0.073
Pig mortality before cross-fostering, %[3]				
Crushed by sow	2.95	2.04	0.745	0.246

Table 6.5. (Cont.)

Low vitality	7.56	7.87	0.830	0.781
Rupture	0.33	0.09	0.127	0.241
Congenital deformity[4]	1.41	0.62	0.290	0.069
Pig mortality after cross-fostering, %[5]				
Crushed by sow/broken legs	2.69	1.50	0.463	0.069
Low vitality/starved/runt	2.62	2.31	0.509	0.642
Rupture[6]	1.24	0.91	0.322	0.475

[1]Least square means for each dependent variable represent 78 and 76 observations for the high-protein and low-protein diet, respectively.

[2]Survival rate was calculated as the number of pigs weaned divided by the number of pigs per litter after cross-fostering.

[3]Calculated as the percentage of liveborn pigs that died before weaning, before cross-fostering.

[4]Congenital deformities also include spraddle leg pigs and hernias.

[5]Calculated as the percentage of live pigs after cross-fostering that died before weaning

[6]Include pigs found dead and pigs with swollen joints.

Table 6.6. Composition of milk samples collected on the day of farrowing and 14 d post-farrowing from sows fed diets containing high or low protein during gestation and lactation[1]

Item	Diet		SEM	*P*-value
Dietary protein:	High	Low		
Colostrum				
Fat, %	5.12	4.80	0.255	0.265
Total solids, %	24.92	24.61	0.484	0.551
IgG[2], mg/mL	81.07	75.47	1.094	< 0.001
Milk				
Fat, %	7.93	7.69	0.044	< 0.001
Free fatty acids, %	0.40	0.30	0.007	< 0.001
Protein, %	4.64	4.50	0.028	< 0.001
MUN[2], mg/dL	45.54	42.36	0.194	< 0.001
Lactose, %	4.70	4.60	0.024	0.002
Other solids, %	6.24	6.25	0.017	0.772
Total solids, %	24.92	24.61	0.484	0.551
SCC[2], × 1,000/mL	98.00	100.53	1.812	0.254
IgG[2], mg/mL	1.25	1.11	0.032	0.003

[1]Least square means for each dependent variable represent 78 and 76 observations for the high-protein and low-protein diet, respectively.

[2]MUN, milk urea nitrogen; SCC, somatic cell count; IgG, immunoglobulin G.

Table 6.7. Serum immune response of sows fed diets containing high or low protein during gestation and lactation[1,2]

Item	Diet		SEM	*P*-value
Dietary protein:	High	Low		
Malonaldehyde, nmol/mL	2.77	2.89	0.042	0.026
Glutathione peroxidase, U/L	1,128.15	1,052.23	11.106	< 0.001
Leukocyte Profile				
White blood cell count, ×10^3/ul	14.31	13.38	0.331	0.039
Neutrophils, %	50.99	50.70	1.208	0.868
Lymphocytes, %	36.57	36.16	0.956	0.757
Monocytes, %	5.95	6.11	0.312	0.715
Eosinophils, %	4.79	5.19	0.276	0.306
Basophils, %	1.06	0.96	0.100	0.458
Cytokines, ng/mL				
IFN-γ	18.03	13.50	2.131	0.071
IL-1α	0.21	0.21	0.026	0.905
IL-1β	0.64	0.83	0.120	0.118
IL-1Ra	1.58	1.44	0.181	0.542
IL-2	0.98	1.32	0.162	0.093
IL-4	15.15	9.66	1.574	0.005
IL-6	0.61	0.65	0.077	0.736
IL-8	0.06	0.08	0.013	0.461

Table 6.7. (Cont.)

IL-10	5.53	4.95	0.566	0.451
IL-12	0.65	0.71	0.099	0.524
IL-18	5.68	5.20	0.752	0.532
TNF-α	0.81	0.24	0.379	0.282

[1]Least square means for each dependent variable represent 78 and 76 observations for the high-protein and low-protein diet, respectively, after the removal of outliers.

[2]IFN-γ, interferon-gamma; IL-, interleukin-; IL-1Ra, interleukin-1 receptor antagonist; TNF-α, tumor necrosis factor-α.

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CHAPTER 7: Impact of soybean meal on nitrogen retention and concentrations of energy in diets fed to growing pigs

Abstract

The objective of this work was to test the hypothesis that adding Val, Ile, and Trp to diets dried grains with solubles (**DDGS**), or high-protein DDGS (**HP-DDGS**) would result in nitrogen retention and digestible energy (**DE**) in the diet without affecting metabolizable energy (**ME**). that is not different from what is obtained from a diet based on corn and soybean meal (**SBM**). The second hypothesis was that pigs fed a diet based on corn and SBM will have greater daily nitrogen retention than pigs fed a diet based on corn, less SBM, and synthetic amino acids (**AA**). Six diets were formulated. Two diets contained corn and 31 or 21% SBM, and four additional diets contained corn, SBM, and DDGS or HP-DDGS, without or with supplementation of Trp, Val, and Ile. A total of 48 pigs (initial weight: 39.91 ± 3.9 kg) were allotted to the six diets in a randomized complete block design. Pigs were housed individually in metabolism crates, with seven days of adaptation and four days of fecal and urine collection. The apparent total tract digestibility (**ATTD**) of energy and nitrogen, retention of nitrogen, and the biological value of nitrogen were calculated for each diet. The DE and ME for each diet were also calculated. Results indicated that fecal output of gross energy and nitrogen were greater ($P < 0.05$) for pigs fed DDGS containing diets compared with pigs fed the SBM or HP-DDGS diets. The ATTD of dry matter, energy, and nitrogen was greater ($P < 0.05$) for pigs fed SBM diets than for pigs fed DDGS or HP-DDGS diets. Absorbed nitrogen was greater ($P < 0.05$) for pigs fed 31% SBM compared with pigs fed the other diets, and retained nitrogen (g/d) was reduced ($P < 0.05$) in pigs fed the 21% SBM or the HP-DDGS diet with added AA compared with pigs fed the 31%

SBM diet or the DDGS diet with added AA. The DE and ME were greater ($P < 0.05$) in SBM and HP-DDGS diets compared with DDGS diets, regardless of addition of AA. In conclusion, feeding SBM increased nitrogen retention and provided greater DE and ME compared with feeding DDGS diets, indicating that supplementation with Ile, Val, and Trp to reduce the negative effects of excess of Leu in corn coproducts did not fully compensate for reduced nitrogen and energy digestibility when SBM was replaced with corn co-products. Likewise, the reduced nitrogen retention in pigs fed the 21% SBM diet compared with the 31% SBM diet indicates that replacing SBM with crystalline AA reduces nitrogen retention.

Keywords: Amino acids, DDGS, energy, HP-DDGS nitrogen retention, pigs, SBM.

Abbreviations: AA, amino acids; ATTD, apparent total tract digestibility; DDGS, corn distillers dried grains with solubles; DE, digestible energy; DM, dry matter; GE, gross energy; HP-DDGS, high-protein DDGS; ME, metabolizable energy; SBM, soybean meal.

Introduction

Soybean meal (**SBM**) is often included in cereal-based diets for growing pigs because SBM provides a well-balanced profile of digestible amino acids (**AA**), which maximizes growth performance and protein synthesis (Stein et al., 2013). However, in recent years, SBM has often been partially replaced by crystalline AA or alternative protein sources such as corn distillers dried grains with solubles (**DDGS**) or corn protein (Stein and Shurson, 2009; Anderson et al., 2012; Rothmund et al., 2026). Depending on processing methods, physical characteristics, chemical composition, and nutrient digestibility of DDGS may vary (Curry et al., 2016). When

oil and solubles are separated, low-oil DDGS is produced (Kerr et al., 2013; Espinosa and Stein, 2018), and fractionation of the protein of the grain results in production of high-protein DDGS (**HP-DDGS;** Rosentrater, 2012; Espinosa and Stein, 2018; Ruiz-Arias et al., 2024). However, DDGS and HP-DDGS have a less balanced AA profile and greater fiber concentrations than SBM, and AA digestibility in HP-DDGS is less than in SBM (Garavito-Duarte et al., 2023).

Modern genotypes of pigs are leaner and have greater requirements for dietary AA to support protein synthesis than older genetic pigs lines (Stein et al., 2024). It is often assumed that replacing SBM with corn and crystalline AA increases the energy of the diet because corn contains more metabolizable energy (**ME**) than SBM (NRC, 2012). However, recent data indicate that SBM may provide more digestible energy (**DE**) and ME than corn (Sotak-Peper et al., 2015), and that pigs fed corn-SBM diets without addition of crystalline AA retain more nitrogen than pigs fed diets based on corn, crystalline AA, and less SBM (Cristobal et al., 2025; Ibagon et al., 2026). Soybean meal has also been replaced in diets for growing and finishing pigs with corn-coproducts and crystalline AA, but growth performance is sometimes reduced in pigs fed diets containing corn-coproducts instead of SBM (Giacomini et al., 2025). It is, however, possible that the reduced growth performance is due to the high concentrations of Leu in corn-coproducts because excess Leu in diets for pigs results in reduced nitrogen retention and brain serotonin concentrations (Kwon et al., 2019). However, negative impacts of excess dietary Leu may be partially overcome by adding extra Val, Ile, and Trp to the diets (Kwon et al., 2019; 2023; Mallea et al., 2024). It is, therefore, possible that the reduction in nitrogen and energy utilization that has been reported for pigs fed diets containing corn-coproducts may be ameliorated by adding extra Val, Ile, and Trp to the diet, but it is not known if this will result in nitrogen retention similar to that of pigs fed corn-SBM diets. Therefore, the objective of this

work was to test the hypothesis that adding Val, Ile, and Trp to diets containing DDGS or HP-DDGS would result in nitrogen retention and DE that is not different from what is obtained from a diet based on corn and SBM. The second hypothesis was that pigs fed a diet based on corn and SBM will have greater daily nitrogen retention than pigs fed a diet based on corn, less SBM, and synthetic AA.

Materials and Methods

The protocol for the experiment was reviewed and approved by the Institutional Animal Care and Use Committee at the University of Illinois.

Experimental diets

A control diet contained corn and 31.2% SBM as the only sources of protein and AA, and a second diet that contained corn, 21.0% SBM, and $_{L}$-Lys·HCl, $_{DL}$-Met, and $_{L}$-Thr was also formulated (Table 7.1). Two additional diets that contained corn, SBM, and 30% DDGS or 15% HP-DDGS, as well as $_{L}$-Lys·HCl, $_{DL}$-Met, $_{L}$-Thr, and $_{L}$-Trp were also formulated. All diets contained AA to meet requirements for 40 kg pigs (NRC, 2012). Two additional diets also contained corn, SBM, and 30% DDGS or 15% HP-DDGS, but these diets were supplemented with additional crystalline AA (i.e., 0.05% $_{L}$-Trp, 0.10% $_{L}$-Val, and 0.10% $_{L}$-Ile) to ameliorate the negative effects of the excess Leu in corn co-products (Kwon et al., 2022; Mallea et al., 2024). Vitamins and minerals were included in all diets to meet or exceed current requirement estimates for growing pigs (NRC, 2012). All diets were fed in meal form. The daily feed allowance was calculated as 3.2 times the maintenance requirement for ME (i.e., 197 kcal ME per kg body weight$^{0.60}$; NRC, 2012). Feed was provided daily in two equal meals that were provided at 0700 h and 1600 h.

Animals, housing and feeding

A total of 48 pigs with an initial body weight of 39.91 ± 3.90 kg were allotted to the six dietary treatments using a randomized complete block design. Pigs were the offspring of Line 800 males mated to Camborough females (Pig Improvement Company, Hendersonville, TN, USA). There were two blocks of 24 pigs with four pigs per diet in each block for a total of eight replicate pigs per diet. The blocking factor was the weaning group and pigs in the two blocks were weaned two weeks apart. Pigs were housed individually in metabolism crates (0.81 × 1.52 m) that were equipped with a self-feeder, a nipple waterer, a fully slatted floor, a screen floor, and urine trays that allowed for the total, but separate, collection of urine and fecal materials from each pig. Throughout the experiment, pigs had free access to water.

Sample collection

Pigs were fed experimental diets for 13 days with feed disappearance recorded daily. The initial seven days were considered the adaptation period to the diet, whereas urine and fecal materials were collected from feed provided during the following four days according to the marker-to-marker approach (Adeola, 2001). Indigo carmine was used to mark the initiation of feces collection and was included in the morning meal on day eight. Fecal collection ceased when the second marker, ferric oxide, which was included in the morning meal on day 12, appeared in the feces. Orts were collected daily and weighed to determine feed intake from day eight to day 12. Urine was collected in urine buckets over a preservative of 50 mL of 6 *N* HCl. Fecal samples, orts, and 20% 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, sub-sampled, and stored at -20 ºC until analysis for nitrogen. A second urine sample was lyophilized to quantify gross energy (**GE**) in urine (Kim et al., 2009). Fecal samples were thawed and mixed within pig and diet, dried in a forced-air oven, and finely ground using a 500 G stainless steel swing-type mill grinder (RRH, Zhejiang, China). Ingredients, diets, and fecal samples were analyzed for dry matter (**DM**; method 930.15; AOAC Int., 2019) and diets and ingredients were also analyzed for ash (method 942.05; AOAC Int., 2019). Diets, ingredients, feces, and lyophilized urine were analyzed for GE using a bomb calorimeter (Model 6400, Parr Instruments, Moline, IL, USA), with benzoic acid as the calibration standard. The concentration of nitrogen was also analyzed in diets, ingredients, fecal and urine samples using the Kjeldahl method on a Kjeltec^{TM} 8400 apparatus (FOSS Inc., Eden Prairie, MN, USA). Crude protein was calculated as analyzed nitrogen × 6.25. Diet and ingredient samples were analyzed for AA on a Hitachi Amino Acid Analyzer, Model No. L8800 (Hitachi High Technologies America, Inc.; Pleasanton, CA, USA) using ninhydrin for post-column derivatization and norleucine as the internal standard (method 982.30 E [a, b, c]; AOAC Int., 2019). Ingredients 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). Total starch was also analyzed in ingredients 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, 2017).

Calculations and statistical analysis

The apparent total tract digestibility (**ATTD**) of nitrogen was calculated using analyzed nitrogen in diets and feces (NRC, 2012). Nitrogen retention was calculated as a percentage of nitrogen intake and in grams per day, and the biological value of nitrogen in each diet was calculated as well. (Rojas and Stein, 2013). The ATTD of DM and GE, as well as the DE and ME for each diet were also calculated (NRC, 2012). Data were analyzed using the MIXED procedure of SAS, and pig was the experimental unit for all analyses. Model assumptions on the residuals were confirmed using the MIXED procedure and the Brown-Forsythe test of the GLM procedure of SAS (version 9.4; SAS Inst. Inc., Cary, NC, USA). The MIXED procedure in SAS was used to generate studentized residuals, and outliers were defined as data points having studentized residuals greater than 3 or less than −3. However, no outliers were detected in the data set. The statistical model included diet as the fixed effect and block and replicate within block as random effects. Least square means were calculated, and if the model was significant, means were separated using the PDIFF option with Tukey's adjustment. Statistical significance and tendencies were considered at $P < 0.05$ and $0.05 \leq P < 0.10$, respectively.

Results

All pigs consumed their diets throughout the experiment without apparent problems, and all 48 pigs completed the experiment. Analyzed concentrations of energy and nutrients in diets and ingredients were in agreement with calculated values (Tables 7.2 and 7.3; NRC 2012).

There were no effects of treatment on feed intake or GE intake (Table 7.4). Daily fecal output and daily fecal GE output were greater ($P < 0.05$) from pigs fed the two DDGS diets compared with pigs fed the other diets, but daily fecal GE output was less in pigs fed the two

SBM diets compared with pigs fed the HP-DDGS diet with no additional AA. Excretion of GE in urine was not different among diets, but a tendency ($P = 0.080$) for greater urine output was observed from pigs fed the two SBM diets compared with pigs fed the other four diets.

The ATTD of DM and GE was greater ($P < 0.05$) for the two SBM diets than for pigs fed the DDGS or HP-DDGS diets, except that the ATTD of DM was not different between the 31% SBM diet and the HP-DDGS diet without additional AA. The ATTD of DM and GE was also greater ($P < 0.05$) for pigs fed the HP-DDGS diets than for pigs fed the DDGS diets. The DE and ME were greater ($P < 0.05$) in diets containing SBM or HP-DDGS compared with the DDGS diets, regardless of the addition of crystalline AA. The ME to DE ratio tended to be greater ($P = 0.072$) for pigs fed the HP-DDGS diet with additional AA compared with pigs fed the DDGS diet without additional AA. The ME to GE ratio was greater ($P < 0.05$) for the 21% SBM diet compared with the two DDGS diets or the HP-DDGS diet without AA addition, and the 31% SBM diet and the HP-DDGS diet with additional AA had greater ($P < 0.05$) ME to GE ratio than the two DDGS diets.

Nitrogen intake of pigs was less ($P < 0.05$) for pigs fed the 21% SBM diet compared with pigs fed the 31% SBM diet, the two DDGS diets, or the HP-DDGS diet containing no additional AA (Table 7.5). Pigs fed the HP-DDGS diets had less ($P < 0.05$) nitrogen intake than pigs fed the DDGS with additional AA diet or the 31% SBM diet, but pigs fed the 31% SBM diet had greater ($P < 0.05$) nitrogen intake than pigs fed the DDGS diet without additional AA. Daily fecal nitrogen excretion was greater ($P < 0.05$) from pigs fed the two DDGS diets compared with pigs fed the other diets, except that daily fecal nitrogen excretion from pigs fed the HP-DDGS diet without extra crystalline AA was not different from that of pigs fed the DDGS diets. Pigs fed the two SBM diets also had less ($P < 0.05$) fecal nitrogen excretion than pigs fed the HP-DDGS diet

without additional AA. However, urine nitrogen excretion was greater ($P < 0.05$) from pigs fed 31% SBM diet than for pigs fed the other five diets. Absorbed nitrogen was also greater ($P < 0.05$) for pigs fed the 31% SBM than for pigs fed the other diets, but absorbed nitrogen was less ($P < 0.05$) for the 21% SBM diet compared with the DDGS diet with additional AA. Retained nitrogen (g/day) by pigs fed the 21% SBM diet or the HP-DDGS diets was less ($P < 0.05$) than by pigs fed the 31% SBM diet or the DDGS diet with additional AA. The ATTD of nitrogen was greater ($P < 0.05$) for pigs fed the two SBM diets than for pigs fed the DDGS or HP-DDGS diets, with the exception that ATTD of nitrogen was not different between pigs fed the 21% SBM and the HP-DDGS with additional AA diets. The ATTD of nitrogen in the HP-DDGS diet with additional AA was also greater ($P < 0.05$) than in the DDGS diet without additional AA. Nitrogen retention (% of intake) was not affected by dietary treatment. The biological value was greater ($P < 0.05$) for the DDGS diet with additional AA compared with the 31% SBM diet, but no differences were observed among the other diets.

Discussion

The lack of an effect of diet on daily feed intake was expected and in agreement with previous data (Garavito-Duarte et al., 2023; Kwon et al., 2023). The ATTD of DM in the diets containing DDGS or HP-DDGS was expected to be less than in the diets containing SBM due to the greater total dietary fiber in corn co-products compared with corn or SBM (NRC, 2012). The ATTD of DM is positively correlated with energy digestibility of diets and feed ingredients (Navarro et al., 2018; Ibagon et al., 2023). Therefore, the observation that the 31% SBM diet had greater ATTD of GE than diets containing corn-coproducts is likely a result of the reduced dietary fiber in the SBM diets, which reduced dry feces excretion and consequently the excretion

of energy in feces. The ME of SBM may be similar to that of corn (Sotak-Peper et al., 2015; Ibagon et al., 2025), and the observation that the 31% SBM diet had DE and ME that were not different from the 21% SBM diet indicates that ATTD of GE is not different between corn and SBM, which is also in agreement with previous data (Sotak-Peper et al., 2015; Cristobal et al., 2025). The observation that both diets containing DDGS had reduced DE and ME compared with diets containing HP-DDGS is likely due to the greater concentration of fiber in DDGS than in HP-DDGS, whereas HP-DDGS contains more protein than DDGS, which likely also contributed to the greater DE and ME (Ruiz-Arias et al., 2024). The lack of differences in ME or DE between diets containing SBM and HP-DDGS diets reflects that there is no difference in ME among corn, SBM, and HP-DDGS (Sotak-Peper et al., 2015; Garavito-Duarte et al., 2023; Ruiz-Arias, et al., 2024). The implication of these observations is that SBM contributes as much DE and ME to diets as corn or HP-DDGS, indicating that SBM is not only a source of digestible AA, but also a significant source of energy in swine feeding programs. The observation that the ME to GE ratio was reduced in diets containing DDGS or HP-DDGS compared with SBM is likely a result of the increased fiber in these diets, which increases the excretion of energy in the feces. The lack of differences in the ME to DE ratio among diets is a result of the lack of differences among diets in the excretion of GE in urine. (Espinosa and Stein, 2018; Garavito-Duarte et al., 2023).

Reduced urine nitrogen excretion by pigs fed diets containing 21% SBM, corn, and crystalline AA compared with pigs fed a corn-SBM diet without crystalline AA has been previously observed (Kerr and Easter, 1995; Cristobal et al., 2025; Ibagon et al., 2026). However, the reduced daily nitrogen retention by pigs fed the 21% SBM diet compared with pigs fed the 31% SBM diet is somewhat surprising because both diets were formulated to meet

requirements for all digestible AA (NRC, 2012). It is not clear why two diets that are formulated to contain similar quantities of digestible AA result in different nitrogen retention, but it may be related to a faster absorption of crystalline AA than protein-bound AA. Theoretically, if crystalline AA arrive at protein-synthesizing cells before other AA, they may be deaminated before they can be used for protein synthesis (Morales et al., 2015; Eugenio et al., 2022) which may result in reduced protein synthesis. However, this hypothesis has not been experimentally verified. In the 21% SBM diet, the replacement of SBM by corn and crystalline AA, a greater proportion of the indispensable AA supply was provided by free AA, which may have increased the asynchrony between the arrival of free and protein-bound AA at the protein-synthesis site and consequently reduced nitrogen retention. This observation is in agreetment with results with recently publish data (Cristobal et al., 2025; Ibagon et al., 2026). Therefore, the hypothesis that a diet containing corn and SBM and no crystalline AA results in a greater nitrogen retention than a diet containing corn, SBM, and crystalline Lys, Thr, and Met was accepted. This observation may have practical implications because the 21% SBM diet used in this experiment is similar to diets often used in practical diets for pigs.

The reduced nitrogen excretion in urine by pigs fed the DDGS and HP-DDGS diets compared with pigs fed the 31% SBM diets is likely a result of the addition of crystalline AA to these diets, which also resulted in less crude protein in the diets compared with the 31% SBM diet. Reduced dietary crude protein and a more balanced indispensable AA supply reduce deamination of AA and, therefore, reduce nitrogen excretion in urine (Duarte et al., 2024; Ibagon et al., 2026).

Soybean meal is the premium source of AA in swine diets due to its well-balanced profile of digestible AA (NRC, 2012). Therefore, the reduced digestibility of nitrogen in diets

containing corn-coproducts or less SBM and more corn is likely the result of the fact that the digestibility of AA in corn-coproducts and corn is less than the digestibility of AA in SBM (Xue et al., 2014; Garavito-Duarte et al., 2023). The digestibility of synthetic AA is close to 100% (Oliveira et al., 2020), but in the diets containing 21% SBM or corn co-products, supplementation with crystalline AA did not fully compensate for the reduced nitrogen digestibility in corn or corn co-products.

Diets based on corn and corn-coproducts are rich in Leu (Kwon et al., 2019). However, Leu stimulates catabolism of Ile and Val in the liver (Harper et al., 1984), and if pigs are fed diets with excess Leu, degradation of not only Leu, but also Val and Ile, may increase, resulting in an imbalance among indispensable AA, which will reduce nitrogen retention (Kwon et al., 2019; 2022). The reduced daily absorbed nitrogen by pigs fed diets containing DDGS, HP-DDGS, or 21% SBM compared with pigs fed the 31% SBM is mainly due to the reduction in the daily nitrogen intake, because crude protein in these diets was reduced compared with the 31% SBM diet. The reduced daily retention of nitrogen by pigs fed both HP-DDGS diets compared with the nitrogen retention of pigs fed the 31% SBM may be due to the imbalance in AA available for protein synthesis generated by excess Leu in HP-DDGS and is in agreement with reported data (Kwon et al., 2019). However, the observation that the nitrogen retention measured in grams per day was not different between the 31% SBM diet and the DDGS diet containing Val, Ile, and Trp indicates that the negative effect of excess Leu in the DDGS diet can be ameliorated with the inclusion of extra Trp, Val, and Ile in the diets. However, the fact that the addition of Trp, Val, and Ile to the HP-DDGS diets to mitigate the effects of excess Leu in these diets did not improve nitrogen retention, measured in grams per day, is likely a result of to the much greater concentration of Leu in HP-DDGS (6.39%) compared with DDGS (2.94%). Excess

of dietary Leu may stimulate the branched AA transferred enzyme and the branched-chain keto acid dehydrogenase complex, which will increase catabolism of all three branched-chain AA (Harper et al., 1984; Kwon et al., 2019). Although supplementation of Val and Ile has been reported to ameliorate the negative effects of excess Leu in diets (Mallea et al., 2024), this response appears to decrease when dietary Leu is excessively high because the increased catabolism of branched-chain AA continuously degrades Val and Ile before they can be utilized for protein synthesis (Kwon et al., 2019). However, the fact that the addition of Trp, Val, and Ile to the HP-DDGS diets did not improve nitrogen retention indicates that the imbalance caused by the excess Leu in HP-DDGS was too high to be neutralized by extra Val and Ile. It may be speculated that the fiber in diets containing HP-DDGS may be as important in reducing nitrogen retention as is the excess Leu, because greater dietary fiber may increase hindgut fermentation and consequently increase nitrogen losses. However, the observation that DDGS diets, which contained more fiber concentrations than HP-DDGS diets, responded positively to Val, Ile, and Trp supplementation indicates that fiber was not the primary limitation in the HP-DDGS diets, and that the excess of Leu was the main factor responsible for the reduced nitrogen retention in pigs fed HP-DDGS diets, regardless of AA supplementation. Therefore, based on the results of the current experiment, the hypothesis that addition of Val, Ile, and Trp to diets containing HP-DDGS will increase nitrogen retention comparable with the 31% SBM diet had to be rejected. However, the fact that nitrogen retention in pigs fed the DDGS diet increased when additional Val, Ile, and Trp were added to the diet indicates that the response to supplementation of branched-chain amino acids may depend on the type of corn coproduct included in the diet.

Conclusions

Pigs fed the 31% SBM diet retained more nitrogen (g/day) than pigs fed the other diets, and in agreement with results of previous research, a diet containing only corn and SBM resulted in greater nitrogen retention than a diet containing corn, SBM, and crystalline AA. Supplementation of crystalline Trp, Val, and Ile to ameliorate the negative effects of high Leu in DDGS and HP-DDGS diets did not improve nitrogen retention (g/day) of HP-DDGS diets. However, nitrogen retention was improved to values comparable to the 31% SBM when Val, Ile, and Trp were added to the DDGS diet. Diets containing SBM or HP-DDGS had greater DE and ME compared with the DDGS diets, regardless of AA supplementation.

Tables

Table 7.1. Ingredient composition of the diets fed to growing pigs, as-fed basis[1]

Item, %	31% SBM	21% SBM + AA	DDGS	DDGS + additional AA	HP-DDGS	HP-DDGS + additional AA
Ingredient composition						
Corn	65.24	74.83	55.73	55.48	75.33	75.08
Soybean meal	31.20	21.00	10.00	10.00	5.00	5.00
Corn DDGS, low oil	-	-	30.00	30.00	-	-
Corn protein	-	-	-	-	15.00	15.00
L-Lys·HCl, 78% Lys	-	0.32	0.57	0.57	0.66	0.66
DL-Met, 99% Met	-	0.06	0.01	0.01	0.02	0.02
L-Thr, 99% Thr	-	0.08	0.09	0.09	0.16	0.16
L-Trp, 99% Trp	-	-	0.04	0.09	0.05	0.10
L-Val, 99% Val	-	-	-	0.10	-	0.10
L-Ile, 99% Ile	-	-	-	0.10	-	0.10
Soybean oil	1.00	1.00	1.00	1.00	1.00	1.00
Dicalcium phosphate	0.87	1.05	0.45	0.45	0.92	0.92
Limestone	0.79	0.76	1.21	1.21	0.96	0.96
Sodium chloride	0.40	0.40	0.40	0.40	0.40	0.40
Vitamin-mineral premix[2]	0.50	0.50	0.50	0.50	0.50	0.50

[1]SBM, soybean meal; DDGS, corn distillers dried grains with solubles; HP-DDGS, high-protein DDGS.

Table 7.1. (Cont.)

[2]The vitamin-micromineral premix provides 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 dihydroiodide; 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 7.2. Analyzed nutrient composition of the diets used in experimental diets, as-fed basis[1]

Item, %	31% SBM	21% SBM + AA	DDGS	DDGS + additional AA	HP-DDGS	HP-DDGS + additional AA
Dry matter	88.65	88.03	88.72	88.57	88.65	88.58
Ash	5.24	4.41	4.93	4.92	3.24	3.22
Nitrogen × 6.25	19.26	15.55	17.74	18.03	17.33	17.29
Gross energy, kcal/kg	3,898	3,873	4,035	4,043	4,071	4,082
Indispensable AA						
Arg	1.13	0.88	0.90	0.88	0.77	0.74
His	0.47	0.40	0.47	0.46	0.45	0.44
Ile	0.80	0.64	0.69	0.80	0.68	0.78
Leu	1.63	1.42	1.84	1.83	1.91	1.85
Lys	0.98	1.02	1.25	1.28	1.23	1.26
Met	0.25	0.30	0.31	0.31	0.33	0.32
Phe	0.92	0.76	0.87	0.86	0.84	0.81
Thr	0.68	0.64	0.78	0.75	0.75	0.73
Trp	0.20	0.15	0.19	0.22	0.16	0.23
Val	0.88	0.72	0.85	0.96	0.83	0.94
Total	7.94	6.93	8.15	8.35	7.95	8.10
Dispensable AA						
Ala	0.94	0.83	1.15	1.13	1.13	1.09
Asp	1.74	1.37	1.34	1.32	1.18	1.11

Table 7.2. (Cont.)

Cys	0.29	0.26	0.33	0.33	0.33	0.32
Glu	3.33	2.78	3.01	2.96	3.02	2.87
Gly	0.73	0.60	0.71	0.69	0.63	0.60
Pro	1.17	1.01	1.32	1.32	1.36	1.34
Ser	0.80	0.67	0.75	0.74	0.71	0.68
Tyr	0.65	0.53	0.63	0.63	0.62	0.61
Total AA	17.59	14.98	17.39	17.47	16.93	16.72
Indispensable AA, as % of total AA	45.14	46.26	46.87	47.80	46.96	48.44
Calculated composition, %						
Crude protein	20.27	16.58	18.33	18.31	16.70	16.68
SID Lys	0.94	0.94	0.94	0.94	0.95	0.95
SID Lys: CP	4.65	5.69	5.13	5.13	5.67	5.67
Metabolizable energy, kcal/kg	3,328	3,338	3,267	3,258	3,369	3,361
STTD of P	0.30	0.30	0.30	0.30	0.30	0.30
Total Ca	0.64	0.64	0.64	0.64	0.64	0.64

[1]SBM, soybean meal; DDGS, corn distillers dried grains with solubles; HP-DDGS, high-protein DDGS.

Table 7.3. Analyzed composition of ingredients used in experimental diets

Item, %	Corn	Soybean meal[1]	Corn DDGS[2]	HP-DDGS[2]
Dry matter	86.89	88.06	87.70	92.55
Ash	1.14	9.89	5.51	0.88
Nitrogen × 6.25	8.09	44.52	27.22	54.53
Starch	62.10	3.55	6.20	1.20
Acid hydrolyzed ether extract	2.51	1.72	3.63	8.21
Gross energy, kcal/kg	3,857	3,922	4,337	5,175
Indispensable amino acids				
Arg	0.33	2.96	1.25	2.19
His	0.22	1.09	0.79	1.49
Ile	0.29	2.03	1.05	2.12
Leu	0.95	3.25	2.94	6.39
Lys	0.25	2.64	1.05	1.74
Met	0.15	0.51	0.50	1.17
Phe	0.38	2.17	1.31	2.72
Thr	0.26	1.60	1.02	1.93
Trp	0.05	0.51	0.15	0.28
Val	0.37	2.11	1.37	2.69
Dispensable amino acids				
Ala	0.58	1.83	1.90	3.74
Asp	0.50	4.57	1.67	3.34

Table 7.3. (Cont.)

Cys	0.17	0.57	0.54	1.09
Glu	1.47	7.66	3.83	9.02
Gly	0.29	1.77	1.14	1.96
Pro	0.70	2.24	1.96	4.27
Ser	0.34	1.78	1.12	2.25
Tyr	0.22	1.57	0.97	2.23
Total amino acids	7.52	40.86	24.56	50.62

[1]Low molecular weight carbohydrates in soybean meal (%): glucose, 0.05; sucrose, 6.26; maltose, 0.21; fructose, 0.05; stachyose, 5.16; and raffinose, 1.04. Trypsin inhibitors (TIU/mg): 1.63.

[2]DDGS, dry distillers garains with solubles; HP-DDGS, high-protein DDGS.

Table 7.4. Apparent total tract digestibility (ATTD) of dry matter and energy, and concentrations of digestible energy (DE) and metabolizable energy (ME) in experimental diets fed to growing pigs, as-fed basis[1,2]

Item	31% SBM	21% SBM + AA	DDGS	DDGS + additional AA	HP-DDGS	HP-DDGS + additional AA	SEM	*P*-value
Intake								
Feed, kg/day	1.69	1.69	1.68	1.73	1.65	1.63	0.06	0.235
Gross energy, kcal/day	6,579	6,538	6,776	6,981	6,707	6,634	240	0.116
Fecal excretion								
Dry feces output, g/day	153[b]	150[b]	301[a]	288[a]	189[b]	187[b]	12	< 0.001
Gross energy, kcal/day	714[c]	711[c]	1,422[a]	1,360[a]	960[b]	932[bc]	60	< 0.001
Urine excretion								
Urine output, kg/day	7.26	8.30	6.54	5.72	5.50	3.16	1.21	0.080
Gross energy, kcal/day	192	163	189	168	166	141	15	0.155
ATTD of dry matter, %	90.29[ab]	90.39[a]	81.01[d]	82.23[d]	87.77[bc]	87.63[c]	0.61	< 0.001
ATTD of gross energy, %	89.13[a]	89.08[a]	79.09[c]	80.59[c]	85.81[b]	85.97[b]	0.71	< 0.001
Energy in diets, kcal/kg								
Digestible energy	3,475[a]	3,450[a]	3,191[b]	3,258[b]	3,493[a]	3,510[a]	29	< 0.001

Table 7.4. (Cont.)

Metabolizable energy	3,361[a]	3,350[a]	3,077[b]	3,163[b]	3,393[a]	3,425[a]	30	< 0.001
Energy efficiency, %								
ME to DE	96.74	97.16	96.48	97.04	97.10	97.55	0.25	0.072
ME to GE	86.22[ab]	86.50[a]	76.27[c]	78.23[c]	83.33[b]	83.90[ab]	0.75	< 0.001

[a-d]Within a row, means without a common superscript differ ($P < 0.05$).

[1]Least square means represent eight ($n = 8$) observations for all treatments.

[2]SBM, soybean meal; DDGS, corn distillers dried grains with solubles; HP-DDGS, high-protein DDGS.

Table 7.5. Apparent total tract digestibility (ATTD) of nitrogen and biological value of experimental diets fed to growing pigs, as-fed basis[1,2]

Item	31% SBM	21% SBM + AA	DDGS	DDGS + additional AA	HP-DDGS	HP-DDGS + additional AA	SEM	*P*-value
Nitrogen intake, g/day	52[a]	42[d]	48[bc]	50[ab]	46[c]	45[cd]	1.69	< 0.001
Nitrogen in feces, g/day	6[c]	5[c]	9[a]	9[a]	8[ab]	7[bc]	0.50	< 0.001
Nitrogen in urine, g/day	14[a]	9[b]	8[b]	8[b]	9[b]	9[b]	0.92	0.001
ATTD of nitrogen, %	89.01[a]	86.96[ab]	80.55[d]	82.03[cd]	83.00[cd]	84.23[bc]	0.86	< 0.001
Nitrogen absorbed, g/day	46[a]	36[c]	38[bc]	41[b]	38[bc]	38[bc]	1.38	< 0.001
Nitrogen retained, g/day	32[ab]	28[c]	30[abc]	33[a]	29[c]	29[c]	1.63	< 0.001
Nitrogen retention, % of intake	62.53	66.30	63.43	66.69	64.16	64.58	1.96	0.377
Biological value[3], %	70.26[b]	76.25[ab]	79.20[ab]	80.80[a]	77.18[ab]	76.96[ab]	2.48	0.019

[a-d]Within a row, means without a common superscript differ ($P < 0.05$).

[1]Least square means represent eight ($n = 8$) observations for all treatments.

[2]SBM, soybean meal; DDGS, corn distillers dried grains with solubles; HP-DDGS, high-protein DDGS.

[3]Calculated by dividing retained nitrogen by absorbed nitrogen and multiplying by 100 (Rojas and Stein, 2013).

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CHAPTER 8: Effects of using soybean meal, canola meal, and distillers dried grains with solubles on net energy, greenhouse gas emissions, and nitrogen balance in group-housed pigs

Abstract

Two experiments were conducted to test the hypothesis that there are no differences in net energy (**NE**), greenhouse gas emissions, nitrogen balance or ileal digestibility of amino acids (**AA**) among pigs fed corn-based diets containing soybean meal (**SBM**), canola meal (**CM**), or corn distillers dried grains with solubles (**DDGS**). Three diets contained corn, and one of the three feed ingredients (i.e., SBM, CM, or DDGS) feed to pigs in experiments 1 and 2. In experiment 1, a nitrogen-free diet was also used to calculate basal endogenous losses of AA and nitrogen, but in experiment 2, no nitrogen-free diet was used. In experiment 1, 8 barrows (initial body weight = 36.75 ± 3.24 kg) that had a T-cannula installed in the distal ileum were allotted to a repeated 4 × 4 Latin square design with 4 diets and 4 periods. In experiment 2, a total of 24 growing pigs (initial weight = 46.84 ± 2.25 kg) were allotted to 6 chambers with 4 pigs per chamber. The 6 chambers were then allotted to the three diets using a repeated 3 × 3 Latin square design with 3 periods. Pigs had free access to water and feed. Feces and urine were quantitatively collected during the collection period, and O_2 consumption, CO_2, and CH_4 production, and urine nitrogen excretion were measured during collection and fasting periods. Results indicated that the apparent ileal digestibility of most indispensable AA AA was greater in the SBM diet than in the CM or DDGS diet. Apparent total tract digestibility (**ATTD**) of dry matter and gross energy were greater ($P < 0.05$) in the SBM diet compared with the CM and DDGS diets. The DDGS diet had greater ($P < 0.05$) NE than the CM diet, but the NE of the SBM diet was not different

from the other two diets. Daily O_2 consumption per kg gain was greater ($P < 0.05$), and CO_2 production per kg gain tended to be greater ($P < 0.10$) by pigs fed the CM diet compared with pigs fed the SBM or DDGS diets. However, production of CH_4 did not differ among the three diets. Intake of nitrogen and total nitrogen excretion were not different among pigs fed the three diets, but the ATTD of nitrogen and absorbed nitrogen were greater ($P < 0.05$), and retained nitrogen (g/d) tended to be greater ($P < 0.10$) for pigs fed the SBM diet compared with the CM or DDGS diets. Retention of nitrogen (% of intake) did not differ among the three diets. Biological value was not different between SBM and CM diets or between CM and DDGS diets, but was less ($P < 0.05$) in the SBM diet than in the DDGS diet. In conclusion, the use of SBM or DDGS increased NE in corn-based diets compared with CM when fed to group-housed pigs. Per kilogram of body weight gain, pigs fed the SBM or DDGS diets had less gas exchange than pigs fed the CM diet. Pigs fed the diet containing SBM had greater absorbed and retained nitrogen (g/d) compared with those fed diets containing CM or DDGS.

Keywords: Greenhouse gases**,** Net energy**,** nitrogen**,** pig, sustainability

Abbreviations: AA, amino acids; AID, apparent ileal digestibility; ATTD, apparent total tract digestibility; CH4, methane; CM, canola meal; CO2, carbon dioxide; DDGS, distillers dried grains with solubles; DE, digestible energy; DM, dry matter; FHP, fasting heat production; GE, gross energy; ME, metabolizable energy; NE, net energy; O2, oxygen; RQ, respiratory quotient; SBM, soybean meal; SID, standardized ileal digestibility; THP, total heat production.

Introduction

The carbon footprint from pork production can be reduced by decreasing emissions of greenhouse gases, including carbon dioxide, nitrous oxide, and methane from pigs (Zhang et al., 2024). It is possible that feeding pigs with diets containing a well-balanced amino acid (**AA**) profile reduces carbon footprint by reducing nitrogen excretion and emissions of greenhouse gases (Pomar et al., 2021). The trend over the last 30 years has been that less and less soybean meal (**SBM**) is used in diets for pigs despite the fact that pigs have become much leaner during that time and, therefore, need more AA for protein synthesis (Stein et al., 2024). Canola meal (**CM**) and corn distillers dried grains with solubles (**DDGS**) are the two most used alternative protein sources in the United States, but these ingredients have a more unbalanced AA composition than SBM, and they also contain more fiber, which results in increased hindgut fermentation in pigs (Urriola and Stein, 2010). Therefore, it is possible that the use of CM and DDGS in pig diets increases nitrogen excretion in manure and carbon footprint by increasing CO_2 and CH_4 emissions due to the increased fermentation but data to confirm this theory have not been reported. Therefore, the objective of these experiments was to test the hypothesis that pigs fed diets based on corn and CM or corn and DDGS excrete more nitrogen in feces and urine and emit more greenhouse gases than pigs fed corn-SBM diets.

Materials and Methods

Two experiments were conducted, and the protocols 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 used in both experiments were the offspring of Line 800 boars mated to Camborough females (Pig Improvement Company, Hendersonville, TN, USA).

Experimental diets, animals, and feeding

For both experiments, the same source of corn, CM, DDGS, and SBM was used (Table 8.1). Three diets were formulated based on corn and CM, DDGS or SBM and used in both experiments (Tables 8.2 and 8.3). Diets were formulated to meet current estimates for nutrient requirements for 50 to 75 kg pigs (NRC, 2012). In experiment 1, a nitrogen-free diet was also used to calculate basal endogenous losses of AA and nitrogen and 0.4% of chromic oxide was mixed into each diet. Synthetic AA, minerals, and vitamins were included in all diets to meet or exceed requirements for growing pigs (NRC, 2012). A sample of the main ingredients and all diets was collected at the time of diet mixing and used for chemical analysis. All diets were fed in a meal form.

In experiment 1, eight barrows with an average initial body weight of 36.8 ± 3.2 kg were allotted in a repeated 4 × 4 Latin square design with four diets and four periods (Kim and Stein, 2009). A T-cannula had been surgically inserted in the distal ileum of pigs for the collection of ileal digesta (Stein et al., 1998), when pigs had a body weight of approximately 21 kg, and pigs had been used in a previous experiment before being fed, a common diet for seven days, and then allotted to diets in the present expeirment. 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 a feeder and a nipple drinker were installed in each pen. Feed allowance was calculated as 3.2 times the maintenance requirement for metabolizable energy (i.e., 197 kcal metabolizable energy/kg body weight$^{0.60}$; NRC, 2012) and feed allowance was adjusted according to the body weight of pigs at the beginning of each period. All pigs had free access to water.

In experiment 2, 24 pigs with an average initial body weight of 46.8 ± 2.2 kg were allotted to 3 diets in a repeated 3 × 3 Latin square design with 6 calorimetry chambers and 3 consecutive periods (Muñoz-Alfonso et al., 2026). Four pigs (i.e., 2 gilts and 2 barrows) were housed in each chamber. The three diets were fed to pigs in each chamber in one period, and the same diet was provided only once to pigs in each chamber. Therefore, there were six replicate chambers per treatment. Each chamber was equipped with a feeder, a nipple waterer, a fully slatted floor, stainless steel screens for the collection of fecal materials, and urine pans, which allowed for the total, but separate, collection of feces and urine. The temperature in the chambers was maintained between 22 and 23 °C, and the relative humidity inside the chambers was 55%, controlled by temperature and humidity control units (Model 9241–2220-B1D0000; Parameter Generation & Control, Parameter, Black Mountain, NC, USA). The air velocity was 1.13 m^3/min, which was controlled 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 deprived of feed during the following 36 h. Throughout the experiment, water was freely available. At the conclusion of the third period, pigs had an average body weight of 87.8 ± 5.7 kg.

Sample collection

In experiment 1, each period lasted seven days, with the initial five days being the adaptation period to the diet, and ileal digesta were collected on days six and seven for nine hours each day (from 0700 to 1600 hours) following standard procedures (Stein et al., 1998). In short, a plastic bag was attached to the open cannula barrel using a cable tie, and the digesta flowing into the bag were collected. Bags were removed and replaced every 30 minutes or when they were filled with ileal digesta and immediately stored at −20 °C to prevent bacterial

degradation of AA in the digesta (Stein et al., 1998). At the conclusion of the experiment, ileal digesta samples were thawed at room temperature, mixed, and a subsample was collected, lyophilized, and finely ground in preparation for chemical analysis.

In experiment 2, the initial seven days of each period was for adaptation to the diet, but from the morning of day 8 to the morning of day 13, gas analyzers (Classic Line, Sable System International, North Las Vegas, NV, USA) measured oxygen (**O_2**) consumption, and carbon dioxide (**CO_2**) and methane (**CH_4**) production for determination of total heat production (**THP**). Fecal and urine samples were also collected quantitatively from days eight to 13. To avoid nitrogen loss in the urine, 50 mL of 6 *N* HCl was added to each urine pan daily. After the last meal, the initial 24 h of fasting are considered the time during which the animals digest and metabolize the remaining feed in the intestinal tract. Therefore, on the morning of day 14, pigs were deprived of feed, and after 24 hours, gas exchange was measured, and urine was collected for the following 12 h, which was considered the actual period during which animals mobilized endogenous nutrients to produce energy (de Lange et al., 2006). Fasting heat production (**FHP**) was calculated using urine nitrogen and measured O_2 consumption, and CO_2 and CH_4 production during this period. All pigs were weighed prior to being moved into calorimetry chambers and also at the conclusion of each collection period. Chambers were opened daily for approximately one hour to check feeders and pigs and collect feces and urine. Calculations of heat production did not include data recorded during this time or during the time required for the chambers to reach the conditions set by the temperature and humidity control unit. Feed spillage on the screens was collected daily during the collection period, and the weight of feed spilled was recorded to determine feed intake. 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) and 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 conclusion of the experiment, urine samples were thawed and mixed within each chamber and period, and two subsamples were collected. One urine subsample was lyophilized (Kim et al., 2009; Ibagon et al., 2026), and the other subsample was stored at −20 °C until analyzed for nitrogen. Likewise, a subsample of the urine collected during the fasting period was stored at −20 °C until analyzed for nitrogen.

Chemical analysis

Diet and ingredient samples were analyzed for dry matter (method 927.05; AOAC Int., 2019) and ash (method 942.05; AOAC Int., 2019). Gross energy was analyzed using an isoperibol bomb calorimeter (Model 6400, Parr Instruments, Moline, IL, USA). Benzoic acid was used for standard calibration. The concentration of nitrogen was analyzed by combustion (method 990.03; AOAC Int., 2019) using a LECO FP628 analyzer (LECO Corp., Saint Joseph, MI, USA) with subsequent calculation of crude protein as nitrogen × 6.25. All diets and ingredients were analyzed for AA (method 982.30 E [a, b, c]; AOAC Int., 2019), and total starch was analyzed using the glucoamylase procedure (method 979.10; AOAC Int., 2019). Diet and ingredient samples were also 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 (method Am 5–04; AOCS, 2013) using petroleum ether (Ankom XT-15 Extractor, Ankom Technology, Macedon, NY, USA). Ingredient samples were analyzed for crude fat by extraction with petroleum ether (method Am 5-04; AOCS, 2024) ether (Ankom XT-15 Extractor, Ankom Technology, Macedon, NY, USA). Low molecular weight carbohydrates, including maltose, sucrose, stachyose, and raffinose, were analyzed in ingredients using high-

performance liquid chromatography (Dionex App Notes 21 and 92). Insoluble dietary fiber and soluble dietary fiber were analyzed in diets and ingredients according to method 991.43 (AOAC Int., 2019) using the Ankom Dietary Fiber Analyzer (Ankom Technology). Total dietary fiber was calculated as the sum of insoluble dietary fiber and soluble dietary fiber.

In experiment 1, ileal digesta samples were also analyzed for dry matter, nitrogen × 6.25, AA, and starch as described for diets and ingredients. Diets and all ileal digesta samples were analyzed for chromium using an inductively coupled plasma atomic emission spectrometric method (method 990.08; AOAC Int., 2019). Samples were prepared for analysis using nitric acid-perchloric acid (method 968.08D(b); AOAC Int., 2019).

In experiment 2, the lyophilized urine samples and dried fecal samples were analyzed for gross energy and dry matter as described for diets and ingredients, and fecal samples and 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).

Calculations

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

In experiment 2, the ATTD of dry matter, gross energy and nitrogen was calculated for each diet (Adeola, 2001), and the DE and ME in the three diets were calculated (NRC, 2012). Data for O_2, CO_2, and CH_4 were averaged within each collection period and also for the last 12 h

of the fasting period. The THP during the collection period was calculated using the following equation (Brouwer, 1965):

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

where O_2, CO_2, and CH_4 are expressed in liters per day, and urine nitrogen is expressed in grams per day . The FHP from pigs during fasting was calculated as described for THP. Heat increment was calculated by subtracting FHP from THP, and the concentration of NE was then calculated using the following equation (modified from NRC, 2012):

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

where ME is in kcal/kg, THP and FHP are in kcal, and feed intake is in kcal during the collection period. The respiratory quotient (**RQ**) was calculated as the ratio of CO_2 production to O_2 consumption. Nitrogen excretion and nitrogen balance were also calculated using weights of feces and urine and analyzed nitrogen in diets, feces, and urine.

Statistical analysis

Model assumptions on the residuals for both experiments were confirmed using the MIXED procedure and the Brown-Forsythe test of the GLM procedure of SAS (SAS Inst. Inc., Cary, NC, USA). The MIXED procedure in SAS was used to generate studentized residuals and outliers were defined as observations with studentized residuals greater than 3 or less than −3. However, no outliers were detected in either experiment. Data were analyzed with the MIXED procedure of SAS. In experiment 1, the statistical model included the diet as the main effect, whereas period and square were random effects. The pig was the experimental unit for all analyses. In experiment 2, the model included diet as the main effect and chamber and period and replicate within period as random effects. The chamber was the experimental unit. Least-

square means were calculated and separated for both experiments and if the model was significant using the PDIFF option with the Tukey's adjustment (Westfall et al., 2011). Results were considered significant at $P \leq 0.05$ and considered a tendency at $0.05 < P \leq 0.10$.

Results

For both experiments, all pigs consumed their diets throughout the experiment without apparent problems. Analyzed concentrations of energy and nutrients in diets were close to calculated values.

Experiment 1

The AID of starch was greater ($P < 0.05$) by pigs fed the CM diet compared with the other two diets (Table 8.4). The SID of nitrogen and most AA were greater ($P < 0.05$) in the SBM diet compared with the other two diets. A tendency of greater ($P < 0.10$) SID of Lys in the SBM diet compared with CM and DDGS diet was observed. However, the SID of Met, His, Trp, Cys, and Glu were not different between the SBM diet and the CM diet, and the SID of Ile, Lys, Phe, Thr, Val, and Asp was reduced ($P < 0.05$) in the DDGS diet compared with the CM diet.

Experiment 2

The RQ of pigs during fasting or during feeding was not affected by dietary treatment (Table 8.5). Likewise, the production of CH_4 was not different among diets. Pigs fed the SBM diet had greater ($P < 0.05$) O_2 consumption and CO_2 production per kilogram of metabolic body weight than pigs fed the DDGS diet, but O_2 consumption and CO_2 production per kilogram of metabolic body weight were not different between pigs fed the SBM and CM diets. The production of CO_2 per gram of nitrogen intake was also greater ($P < 0.05$) in pigs fed the SBM diet than in pigs fed the DDGS diet, but was not different from pigs fed the CM diet.

Daily feed intake and daily gross energy intake of pigs were not affected by dietary treatment (Table 8.6). The ATTD of dry matter and gross energy were greater ($P < 0.05$) for pigs fed the SBM diet compared with pigs fed the CM or the DDGS diet, but pigs fed the DDGS diet had reduced ($P < 0.05$) ATTD of dry matter and gross energy than pigs fed the CM diet. The weight of feces was greater ($P < 0.05$) in pigs fed the DDGS diet than in pigs fed the CM diet, and the weight of feces in pigs fed the CM diet was greater ($P < 0.05$) than in pigs fed the SBM diet. Fecal gross energy output was greater ($P < 0.05$) in pigs fed the DDGS diet than in pigs fed the SBM or the CM diet, but no difference in fecal gross energy output was observed between pigs fed the SBM and CM diets. The concentrations of DE and ME were greater ($P < 0.05$) in the SBM diet than in the CM or the DDGS diet, but no differences in DE or ME were observed between the CM and DDGS diets. The FHP by pigs fed the SBM diet was greater ($P < 0.05$) than that of pigs fed the CM diet, but no difference in FHP was observed between pigs fed the SBM and DDGS diets, whereas NE was greater ($P < 0.05$) in the DDGS diet than in the CM diet, but no differences in NE between the SBM and DDGS diets or between the SBM and CM diets were observed.

Daily nitrogen intake, nitrogen in feces and urine, and nitrogen retention as percentage of intake were not influenced by the diet (Table 8.7). There was a tendency for greater ($P < 0.10$) nitrogen retention in grams per day by pigs fed the SBM diet compared with pigs fed the other two diets. The ATTD of nitrogen, absorbed nitrogen in grams per day, and urine nitrogen excretion was greater ($P < 0.05$) by pigs fed the SBM diet compared with the other two diets. However, the ATTD of nitrogen was reduced ($P < 0.05$) in pigs fed the DDGS diet compared with pigs fed the CM diet. Fecal nitrogen excretion and biological value were greater ($P < 0.05$) in pigs fed the DDGS diet compared with pigs fed the SBM diet. However, fecal nitrogen

excretion was not different between pigs fed the SBM diet and pigs fed the CM diet, and biological value was not different between pigs fed the DDGS diet and the CM diet.

Discussion

Concentrations of gross energy in corn, SBM, CM, and DDGS were in agreement with reported values (NRC, 2012; Stein et al., 2016). Soybean meal has been the most common protein and AA source used in diets for pigs in the United States because of its well-balanced AA profile (NRC, 2012). However, production of ethanol and its co-products such as DDGS, along with the availability of alternative ingredients such as CM, has increased their use in diets for growing-to-finishing pigs (Stein and Shurson, 2009; Woyengo et al., 2014). These alternative ingredients contain more fiber than SBM, which may potentially reduce nutrient digestibility and influence nitrogen excretion and greenhouse gas production (Zhang et al., 2013; Acosta et al., 2020).

The SID of AA that were calculated for CM, SBM, and DDGS were in agreement with reported values (Berrocoso et al., 2015; Cristobal et al., 2020). The observation that the SID of nitrogen and most AA was greater for the SBM diet than in the CM or DDGS diets is also in agreement with previous results (Berrocoso et al., 2015; Maison et al., 2014; Cristobal et al., 2020). Greater concentrations of insoluble dietary fiber in some ingredients result in a faster rate of passage, which reduces the time for digestion and absorption of AA in the small intestine (Kim et al., 2007; Ibagon et al., 2021). Additionally, insoluble fiber may increase encapsulation of nutrients, including AA, which reduces the substrate available for digestive enzymes and, therefore, reduces the SID of AA (Kerr and Shurson, 2013). Although synthetic Lys, Met, Thr, and Trp were added to the CM and DDGS diets to meet the requirements for digestible AA, the overall SID of nitrogen and most AA remained less for these diets than in the SBM diet,

indicating that supplementation with synthetic AA did not fully compensate for the lower digestibility of protein-bound AA in CM and DDGS. Therefore, the hypothesis that ileal digestibility of AA would not be affected by protein source was rejected.

The observation that the calculated RQ during the fed or fasting period was not affected by dietary treatment is likely a result of similar starch concentrations among the three diets, indicating similar carbohydrate oxidation, which indicates a similar metabolic energy usage pattern among the diets (Richardson, 1929). Because feces and urine were collected and removed from chambers daily, CH_4 measured in the current experiment was considered to originate primarily from hindgut fermentation rather than manure decomposition (Petersen et al., 2016). Although greater dietary fiber inclusion has been associated with increased enteric CH_4 production in pigs (Jarret et al., 2012; Montalvo et al., 2013), the greater amount of fiber from CM and DDGS was apparently insufficient to stimulate a measurable increase in hindgut fermentation, which agrees with results from other experiments indicating that moderate increases in dietary fiber do not always result in greater CH_4 emissions from pigs (Pepple et al., 2011; Kerr et al., 2020). However, the tendency for greater daily CO_2 production per kilogram of body weight gain in pigs fed the CM diet compared with pigs fed the SBM or DDGS diets indicates that more gas exchange was required per unit of gain, and, therefore, a less efficient utilization of energy for growth in pigs fed the CM diet. Likewise, the observation that O_2 consumption per kg of metabolic body weight was greater by pigs fed SBM than by pigs fed DDGS may reflect the greater metabolic demand associated with greater rates of protein synthesis and tissue deposition in pigs fed SBM, beacuse protein deposition is a high-energy requiring process that increases the whole body oxygen consumption (Yen, 1997; Nyachoti et al.,

2000).It is therefore, likely that the greater nitrogen retention by pigs fed the SBM diet resulted in increased need for O_2 and increased production of CO_2.

The greater ATTD of dry matter and gross energy by pigs fed the SBM diet compared with pigs fed the CM or DDGS diets is consistent with the greater total dietary fiber concentration of CM and DDGS, which reduces the digestibility of nutrients by increasing fecal bulk and energy excretion (Moeser and van Kempen, 2002; Acosta et al., 2020). Consequently, the greater DE and ME observed in the SBM diet reflect the greater digestibility of energy in that diet, and these values are in agreement with previously published values for these ingredients (NRC, 2012; Kim et al., 2018). The DE and ME in the SBM, DDGS, and CM diets calculated in the current experiment were also in agreement with previous values (NRC, 2012; Kim et al., 2018; Ibagon et al., 2024). However, despite lower DE and ME in the DDGS diet compared with the SBM diet, NE in the DDGS diet was not different from NE in the SBM diet, which may be partly explained by the greater concentration of AEE in DDGS compared with SBM and CM, because fat is the most efficiently utilized macronutrient for NE in pigs (Noblet et al., 1994). This observation indicates that differences in DE and ME among diets are not directly reflected in NE, and that the form of the energy-yielding nutrients may also influence energy utilization.

It has been hypothesized that using co-products such as DDGS or CM may reduce ammonia emissions and, therefore, the environmental impact of pig production (Jarret et al., 2011). The inclusion of high-fiber ingredients in the diets of growing to finishing pigs will result in a greater excretion of nitrogen in feces rather than in urine, resulting in a reduction of emissions of ammonia (Jarret et al., 2011). The observation that nitrogen intake did not differ among diets indicates that the differences in nitrogen balance were not a consequence of differences in nitrogen consumption, which is in agreement with the fact that there was no

difference among diets in crude protein concentrations. The greater fecal nitrogen excretion by pigs fed the DDGS diet compared with pigs fed the SBM or CM diet was likely due to the reduced nitrogen digestibility in DDGS, and also due to the fact that greater fiber inclusion in the diet will increase fecal nitrogen output due to an increased rate of passage in the small intestine (Jarret et al., 2011; Zhang et al., 2013). As a consequence, the ATTD of nitrogen is also reduced in diets with greater concentration of fiber. The ATTD of nitrogen in the SBM, DDGS, and CM diets was in agreement with reported values (Kim et al., 2018; Acosta et al., 2020; Cristobal et al., 2025). The greater ATTD of nitrogen and absorbed nitrogen by pigs fed the SBM diet compared with pigs fed the CM or DDGS diets agrees with the greater AID of nitrogen observed in experiment 1 and indicates that nitrogen in the SBM diet was more available for digestion and absorption than nitrogen in the CM or DDGS diets.

The greater urine nitrogen excretion in pigs fed the SBM diet compared with pigs fed the CM and DDGS diets may indicate that, although SBM increased nitrogen digestibility and nitrogen absorption, this diet may have provided digestible AA in excess of the anabolic capacity of the pig resulting in greater metabolism of AA and increased urea synthesis (Fuller et al., 1989), whereas the CM and DDGS diets, because they were supplemented with synthetic AA, may have supplied the limiting indispensable AA in a more balanced ration and, therefore, reduced AA deamination and urinary nitrogen excretion. The observation that there was a tendency for greater nitrogen retention measured in grams per day by pigs fed the SBM diet than by pigs fed the CM or DDGS diets may be due to the fact that synthetic AA may have a different rate of absorption at the site of protein synthesis than intact protein (Eugenio et al., 2022), and metabolism of free AA may have occurred before the AA from the intact protein arrived at the cell, which may have reduced protein synthesis (Wang et al., 2021).

In conclusion, the inclusion of CM or DDGS in corn-based diets for growing pigs resulted in greenhouse gas emissions, and NE values that were similar to those observed for pigs fed SBM-based diets. The greater fiber in CM and DDGS reduced the digestibility of nutrients, but the greater concentration of AEE in DDGS contributed to a NE in the DDGS diet that was not different from the NE in the SBM diet. However, SBM resulted in greater AA digestibility and tended to support greater daily nitrogen retention, indicating that pigs fed a SBM based diet likely had greater protein synthesis.

Conclusions

In conclusion, the inclusion of CM or DDGS in corn-based diets for growing pigs resulted in greenhouse gas emissions, and NE values were similar to those observed in pigs fed SBM-based diets. The greater fiber content of CM and DDGS reduced the digestibility of nutrients, but the greater concentration of AEE in DDGS contributed to a NE in the DDGS diet similar to that of NE of the SBM diet. Therefore, the inclusion of CM or DDGS in diets for growing pigs may be an alternative from an environmental perspective, because major differences in greenhouse gas emissions were not observed among diets. However, SBM resulted in greater AA digestibility and tended to support greater daily nitrogen retention, indicating that SBM remained the better protein source to maximize nutrient utilization.

Tables

Table 8.1. Analyzed nutrient composition of ingredients, as-fed basis

Item	Corn	Soybean meal	Canola meal	DDGS[1]
Dry matter, %	87.46	89.66	88.66	85.39
Ash, %	1.26	6.41	6.20	5.94
Gross energy, kcal/kg	3,784	4,101	4,223	4,558
Nitrogen × 6.25, %	6.44	46.85	38.99	25.02
Ether extract, %	2.82	1.61	2.28	8.02
Acid hydrolyzed ether extract, %	3.17	2.05	2.66	7.72
Insoluble dietary fiber, %	7.02	11.18	26.25	29.92
Soluble dietary fiber, %	2.80	1.93	5.13	2.94
Total dietary fiber, %	9.82	13.11	31.38	32.86
Starch, %	65.67	2.10	1.80	6.24
Low molecular weight carbohydrates, %				
Fructose	0.93	0.09	0.26	0.11
Glucose	0.51	ND[1]	0.13	0.31
Sucrose	1.09	8.78	7.24	0.10
Maltose	ND	0.79	ND	0.10
Raffinose	0.21	1.55	0.49	ND
Stachyose	0.02	7.69	0.01	0.03
Verbascose	ND	0.27	ND	ND
Indispensable amino acids, %				
Arg	0.26	3.47	2.43	1.20

Table 8.1. (Cont.)

His	0.19	1.39	1.19	0.75
Ile	0.22	2.36	1.71	0.91
Leu	0.65	3.78	2.88	2.72
Lys	0.24	3.14	2.36	0.76
Met	0.14	0.69	0.81	0.45
Phe	0.28	2.51	1.67	1.16
Thr	0.22	1.90	1.72	0.93
Trp	0.04	0.71	0.53	0.18
Val	0.29	2.43	2.14	1.24
Dispensable amino acids, %				
Ala	0.42	2.08	1.76	1.65
Asp	0.42	5.48	2.90	1.43
Cys	0.14	0.71	1.00	0.49
Glu	1.07	8.80	6.16	2.94
Gly	0.26	2.04	2.05	0.97
Pro	0.47	2.28	2.34	1.80
Ser	0.28	2.12	1.52	1.09
Tyr	0.11	1.77	1.11	0.88
Total amino acids, %	5.70	45.89	36.28	21.55

[1]ND, not detected.

Table 8.2. Ingredient composition of experimental diets, as-fed basis

Item	Soybean meal	Canola meal	DDGS[1]	Nitrogen-free diet[2]
Ingredient, %				
Ground corn	77.40	71.50	65.70	-
Soybean meal, 48% crude protein	20.00	-	-	-
Canola meal	-	26.00	-	-
DDGS, low-oil	-	-	31.00	-
Soybean oil	-	-	-	4.00
Corn starch	-	-	-	68.15
Sugar	-	-	-	20.00
Solca Floc[3]	-	-	-	4.00
L-Lys-HCl	0.16	0.33	0.70	-
DL-Met	-	-	0.01	-
L-Thr	0.01	0.06	0.14	-
L-Trp	-	0.03	0.07	-
Dicalcium phosphate	0.82	0.66	0.32	1.75
Ground limestone	0.71	0.52	1.16	0.30
Sodium chloride	0.40	0.40	0.40	0.40
Magnesium oxide	-	-	-	0.10
Potassium carbonate	-	-	-	0.40
Vitamin-mineral premix[4]	0.50	0.50	0.50	0.50
Calculated composition				

Table 8.2. (Cont.)

Metabolizable energy, kcal/kg	3,294	3,229	3,233	-
Crude protein, %	13.56	14.84	13.79	-
Total Ca, %	0.66	0.66	0.66	-
Digestible P[5], %	0.31	0.31	0.31	-

[1]DDGS, distillers dried grains with solubles.

[2]The nitrogen-free diet was used only in experiment 1.

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

[4]The vitamin-mineral premix provided the following quantities of vitamins and micro-minerals per kg of complete diet: Vitamin A as retinyl acetate, 11,136 IU; vitamin D3 as cholecalciferol, 2208 IU; vitamin E as $_{DL}$-alpha tocopheryl acetate, 66 IU; vitamin K as menadione dimethylprimidinol bisulfite, 1.42 mg; thiamin as thiamine mononitrate, 0.24 mg; riboflavin, 6.59 mg; pyridoxine as pyridoxine hydrochloride, 0.24 mg; vitamin B_{12}, 0.03 mg; $_{D}$-pantothenic acid as $_{D}$-calcium pantothenate, 23.5 mg; niacin, 44.1 mg; folic acid, 1.59 mg; biotin, 0.44 mg; Cu, 20 mg as copper chloride; Fe, 126 mg as ferrous sulfate; I, 1.26 mg as ethylenediamine dihydriodide; Mn, 60.2 mg as manganese hydroxychloride; Se, 0.3 mg as sodium selenite and selenium yeast; and Zn, 125.1 mg as zinc hydroxychloride.

[5]Standardized total tract digestible P.

Table 8.3. Analyzed composition of experimental diets, as-fed basis

Item,	Soybean meal	Canola meal	DDGS[1]
Dry matter, %	87.06	88.62	86.93
Ash, %	3.85	3.82	4.38
Gross energy, kcal/kg	3,740	3,835	3,920
Starch, %	49.48	48.62	47.56
Nitrogen × 6.25, %	14.86	14.56	13.78
Acid hydrolyzed ether extract, %	2.31	2.64	4.97
Indispensable amino acids, %			
Arg	0.95	0.82	0.68
His	0.44	0.44	0.42
Ile	0.67	0.60	0.52
Leu	1.32	1.20	1.50
Lys	0.98	1.04	1.04
Met	0.25	0.29	0.29
Phe	0.75	0.63	0.66
Thr	0.57	0.66	0.63
Trp	0.17	0.19	0.14
Val	0.75	0.76	0.68
Dispensable amino acids, %			
Ala	0.78	0.75	0.95
Asp	1.48	1.03	0.94
Cys	0.25	0.35	0.28

Table 8.3. (Cont.)

Glu	2.72	2.61	2.25
Gly	0.64	0.71	0.57
Pro	0.86	0.94	1.02
Ser	0.65	0.56	0.56
Tyr	0.48	0.39	0.45
Total amino acids, %	14.71	13.58	13.13

[1]DDGS, distillers dried grains with solubles.

Table 8.4. Apparent ileal digestibility (AID) of dry matter and starch and standardized ileal digestibility (SID) of amino acids (AA) in diets fed to growing pigs[1]

Item, %	Soybean meal	Canola meal	DDGS[2]	SEM	*P*-value
AID of starch	93.49[b]	96.18[a]	93.07[b]	0.74	0.001
SID[3] of nitrogen	87.62[a]	75.65[b]	71.14[b]	2.62	0.001
SID[3] of indispensable AA					
Arg	95.66[a]	87.59[b]	84.83[b]	1.14	< 0.001
His	87.00[a]	83.82[a]	71.16[b]	2.67	< 0.001
Ile	85.21[a]	78.44[b]	70.72[c]	3.01	< 0.001
Leu	85.72[a]	82.23[ab]	78.49[b]	2.85	0.001
Lys	86.66	81.32	83.26	2.71	0.054
Met	87.71[a]	85.95[a]	77.65[b]	2.42	< 0.001
Phe	86.14[a]	81.36[b]	75.15[c]	2.82	< 0.001
Thr	81.25[a]	72.10[b]	70.45[b]	3.23	< 0.001
Trp	88.55[a]	85.93[a]	80.76[b]	1.95	0.001
Val	82.67[a]	75.40[b]	69.05[c]	3.23	< 0.001
Total	86.64[a]	80.91[b]	76.79[c]	2.56	< 0.001
SID of dispensable AA					
Ala	83.95[a]	77.70[b]	75.06[b]	2.62	0.008
Asp	84.41[a]	75.48[b]	64.05[c]	3.79	< 0.001
Cys	78.04[a]	73.92[a]	57.96[b]	3.64	< 0.001
Glu	88.08[a]	86.40[a]	76.36[b]	2.25	< 0.001

Table 8.4. (Cont.)

Gly	85.44	66.08	63.64	5.14	0.010
Ser	86.34[a]	76.33[b]	73.13[b]	2.56	< 0.001
Tyr	85.98[a]	76.28[b]	77.41[b]	2.80	0.001
Total	90.18[a]	79.12[b]	77.08[b]	1.86	0.008
Total AA	88.37[a]	80.00[b]	76.96[b]	1.84	< 0.001

[1]Least-mean squares represent 8 replicates per dietary treatment (n = 8).

[2]DDGS, distillers dried grains with solubles.

[3]Values for the SID were calculated by correcting the AID for basal ileal endogenous losses. Basal ileal endogenous losses (%) were determined as: nitrogen, 3.81; Arg, 0.84; His, 0.32; Ile, 0.59; Leu, 1.07; Lys, 0.72; Met, 0.16; Phe, 0.63; Thr, 0.90; Trp, 0.15; Val, 0.82.

Table 8.5. Oxygen (O_2) consumption and greenhouse gas emissions as carbon dioxide (CO_2) and methane (CH_4) from growing pigs fed experimental diets[1]

Item	Soybean meal	Canola meal	DDGS[2]	SEM	*P*-value
Initial body weight, kg	58.55[b]	60.43[a]	59.25[ab]	7.47	0.034
Final body weight, kg	80.57	80.05	79.84	1.13	0.132
Respiratory quotient, fasted	0.75	0.78	0.82	0.03	0.130
Respiratory quotient, fed	1.20	1.13	1.20	0.06	0.251
Gas exchanges, L					
O_2 consumption[3]	789.13	712.29	706.61	72.77	0.040
CO_2 production	914.31	814.65	808.74	90.45	0.055
CH_4 production	-6.12	-4.90	-4.94	1.64	0.796
Gas exchanges, L/kg of body weight					
O_2 consumption	9.81	8.91	8.86	0.99	0.062
CO_2 production	11.36	10.18	10.14	1.21	0.078
CH_4 production	-0.08	-0.06	-0.06	0.02	0.793
Gas exchanges, L/kg of body weight$^{0.60}$					
O_2 consumption	63.93[a]	57.62[b]	57.36[b]	3.61	0.008
CO_2 production	73.97[a]	65.97[ab]	65.53[b]	4.59	0.034
CH_4 production	-0.35	-0.26	-0.26	0.15	0.895
Daily gas exchanges, L/kg of body weight gain					
O_2 consumption[3]	751.3	857.18	759.11	66.35	0.038
CO_2 production	868.63	981.84	867.99	79.95	0.088

Table 8.5. (Cont.)

CH_4 production	-5.42	-5.39	-4.69	1.93	0.695
Gas exchanges, L/g of nitrogen intake					
O_2 consumption	12.39	12.30	11.77	0.75	0.158
CO_2 production	14.34[a]	14.06[ab]	13.43[b]	0.87	0.045
CH_4 production	-0.09	-0.08	-0.07	0.03	0.980

[1]Least mean squares represent six replicates per dietary treatment (n = 6), except for the CH_4 measurements (n = 5).

[2]DDGS, distillers dried grains with solubles.

[3]Although the model was significant for the daily O_2 consumption in L, and for the daily O_2 consumption in L/kg of body weight gain, the adjusted P-value of the pairwise multiple comparison was not significant.

Table 8.6. Apparent total tract digestibility (ATTD) of dry matter and gross energy, and concentrations of digestible energy, metabolizable energy, and net energy in diets fed to growing pigs[1,2]

Item	Soybean meal	Canola meal	DDGS	SEM	*P*-value
Feed intake, kg/d	2.87	2.50	2.83	0.24	0.063
Dry feces output, kg/d	0.30[c]	0.37[b]	0.44[a]	0.04	0.001
ATTD of dry matter, %	89.66[a]	85.28[b]	84.46[c]	0.32	< 0.001
Gross energy intake, Mcal/d	10.73	9.59	11.10	0.92	0.062
Fecal gross energy output, kcal/d	1,351[b]	1,640[b]	2,069[a]	165.16	< 0.001
ATTD of gross energy, %	87.40[a]	82.85[b]	81.41[c]	0.39	< 0.001
Digestible energy, kcal/kg	3,269[a]	3,177[b]	3,190[b]	15.13	< 0.001
Urine output, kg/d	5.71[a]	3.34[b]	4.28[ab]	0.65	0.039
Urine gross energy output, kcal/d	196	195	193	29.83	0.994
Metabolizable energy, kcal/kg	3,201[a]	3,099[b]	3,123[b]	16.73	< 0.001
THP[3], kcal/d	4,133	3,721	3,693	386.78	0.044
FHP, kcal/d	2,359[a]	2,163[b]	2,305[ab]	160.22	0.031
Net energy, kcal/kg	2,584[ab]	2,479[b]	2,639[a]	73.33	0.030

[a-c]Within a row, means without a common superscript letter are different ($P < 0.05$).

[1]Least square means represents six observations per treatment ($n = 6$).

[2]DDGS, distillers dried grains with solubles; FHP, fasting heat production; THP, total heat production.

Table 8.6. (Cont.)

[3]Although the model was significant for THP, the adjusted *P*-value of the pairwise multiple comparison was not significant.

Table 8.7. Nitrogen excretion and nitrogen balance in diets fed to growing pigs[1]

Item	Soybean meal	Canola meal	DDGS[2]	SEM	*P*-value
Nitrogen intake, g/d	63.66	57.91	60.62	5.26	0.228
Nitrogen in feces, %	3.22	3.09	3.25	0.07	0.190
Fecal nitrogen excretion, g/d	9.54[b]	11.31[b]	14.41[a]	1.16	0.005
ATTD of nitrogen, %	85.00[a]	80.33[b]	76.37[c]	0.69	< 0.001
Absorbed nitrogen, g/d	54.12[a]	46.60[b]	46.21[b]	4.22	0.012
Nitrogen in urine, %	0.25	0.29	0.21	0.05	0.157
Urine nitrogen excretion, g/d	12.84[a]	9.40[b]	8.47[b]	2.31	< 0.001
Retained nitrogen, g/d	41.29	37.20	37.75	2.49	0.077
Nitrogen retention, as % intake	65.16	64.30	62.83	2.56	0.261
Biological value, %	76.64[b]	80.15[ab]	82.17[a]	3.07	0.009

[a-c]Within a row, means without a common superscript letter are different ($P < 0.05$).

[1]Least square means represents six observations per treatment ($n = 6$).

[2]DDGS, distillers dried grains with solubles.

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CHAPTER 9: Conclusion

In this dissertation, soybean meal was demonstrated to be a very valuable ingredient in diets for both growing pigs and reproducing sows because it contributes not only of digestible amino acids, but also energy to the diets. Results indicated that the net energy value of soybean meal is greater than what is currently reported in the feed tables, and that modern pigs may retain more than 60% of the ingested nitrogen, which further supports the contribution of soybean meal to energy efficiency and protein deposition. Additionally, the productive energy value of soybean meal was estimated to be greater than 100% of the energy value of corn, indicating that the energy contribution of soybean meal has indeed been underestimated in current feeding systems.

Reducing dietary crude protein by decreasing soybean meal inclusion and replacing intact protein with crystalline amino acids did not fully maintain nutrient utilization. Although all diets were formulated to meet requirements for digestible amino acids, and metabolizable energy was not affected, reductions in soybean meal consistently resulted in decreased digestibility of energy and nitrogen, reduced daily nitrogen retention measured in grams per day, reduced carcass leanness, and increased fat deposition in growing pigs. Although extra nitrogen and soy isoflavones were added to the reduced-protein diets, neither treatment recovered the reduction in nitrogen retention or carcass leanness observed when soybean meal was reduced. In reproducing sows, reducing dietary protein did not affect reproductive performance, but decreased daily nitrogen retention during gestation and reduced the milk quality of sows during lactation. Therefore, these results indicate that there must be something in soybean meal beyond digestible indispensable amino acids and energy that supports protein deposition and nutrient utilization in pigs, which indicates that formulation based only on digestible amino acid requirements using

free amino acids does not fully compensate for the nutritional and metabolic value of intact protein sources such as soybean meal.

Replacing soybean meal with alternative proteins is not an effective strategy to reduce greenhouse gas emissions and may reduce the efficiency of nitrogen utilization. In diets containing distiller dry grains with solubles and soybean meal, nitrogen retention could be maintained when additional Val, Ile, and Trp were included, but this response was not observed in diets containing corn protein, likely because the greater excess of Leu limited the beneficial effect of the supplemental amino acids. In addition, replacing soybean meal with canola meal or dry distiller grains with solubles did not reduce greenhouse gas emissions compared with soybean meal-based diets. Although net energy in distiller dried grains with solubles-based diets was not different from that of soybean meal-based diets, pigs fed soybean meal had greater amino acid digestibility, greater absorbed nitrogen, and greater daily nitrogen retention measured in grams per day, whereas fecal nitrogen excretion was greater from pigs fed diets containing canola meal or distiller dried grains with solubles. Therefore, soybean meal remained the most efficient protein source for supporting nitrogen retention.

Overall, these results demonstrate that the value of soybean meal in swine diets extends beyond its role as a source of digestible amino acids. Soybean meal contributes to improved nutrient digestibility, greater retention of nitrogen, and greater overall efficiency of energy utilization. Therefore, although alternative ingredients and crystalline amino acids can be used in diet formulation, replacement of soybean meal is possible, but it may reduce the efficiency of protein and energy utilization in pigs.