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High Protein Yeast-Based DDGS as an Alternative to Commonly Used Protein Sources in Pig Diets

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HIGH PROTEIN YEAST-BASED DDGS AS AN ALTERNATIVE TO COMMONLY USED PROTEIN SOURCES IN PIG DIETS

BY

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THESIS ACCEPTANCE PAGE

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This thesis is approved as a creditable and independent investigation by a candidate for the master's degree and is acceptable for meeting the thesis requirements for this degree. Acceptance of this does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

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CONTENT

ABBREVIATIONS

AA amino acid

ADFI average daily feed intake

ADG average daily gain

AID apparent ileal digestibility

ANFs anti-nutritional factors

ATTD apparent total tract digestibility

BW body weight

CF crude fiber

CP crude protein

DDG dry distiller grains

DDGS dry distillers grain with solubles

DE digestible energy

DM dry matter

DMI dry matter intake

DSAT differential sugar absorption test

EAA essential amino acids

ESBM enzymatically treated soybean meal

FM fish meal

GE gross energy

G:F gain to feed ratio

HP-GDDY high protein yeast-based DDGS

IGA immunoglobulin A

ISO isoleucine

LYS lysine

ME metabolizable energy

MET methionine

NDC non-digestible carbohydrates

NDF neutral detergent fiber

NSP non-starch polysaccharides

P phosphorus

SBM soybean meal

SID standard ileal digestibility

THR threonine

TI trypsin inhibitor

TRP tryptophane

VAL valine

WDG wet distillery grains

WDGS wet distillery grains with soluble

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ABSTRACT

HIGH PROTEIN YEAST-BASED DDGS AS AN ALTERNATIVE TO COMMONLY USED PROTEIN SOURCES IN PIG DIETS

RICARDO GARAVITO DUARTE

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Alternative ingredients and co-products from ethanol and biofuel industries represent an opportunity for utilization in swine diets. In this context, understanding nutritional value to practical swine feeding begins with product characterization and determination of nutrient digestibility. The objective of this research was to test the hypothesis: yeast-based high-protein DDGS (HP-GDDY) has comparable AA and metabolizable energy (ME) digestibility to feedstuffs commonly used in swine diets and that it could be included as an alternative protein source in nursery diets without negative impact on growth performance. Cornstarch-based diets were formulated using six ingredients as the sole source of AA: spray dried HP-GDDY, ring dried HP-GDDY, conventional DDGS, soybean meal (SBM), enzymatically treated SBM (HP300) and fishmeal (FM) to determine SID of AA (Experiment 1) and ME content (Experiment 2) and to evaluate the growth performance and health status (Experiment 3). The experiment 1 was conducted as a 7×7 Latin square design with 7 collection periods of 7days using seven canulated barrows (25 ± 0.8 kg BW). The SID of all analyzed AA was greater ($P < 0.05$) in HP300 than in the other protein sources. Among the other ingredients, the SID of AA were generally similar ($P > 0.05$) in SBM, FM, spray and ring HP-GDDY samples. With respect to the most common first limiting AA (Lysine), the SID was greater ($P < 0.05$) in spray HP-GDDY than ring HP-GDDY and DDGS. In Exp. 2 fecal and urine were collected from 28 barrows (28.8 ± 1.4 kg BW). The ME in SBM, HP300 and FM was similar ($P > 0.05$) to spray and ring GDDY.

In a third experiment, 594 weaned pigs were assigned to pens using a randomized complete block design. Pens were assigned to one of four dietary treatments, replacing a percentage of the main protein sources by HP-GDDY. Dietary treatments had no significant effects (P<0.05) on growth performance parameters during the first two weeks. Collectively, this project demonstrates that HP-GDDY products are potential alternative ingredients for swine diets, in particular SBM, one of the main protein resources in pig diets, and the data obtained allows their inclusion in practical diet formulations.

Keywords: amino acids, digestibility, metabolizable energy, growth performance, postweaning period, protein source.

LITERATURE REVIEW

1. GENERAL INTRODUCTION

Feed accounts for up to 70% of the variable cost in production and is crucial because it influences factors such as growth, health, well-being, and even environmental emissions (Parsons, et al., 2007). The formulations in swine diets are primarily intended to meet the requirements at the lowest possible cost.

In general, vegetable crop seeds contain some of the primary nutrients that provide energy, carbohydrates, proteins, and fats. These crops have been commercially fractionated using a variety of technologies, with the primary goal of producing human food and feed (Zijlstra et al., 2004) and, more recently, biofuels. The current production of first-generation biofuels, primarily from corn crops, has provided a chance for mitigating climate change by replacing conventional fossil fuels (Garran et al., 2016). Interest in biofuels has grown due to interest in the impact of expanding corn ethanol production on feed prices and the increased potential of cellulosic biofuels to mitigate climate change (Tyner and Taheripour, 2007). Most high protein biorefinery co-products, such as soybean meal (SBM), are intended for monogastric animals, though the inclusion of many co-products may be hampered in some cases due to the sensitivity of commercial pig and poultry breeds to fluctuations in feed quality and protein and energy density (Burton, et al., 2013). For example, despite the fact distillers dried grain with solubles (DDGS) has historically been used as a protein supplement in livestock diets, the demand for DDGS tends to be more sensitive to price changes in feedstuff used as an energy source such as corn (Beckman, et al., 2011). Although its inclusion is limited due to the high fiber content, which decreases feed intake and limits the use of nutrients in both pigs and poultry (McDonnell, et al., 2011; Youssef, et al., 2008).

The identification and characterization of alternative ingredients, as well as their proper incorporation into the different stages of production, can benefit growth performance and the economic component of pig production systems. Weaning is one of the critical phases because piglets face a variety of stressors such as a new environment, social interactions, and a change in diet (Zheng, et al., 2021). In addition to these stressors, during the weaning transition approximately 50% of piglets take up to 24 hours before consuming feed after weaning, and approximately 10% of anorexia persists for up to 48 hours after weaning; these stressors are undoubtedly a source of concern in production. It has also been demonstrated that weaning and stress factors alter the developmental trajectory of gastro-intestinal barrier functions, which can result in negative consequences to intestinal health throughout the productive life (Moeser, et al., 2017). As a result, it is critical to pay close attention to nutritional strategies that contribute to the digestive tract during its development, including ingredients and additives that promote better intestinal development (Heo, et al., 2013). Weaned pig diet supplementation and ingredient management can improve growth performance by reducing intestinal inflammation and oxidative stress caused by diet change and improving intestinal villus structure and nutrient digestibility (Jang and Kim, 2019). Some options used in weaned piglets that benefit growth performance and gut health during this stage include co-product ingredients such as whey protein or ingredients treated with enzymatic processes such as HP300 (Jang, et al., 2021; Zhu, et al., 1998). According to Silva Junior et al. (2020), the use of phytogenic additives such as organic oils improve growth performance, nutrient digestibility, and intestinal health. Luciano et al. (2021) used 30% former food products (leftovers from the food industry, e.g., biscuits, bread, breakfast cereals, chocolate bars, pasta, and sweets) in weaned pig diets and found no negative effects on metabolic profile and concluded that former feed products can be included in post-weaning pig diets as alternative ingredients.

Therefore, one of the main objectives of swine nutrition research is to meet the nutritional requirements of pigs with the adequate supply of dietary nutrients in an efficient and profitable way. As precision animal production practices are adopted, profitability, efficiency, environmental sustainability, animal health and welfare also need to be improved (Banhazi, et al., 2012).

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1.1 COMMON FEED INGREDIENTS FOR SWINE

Currently, ingredients for pig production are being selected and procured in such a way that they can be incorporated into biosafe, eco-nutritional, and precision pig feeding programs without the use of antibiotic growth promoters (Shurson, et al., 2021). As technology advances, research and commercial applications shift away from feeding practices based on simple nutrient composition and toward modeling the dynamics of nutrient digestion, balance, and utilization (Coffey, et al., 2016). The selection of ingredients and the design of diets to approach precision feeding in pigs represents a great impact on the profitability of production due to the ability to feed pigs with diets adapted to their nutrient needs, and in turn can decrease the environmental impacts by optimizing dietary nutrient utilization, which results in decreased nutrient excretion (Pomar, et al., 2019).

Soybeans and corn are the two most important ingredients in pig nutrition due to their nutritional composition (Table 1.1). Swine diets based primarily on corn and SBM have become common in many countries around the world and are now the most commonly used ingredient combination in pig production in the Americas, Asia, and some European countries. However, in areas where corn production is not feasible due to economic or environmental constraints, other cereal grains such as wheat, barley, triticale, sorghum, and rye are used in conjunction with SBM or other protein sources (Stein, et al., 2016). Corn and soybeans have two main price links: the first is competition for acreage due to similar soil and climate requirements, and the second is corn and soybeans share various industrial uses. In addition, the potential substitution of corn crops for soybean crops when the corn crop is more expensive is another factor that influences the demand for soybeans and, ultimately, the price of these crops (Avalos, 2014).

An alternative to dealing with the complications of competition with human food, competition with other crops, the use of large areas, market price fluctuations, and environmental consequences is to use new ingredients such as the co-products of industries related to agriculture that lead to greater sustainability and productive efficiency in the feeding of monogastrics. However, the inclusion of co-products can change the density of the diet and affect food consumption (Avelar et al., 2010). Therefore, it is important to take these aspects into account to cover the requirements of the animal according to its productive stage and its physiological state (Nyachoti et al., 2004). Jha et al. (2013) found that while using oilseed co-products in the diet provides some beneficial nutrients (omega 3 fatty acids), it has a negative impact on weight gain and feed intake, most likely as a result of dietary fiber, fat content, or a possible AA imbalance. Similarly, Stein and Shurson (2009) concluded that acceptable growth performance can be obtained by including up to 30% DDGS in nursery and grower-finisher pig diets and inclusions greater than 30% negatively decrease growth performance due to fiber content.

1.2 CEREAL GRAINS FOR PIGS

The main sources of energy used in diets for pigs come from cereal grains, like corn. Although there is no requirement for cereal grains in the diet, most diets include a portion of these cereal grains (Stein, et al., 2016). The largest component in cereal grains is starch, such corn (Table 1.1), however it differs in terms of structure, due to the proportion of amylose and amylopectin. Amylopectin is a highly branched molecule with (1 to 4) α-D-glycosyl units linked in chains linked by 1 to 6 bonds. Amylose is mainly linear with glycosyl units attached with α-1-4 linkages. Amylose can have 10 or more branches (Magallanes-Cruz, et al., 2017), this characteristic directly affects the speed and digestion of starch in the intestine (Tan, et al., 2021). Starch is primarily digested by pancreatic α-amylase and brush border disaccharide hydrolases in the small intestine (Regmi, et al., 2011). Starch is largely digested in the small intestine by pancreatic α-amylase and brush border disaccharide hydrolases (Regmi, et al., 2011). Resistant starch is starch that cannot be digested in the small intestine and thus reaches the large intestine and is fermented in the digesta of the colon, where it increases the production of different substrates like butyrate, improving the integrity of the mucosa, and generating benefits in intestinal health (Nofraras et al., 2007). These changes in substrate degradation by the intestinal bacterial population cause changes in the microbial and fermentative profiles; however, starch fermentation rather than starch digestion can increase heat production and loss, decrease energy efficiency, and therefore reduce weaned piglet growth. (Fouhse, et al., 2015).

1.3. CEREAL CO-PRODUCTS

Cereal grains are processed into ethanol biofuel, flour, and other products for human food or industrial applications, resulting in co-products that can be fed to livestock. In the case of corn, different products are obtained by processing methods such as dry milling and wet milling (Rojas, et al., 2013). Some examples of the products obtained from corn are found in Figure 1.1. The wet processing (Fig.1) begins with soaking the maize kernel to soften it. This is done to make separation of the individual components easier before they are processed into ethanol to obtain different products such as corn bran, starch, corn gluten meal (protein). While in the dry milling process, the corn goes through grinding and cooking process before the fermentation process to produce the stillage which can be dried to obtain distiller's grains, wet distillers grains and dried distillers grains (DDG), or the stillage can also be processed to obtain dried distillers solubles. Finally, DDGS and wet dry distillers grains with solubles can be produced from previously obtained co-products, which can be sold as wet or dry feeds. The dryer process affects the quality of different co-products, for example dry corn gluten feed contains less energy than wet corn gluten feed (Erickson et al., 2005). The main co-product of ethanol production is DDGS (Woyengo, et al., 2014). The high availability of this co-product is tied to ethanol production; government subsidies and legislation require a minimum amount of ethanol in gasoline increasing ethanol production and thus DDGS production (Tyner and Taheripour, 2007).

1.3.1. DISTILLER'S DRIED GRAINS WITH SOLUBLES

Bioethanol production uses enzymatic liquefaction and saccharification of starch to produce glucose, which is fermented by the yeast Saccharomyces cerevisiae to obtain ethanol (Fig. 1.2). The residues from this process are decanted into a solid and fibrous component and a liquid component. The liquid component contains most of the yeast protein and soluble components, this liquid fraction is evaporated into a syrup, re-mixed with the solid component and dried to form DDGS (Burton, et al., 2013).

Several studies on the nutritional value of DDGS have been conducted over the last few decades, and it has been demonstrated that DDGS contains approximately three times more protein, AA, fat, fiber, and minerals than the main cereal (Stein and Shurson, 2009), though it has also been demonstrated that the content of mycotoxins increases in the same manner (Abudabos, et al., 2017). It has been noted that the chemical composition of DDGS varies due to factors such as processing methods utilized by bioethanol plants, as well as the variety and content of the grains used (Liu, 2011). In the case of pigs, the nutritional value of a nutrient in an ingredient (i.e. energy and AA) is hampered by the high concentration of insoluble fiber compared to soluble fiber; in DDGS, insoluble fiber can reach 31% while soluble fiber content is approximately 2% (Widyaratne and Zijlstra, 2007). The use of fiber-degrading enzymes, on the other hand, reduces the fiber fractions in DDGS and can be introduced throughout the fermentation process to obtain a DDGS with higher nutritional properties (Pedersen, et al., 2014).

Acceptable growth performance has been reported when up to 30% DDGS is included in diets fed to weaned pigs and grower-finisher pigs (Stein and Shurson, 2009), but reduced firmness of the pork belly has been reported when more than 20% DDGS is included in growing-finishing diets due to the unsaturated fatty acid content of DDGS combined with lean biological types of pigs (Graham et al., 2014; Shircliff et al., 2015). As the value of corn oil has increased, different oil extraction processes to acquire a higher proportion of corn oil have been developed, resulting in DDGS with oil content ranging from 4% to 12% (CEPA, 2011). Likewise, using the technology and separation processes of the DDGS protein fraction, high protein yeast-based DDGS (HP-GDDY) was obtained, with 20% more CP compared to conventional DDGS, and the ME is approximately 35% higher than conventional DDGS (Garavito et al., 2022).

1.4. SOYBEAN MEAL AND ALTERNATIVE INGREDIENT SOURCES

The United States is the world's largest soybean producer, with an annual production of approximately 122 million tons in 2021, only surpassed by Brazil's 124 million tons, and the majority of soybeans are destined for oil and SBM production, with nearly 6 million tons of SBM fed to pigs in the United States in 2020/2021 (ASA, 2021). Soybeans are important because they are one of the main sources of vegetable protein in animal feed and human food production (LIU, 2000). Soybeans are typically included at a lower level in the first few phases of nursery pig diets, with diet inclusion increasing over time from around 18% during the first phases up to 30% in last periods of nursery phase (Zeamer et al., 2021). Soybean meal is regarded as the most important vegetable protein source fed to pigs, with a crude protein content of 45.13% (NRC, 2012), owing to its excellent balance of essential AA (Table 1.1), in particular lysine (Lys), methionine (Met), and threonine (Thr), as well as its low fiber concentration, which contributes to a higher concentration of energy when compared to other oilseed-based meals (Stein et al. 2008)

1.4.1. ANTINUTRITIONAL FACTORS IN SBM

Conventional diets used in swine production commonly include SBM as the main protein source; however, SBM contains a variety of antinutritional factors (ANFs) that decrease digestibility and absorption based on young animal studies (Shi, et al., 2017; Goebel and Stein, 2010). For example, in weaned pigs, soybean antigenic proteins caused a transient hypersensitivity associated with abnormal morphology of the small intestine (Friesen et al., 1993) and therefore, the use of SBM in diets of weaned pigs is typically restricted and gradually introduced into the diets of weaned pigs (Engle, 1994). This gradual increase in inclusion is related to their abrasiveness within the gastrointestinal tract, impact on intestinal morphology, and suppression of immune response (Zarkadas and Wiseman, 2005). Trypsin inhibitor (TI), one of the most common protein-based ANFs in SBM, has a significant effect in swine and poultry (Zheng, et al., 2017). The higher the TI and lectin inhibitor content in soybeans, the lower the digestibility of nutrients, which directly affects nitrogen balance and retention (Gu et al., 2010) and has a negative impact on growth performance. Decreased growth in pigs, particularly in nursery pigs, is caused by a combination of two factors: endogenous losses of essential AA (particularly those high in sulfur, which are important components of trypsin) and decreased proteolysis of dietary protein

(Zarkadas and Wiseman, 2005). Because TI forms complexes with enzymes that reduce the activities of trypsin and pancreatic chymotrypsin during digestion, it also causes hypertrophy and pancreatic hyperplasia, as well as excessive secretion by the exocrine pancreas. Feng, et al. (2007) suggest that the use of fermented SBM improves intestinal morphology and digestive enzyme activity of weaned piglets and in addition, fermentation increases the protein content (55.3%) and reduces the size of peptides in soybeans and SBM (Kee-Jong, et al., 2004). In addition, enzyme-treated soybean meal (ESBM) has a higher crude protein content and greater AA digestibility than SBM and FSBM, as well as a lower ANF content (Cervantes-Pahm and Stein, 2010).

1.4.2. ALTERNATIVE INGREDIENTS PROTEIN

As previously stated, appropriate nutrients must be provided in pig diets to ensure efficient animal performance and profitable production (Velayudhan and Nyachoti, 2016). Given the volatility of the pricing of feedstuffs typically used in pig production, the search for alternatives to these ingredients provides a chance to mitigate the impact of this volatility. Some alternatives include the use of potential components such as canola, which are abundant. However, due to considerations such as nutritional value and even availability, the addition of alternative feeds may have some limitations (Woyengo, et al., 2014).

The field pea is a cold season alternative for regions not suitable for soybean cultivation due to climatic conditions; however, the field pea contains less CP and Lys than SBM, less starch than corn or wheat (Table 1.2) and also contains some tannin and antinutritional factors similar to other legumes (Jezierny, et al., 2010). However, Stein et al (2010) showed no negative effects on growth performance in weaned pigs using 36% raw field peas. Gatta et al (2013) observed no negative effects on growth performance and meat quality in finishing pigs when replacing SBM with 20% field peas in diets with similar DE and CP content.

Compared to peas, faba bean contains more CP, Lys and fiber and less starch (Table 1.2). Beltranena et al. (2009) concluded that young pigs can be fed with up to 40% raw beans with zero tannin content as a substitute for SBM in starter diets. Faba bean can be included at 30% in diets for growing and finishing pigs without affecting growth performance, however research is required to achieve greater efficiency and carcass yield (Woyengo, et al., 2014).

Oilseeds are an important source of essential amino acids (EAA) and an important alternative protein source. For example, SBM is generally used due to its protein content and EAA profile (Bruce, et al., 2006). However, there are other alternatives within oilseed meals, such as canola meal, cottonseed meal and sunflower meal. The nutritional quality of these alternatives depends largely on the oil extraction process, for example, the concentration of nutrients in sunflower meal would increase proportionally to the amount of oil extracted from the sunflower seeds, as is the case with the content of CP in sunflower seed meal is 22% while in sunflower meal the CP content is 29% (González-Vega and Stein, 2012).

Generally, canola meal contains less CP and AA and more fiber than SBM, and also contain glucosinolates that can affect the feed intake and nutrient utilization in pigs (Woyengo, et al., 2011). Replacing SBM with 20% solvent extracted canola meal or expeller-pressed canola meal did not affect growth performance in weaned pigs (Landero et al., 2011) and the inclusion of 22.5% of solvent extracted canola meal did not reduce growth performance of grower pigs (Montoya and Leterme, 2010). In the case of cottonseed meal, the CP content is also lower in comparison with SBM

(Table 1.2), however, the inclusion of cottonseed meal is limited by fiber content and by the presence of gossypol, which is toxic in monogastric animals (Gadhela, et al. 2011; Kamga, et al. 2000). Reported cottonseed meal inclusion levels in diets for grower and finishing pigs range from 8% to 15% without affecting animal performance (Li, et al., 2000; Rostagno, et al., 2005; da Silva, et al., 2021). Similar to canola meal and cottonseed meal, sunflower meal has a higher content of fiber than SBM (Table 1.2), however, more research is needed related to the use of sunflower meal in pigs to improve the use of its nutrients and the precision in the prediction of its nutritional value in feed formulations (Lannuzel, et al., 2022).

Triticale is a hybrid of wheat and rye that has superior agronomic characteristics to wheat and has potential for use in livestock (McGoverin et al., 2011). Although its nutritional potential requires more research, some authors discovered that triticale can be mixed in diets for growing pigs, achieving similar or slightly better ADG and F: G. (Eneva, et al., 2022; Myer and Brendemuhl, 2009). Triticale has a similar starch and fiber composition to corn; thus, their NE values are comparable (Table 1.2).

The sorghum crop is considered a substitute for energy feedstuffs due to its energy value (Table 1.2) and its price compared to wheat, barley, or oats, and can be used for ethanol production (Rooney, et al., 2007). Although the content of CP, starch and fiber is similar to corn, sorghum has a higher content of tannins (3.7%), depending on the varieties grown, which negatively affect digestibility and the use of energy and nitrogen in pigs (Pan, et al., 2022).

Hybrid rye production has increased in North America, primarily in western Canada and parts of the midwestern United States. The increase in production is due to increased yields, hibernation capacity, and drought tolerance. The ME content of hybrid rye is comparable to that of barley and sorghum (Table 1.2), but lower than that of corn and wheat (McGhee and Stein, 2020).

Field peas, faba beans, and chickpeas are examples of legumes that have been grown extensively for human use. Pea grains contain a high content of starch, however, due to the conformation of the starch and the presence of components associated with starch, they are less digestible corn (Tan, et al., 2021). Also, compared to other protein sources such as SBM, legume grains have low amounts of the sulfur AA, which requires the use of crystalline AA (Jezierny, et al., 2010).

1.5. DIETS FOR NURSERY PIGS

The weaning phase is one of the most critical periods during pig production due to the combined stressors faced by pigs such as new environment, social interactions, and change of diet (Zheng, et al., 2021). All these stressors lead to an increased susceptibility to intestinal disorders, which, in concert with an immature digestive and immune system, results in increased incidences of diarrhea, poor growth rate, increased mortality and (or) morbidity, and subsequent economic losses for swine producers (Lallés et al., 2004; Smith et al., 2010; Pohl et al., 2017). In general, there is a link between gastrointestinal immaturity and feed allergies observed during the first week after weaning. Because the intestinal barrier is altered, there may be increased transport of dietary antigens across the barrier, resulting in a stronger immune response (Groschwitz and Hogan, 2009). Weaning age significantly affects the function of the intestinal barrier; the different stressors during this stage can cause an increase in the passage of luminal antigens and toxins through the intestinal epithelium, causing inflammatory processes and systemic diseases (Moeser, et al., 2007). Therefore, it is important to implement different management strategies in order to reduce the stress associated with the weaning process, promote the physiological development of pigs and make this transition period easer. One of the commonly used strategies during this period is related with diet formulation, especially the use of ingredients with high protein content and digestibility, high-value ingredients for example, plant-based ingredients such as isolates of soy protein are included to provide a rapid increase in voluntary feed intake, thereby driving an adequate transition to solid diet, (Zijlstra, et al., 2004).

The content of other components in the diet, such as dietary fiber, defined as the dietary components resistant to degradation by mammalian enzymes and composed of non-digestible carbohydrates (NDC) and lignin, can also affect the development and integrity of the intestinal mucosa (Li, et al., 2021). Non-starch polysaccharides (NSP), which are primarily derived from plant cell walls such as cellulose, hemicellulose, and pectin, are one of the components of NDC and have been identified as anti-nutritional factors in the major cereals that make up a significant portion of pig diets. (Williams, et al., 2017; Wu, et al., 2018). Generally, amounts of fiber greater than 5% in the diet can affect growth performance in weaned piglets (Wu, et al., 2018). However, the beneficial properties of dietary fiber and other carbohydrates have been discovered when included in diets at adequate levels, and this may be a solution in intestinal health when replacing the use of antibiotics as growth promoters in pig food (Zijlstra, et al., 2019). For example, dense hydrogen bonding fibers form a hydrophobic and crystalline structure, resulting in an insoluble fiber that resists hydrolysis by enzymes. This type of fiber in nursery diets increases digesta passage rate and prevents pathogenic bacteria colonization (McDonald, et al., 2001; Guan, et al., 2021).

1.5.1. PROTEIN SOURCES IN NURSERY PIGS

To achieve a successful transition at weaning, to improve poor feed intake and post-weaning diarrhea and to promote better pig performance, ingredients such as high-quality protein sources that are easy to digest and absorb without limited ANF are generally used. Animal protein sources are generally used although they tend to be more expensive than vegetable protein sources in part due to nutrient content, for example, vegetable protein sources are lower in some essential AA, energy, and minerals such as phosphorus (P) compared to animal protein sources. Although the cost of animal protein is typically higher, the inclusion rates are lower, and nutrient digestibility and intestinal morphology in weaned pigs are improved compared to plant protein sources (Yun, et al., 2005).

1.5.2. MILK CO-PRODUCTS

Milk co-products are generally used for nursery pigs to provide lactose as highly digestible energy source in starter feeds (Jang, et al., 2021). The development of new technologies and methods allow obtaining a wide variety of products derived from milk that can be included in pig diets (dehydrated whey, crystalline lactose, deproteinized whey and whey permeate) (Nessmith, et al., 1997). The use of lactose sources in the first few phases of nursery pig diets results in significant increases in BW, feed intake and growth rate (Cromwell, et al., 2008; Naranjo, et al., 2010). An example of a source of lactose is whey powder (Table 1.3), which contains around 10 to 25% protein with 85 to 95% lactose and is a great source of energy in pig diets (Kim, et al., 2012). However, economic concerns could limit the use of milk co-products in nursery feeds, because price trends for dairy products are very unstable, and their price is high compared to grains. Therefore, diets containing high levels of milk co-products could cause an increase in the cost of pork production (Mahan, et al., 2004). The improvement in the growth performance of piglets can be stimulated mainly by two things, the easy adaptation of the intestinal tract to the diet and the stimulation of appetite and feed intake, which can be achieved with diets containing dairy products (Yoo, et al., 2018).

Similarly, the use of milk co-products stimulates intestinal immune responses and favors the proliferation of enterocytes and, therefore, the improvement of the intestinal mucosa (Jang, et al., 2021). Optimum performance results have been obtained with total lactate inclusion levels between 25% and 30% during the initial week after weaning, with a gradual decrease during the nursery period (Mahan, et al., 2004).

1.5.3. FISH MEAL AND SPRAY-DRIED PLASMA

Fishmeal (FM) is traditionally recognized as a highly digestible protein with a crude protein content of 63.28% (NRC, 2012), good profile of amino acids (Table 1.3), vitamins, and minerals. The use of FM in weaned animal diets has shown greater ileal amino acid digestibility and growth performance when compared to diets containing spray-dried plasma protein. (Kim and Easter, 2001). The use of spray-dried animal plasma (SPD) in diets for weaned piglets stimulates feed intake and improves the rate and efficiency of gain during the first two weeks after weaning (Coffey and Cromwell, 2021) due to good nutrient profile (Table 1.3). The immunoglobulin G fraction of animal plasma is responsible for the improvement in pig performance that occurs when spray-dried plasma of either bovine or porcine is included in the diet (Pierce, et al., 2005).

However, FM is about three times more expensive than vegetable protein sources such as SBM but could be an alternative for spray-dried plasma, which makes it important to think about ingredient alternatives with similar protein content and easily digestible characteristics to reduce costs (Jeong, et al., 2016).

1.5.4. ENZYMATIC TREATED SOYBEAN MEAL

During nursery period, soy is generally used as a protein source, which has a high concentration of carbohydrates, mainly NSP and free sugars, as well as oligosaccharides. These components are one of the main factors responsible for the antinutritional effect of soy, although these antinutritional factors can be reduced with ethanol extraction processes, improving the qualities of this protein ingredient (Choct, et al., 2010). To improve the quality of vegetable protein sources, the use of enzymes in SBM improve soy protein values (Table 1.3), through a process of enzymatic hydrolysis, the ANFs of soybean are broken down and the proteins are separated into more digestible peptides compared to standard SBM, the selection of enzymes must be based on the chemical composition and anatomy of cell wall (Liu, et al., 2021; Dierick, et al., 2004; Nadar, et al., 2018). Performance improved in weaned pigs from d0 to d14, plus ESBM improved immune function and antioxidant capacity with the same efficacy as fishmeal and improved compared to soybean meal, soybean protein concentrate, and fermented soybean meal (Ma, et al., 2019).

1.5.5. SYNTHETIC AMINO ACIDS

Due to the high cost of the protein sources used during the nursery period and to better meet requirements, AA supplements such as Lys, Met, Thr, Trp, Val, Gln, and in some cases Ile have been used in swine diets as additives to meet dietary targets of essential AA and in some cases to replace high-cost protein sources and reduce the CP content in the diets (Zhao, et al., 2015). The addition of synthetic AA to the diet in combination with reduced CP can also decrease the amount of nitrogen excretion. For each percentage unit of CP that is reduced in the diet, there is a reported 8.5 percenrt reduction in nitrogen excretion (Sutton and Richert, 2004). Because AAs regulate key metabolic pathways that are critical for animal maintenance, health, and growth, excessive CP reduction can have a negative effect on growth performance. This may be due to inaccurate AA requirements estimates as well as the use of synthetic AAs, which can result in AA deficiency if one or more AA are not properly balanced (Wu, et al., 2015; Vonderohe, et al., 2022). Therefore, it is necessary to consider carefully levels of synthetic AA in diet formulations.

To achieve an adequate transition where the newly weaned pig can complete its physiological and digestive development, several nutritional strategies can be used with different options as mentioned above. The proper use of ingredients during the weaning period will aid in reducing the impact of post-weaning stress and will facilitate the transition from weaned to growth period. It is also critical to identify undesirable components such ANF in feed ingredients that can impair young pig performance. As mentioned previously, the search for new strategies to promote intestinal health in pigs is important because there is a need to reduce the use of antibiotics in production animals, reduce the risk of bacterial resistance to antibiotics and reduce post-weaning diarrhea (Fabà, et al., 2019).

1.6. EVALUATING DIGESTIBLITY OF ENERGY, AMINO ACIDS, AND PHOSPHORUS IN FEED INGREDIENTS FOR PIGS

Providing adequate amounts of available energy and nutrients is crucial to optimize pig production, therefore it is necessary to have adequate information on the energy and nutrient requirements of pigs and, at the same time, to know the nutritional values of the ingredients used for pig diets. Therefore, the provision of diets that more accurately meet the daily requirements of pigs is achieved through precise quantification of the energy and nutrient composition of feed ingredients, coupled with greater accuracy of the nutritional requirements of pigs, evolving from a total concentration basis to a digestible content basis, and then to a net or bioavailable content basis (Pomar, et al., 2009). The actual nutritional and economic value of feed ingredients is only realized during least-cost diet formulation using metabolizable energy (ME) or net energy (NE), digestible amino acids (AAs), and digestible phosphorus content (Shurson, et al., 2021).

1.6.1. AMINO ACIDS DIGESTIBILITY

The enzymatic hydrolysis and microbial fermentation of proteins and peptides contained in the animal's diet, as well as the absorption of AA and peptides from the gastrointestinal lumen, are expressed as AA digestibility (Fuller, 2003). When compared to total tract digestibility, ileal digestibility estimates AA digestibility more accurately to what is available for metabolic use, allowing for more reliable values of AA bioavailability because AA are only absorbed in the small intestine and because AA are fermented in the hindgut (Sauer and Ozimek, 1986). Generally, the determination of AA digestibility values can be apparent, although apparent digestibility does not differentiate AA losses of dietary and endogenous origin in ileal digesta. While standard digestibility values correct endogenous losses in ileal digesta (Stein, et al., 2017). Digestibility values can be calculated according to the method of Lange et al. (1998). Apparent ileal digestibility (AID) values are calculated from the difference between the dietary intake of AA and the composition of AA in the digesta present in the distal ileum of pigs according to the equation (Stein, et al., 2007):

$$AID, \% = AA\ intake - ileal\ AA\ out\ flowAA\ intakex\ 100$$

Standardized ileal digestibility (SID) values are calculated as the difference between the amount of AA ingested, the amount of AA from the digesta in the ileum, and the endogenous loss of AA, according to the equation (Stein, et al., 2007):

$$SID, \% = AA\ intake - ileal\ AA\ outflow - basal\ IAAend\ AA\ intakex\ 100$$

Once the AA digestibility values of the ingredients have been determined, the additivity of the digestible contents of each AA in the individual ingredients when mixed in a diet can be assumed, and the sum represents the amount of digestible AA in the diet. However, this assumption is not entirely valid for AID, because the relative contribution of endogenous AA losses to the AA digestibility value is greater for lower crude protein ingredients (eg. cereal grains, ≤10% CP) compared to typical protein sources (eg. SBM, ≥40% CP). Therefore, the correction of the basal ileal endogenous losses of AA in SID allows to obtain digestibility coefficients with higher additivity in diets compared to the values based on AID (Stein, et al., 2005).

1.6.2. ENERGY DIGESTIBILITY

Although energy is not a nutrient, it is required for all biological processes in pigs. Energy is utilized for maintenance and production; when pigs consume feeds, dietary energy is either absorbed or excreted in feces, urine, or heat. Dietary energy absorbed by pigs is then utilized for maintenance or retention of protein or lipids (Van Milgen and Noblet, 2003). As a result, it is necessary to calculate the energy available in pig diets by subtracting the energy not available to pigs (excreted through feces, urine, gases, and increased heat) from the total energy content of the diet (Kil, et al., 2013).

Values for DE and ME are calculated by subtracting fecal energy and both fecal and urinary energy, respectively, from energy intake, according to the next equations (Adeola, 2001).

$$DE = 100\ x\ GEintake - GEoutputGEintake$$

$$ME = 100\ xGEintake - GEoutput - GEurineGeintake$$

The importance of providing adequate amounts of energy in pig diets is because energy, although not a nutrient, is an important resource for multiple biological processes (Kil, et al., 2013). Carbon-containing compounds in the feed, including fat, carbohydrate and protein provide energy when they are oxidized by the animal to produce the energy necessary to carry out biochemical processes, biosynthesis of proteins and lipids, transport of active ions, regulation of membranes and mechanical work or muscle movement (Gutierrez and Patience, 2012). Also, the concentration of energy in the ingredients of the diet can affect pig voluntary feed intake because the pig will stop consuming feed once daily energy requirement has been met (Nyachoti, et al., 2004). However, there are other factors that can affect energy intake, for example, young pigs try to consume enough feed to meet energy requirements for maintenance and growth, but in many cases, feed intake is affected by social restrictions, physiological or environmental factors, causing a daily energy intake lower than that required for maximum weight gain (Patience, et al., 2015).

The content of poorly digestible nutrients in diets and the high content of NSP are indigestible by pig digestive enzymes, can reduce the dietary nutrient utilization and increase endogenous nutrient losses (Jerez-Bogota, et al., 2021), resulting in higher fecal output and greater energy loss in the feces. Espinosa and Stein, (2018) also observed a lower output of feces and urine in pigs fed HP-DDGS compared to pigs fed DDGS, which concluded that pigs fed HP-DDGS can absorb and use more energy than DDGS-fed pigs.

1.6.3. PHOSPHORUS DIGESTIBILITY

Phosphorus is a macromineral of great importance to animals and the inclusion of adequate amounts of P in the diet is necessary for normal development, growth, and health (Mutucumarana, et al., 2015). Generally, pigs use plant-based P inefficiently, excreting between 60% and 80% of what they consume. Therefore, a large amount of the P supplied in the ingredients and in the diets is not used efficiently for meat production (Knowlton, et al., 2004). In the case of cereals used in pig diets, there is a high content of P bound to phytate, which makes it difficult for pigs to digest P, this poor digestion of P is due to the limited production of phytase, an enzyme which is capable of releasing P from phytate (Adeola and Cowieson, 2011). The digestibility of P represents a major challenge today due to the potential negative impact on groundwater and surface water and air quality. The P that is not used by the animal is excreted and accumulates in the soil; the main concern with the accumulation of P in the soil is that it accumulates and contaminates surface and ground water sources (Zhou, et al., 2016). Therefore, to avoid P losses, management practices such as avoiding P surpluses and improving P use efficiency should be implemented and should always have priority in the preparation of diets that have P sources (O' Flynn, et al., 2018).

Digestibility studies indirectly estimate P availability by measuring its digestive utilization. To estimate the digestibility of P, different procedures are used: the direct method, the difference method, or the regression method (Zhai and Adeola, 2013). The direct method requires a P-free diet and a diet where the evaluated ingredient is the only source of the component of interest; the difference method requires a basal diet and a test diet in which a portion of the basal diet has been replaced by the test ingredient (Kong and Adeola, 2014). The regression analysis method establishes linear relationships between P outputs in ileal digesta or feces and their dietary inputs (Fan, et al., 2001).

Total tract apparent digestibility (ATTD) and relative bioavailability are used to determine P digestibility in porcine feed ingredients (Jondreville and Dourmad, 2005). The ATTD of P is calculated as the difference between P intake and P excretion in feces. The sample collection methods are performed by means of the total collection method and the indicator procedure (She, et al., 2017). To determine ATTD on nutrients and P, the following equation described by Adeola (2001) was used:

$$ATTD\ (\%) = 100\ XNintake - NoutputNintake$$

However, estimates of apparent P digestibility do not take into account the animal's endogenous P loss (EPL). By quantifying the EPL resulting from the feed it is possible to determine the standardized total tract digestibility and the true total tract digestibility n individual feeds, and thus the inclusion of P in diets is more accurate based on requirements of animals and reduces the use of mineral sources that add additional cost to diets (Dilger and Adeola, 2006).

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Table 1.1 The primary energy and protein source used in swine production around the world[1]

	Corn	SBM
DM %	90.87	95.57
CP %	14.79	45.13
Starch %	23.51	1.89
Essential AA		
Arg	1.11	3.02
His	0.42	1.14
Ile	0.43	1.90
Leu	1.05	3.21
Lys	0.78	2.79
Met	0.26	0.60
Phe	0.57	2.15
Thr	0.52	1.73
Trp	0.10	0.69
Val	0.72	2.01

[1] Values obtained from NRC (2012).

Table 1.2. Nutrient composition, energy value, and standardized ileal digestibility (SID) of Lys for alternative feedstuffs for pigs compared with corn and SBM[1].

Feedstuff[2]	**Content, %DM**					**Energy, Kcal/Kg DM**		**SID of**
	CP	EE	Starch	ADF	Lys	DE	NE	**Lys, %**
Corn	9.33	3.94	70.8	3.20	0.28	3,908	3,026	83.8
Sorghum	10.5	3.83	78.4	5.48	0.22	4,023	3,110	82.8
Triticale	15.4	2.00	72.7	3.84	0.52	3,752	2,833	88.2
Wheat	16.3	2.05	67.1	3.95	0.44	3,736	2,788	92.5
H. rye[3]	10.81	1.28	56.6	2.58	0.41	3,682	-	62.1
Field pea	25.2	1.36	49.3	7.68	1.85	3,977	2,746	96.5
Faba bean	30.8	1.48	44.5	11.5	1.87	3,682	2,432	96.5
SBM	53.0	1.69	2.10	5.87	3.29	4,022	2,319	98.9
EPCM	37.8	10.7	4.08	19.2	1.70	4,059	2,525	76.3
SECM	41.1	3.53	6.65	17.2	2.27	3,584	2,069	98.9
CSM	90.69	5.50	1.95	17.92	1.50	2,912	1,624	63.0
SFM	39.86	2.90	2.08	23.0	1.45	2,840	1,482	78.0

[1]Values obtained from NRC, 2012
[2] EPCM: expeller-pressed canola meal, SECM: Solvent extracted canola meal, CSM: cottonseed meal, SFM: sunflower meal.
[3]McGhee and Stein, 2020.

Table 1.3. Common high-protein sources used in weaned pig diets.

	Fish Meal[1]	ESBM[2]	SDP[3]	Whey powder[4]
Dry matter	93.7	91.48	90.8	95.82
CP	63.28	53.74	78.12	13.20
Lactose	-	-	-	66
C. Fiber	0.24	3.31	-	-
Essential AA				
Arg	3.84	3.50	3.35	0.22
His	1.44	1.35	4.99	0.17
Ile	2.56	2.31	0.94	0.52
Leu	4.47	3.98	9.98	0.91
Lys	4.56	3.06	6.94	0.75
Met	1.73	0.71	0.94	0.16
Phe	2.47	2.74	5.38	0.29
Thr	2.58	2.02	1.87	0.62
Trp	0.63	0.69	1.17	0.18
Val	3.06	2.40	6.71	0.50

[1] NRC, 2012
[2] Cervantes-Pahm and Stein, 2010
[3] Zhang, et al., 2019
[4] Kim, et al., 2012; Nessmith, et al., 1997

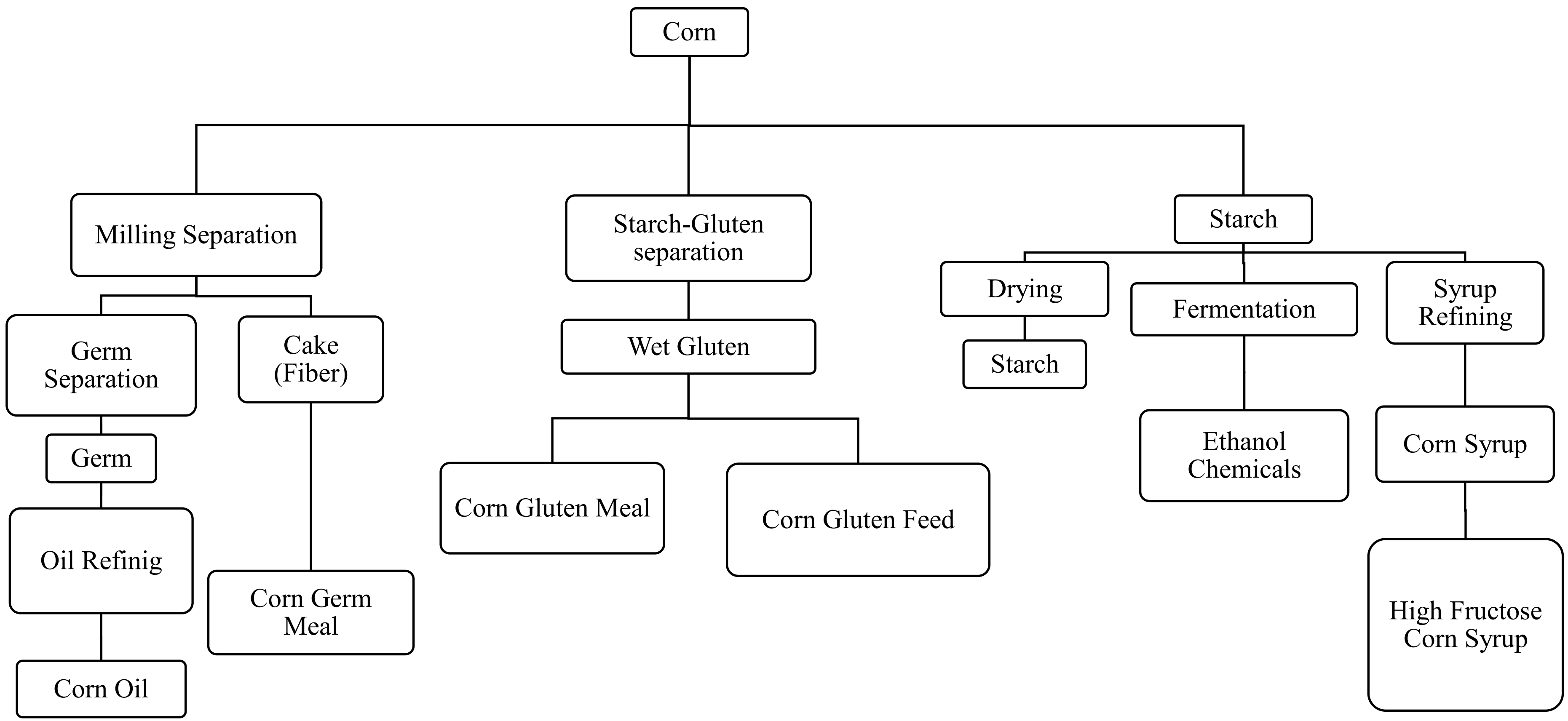

Figure 1.1. By-products obtained from the milling industry. Adapted from Erickson et al. (2005)

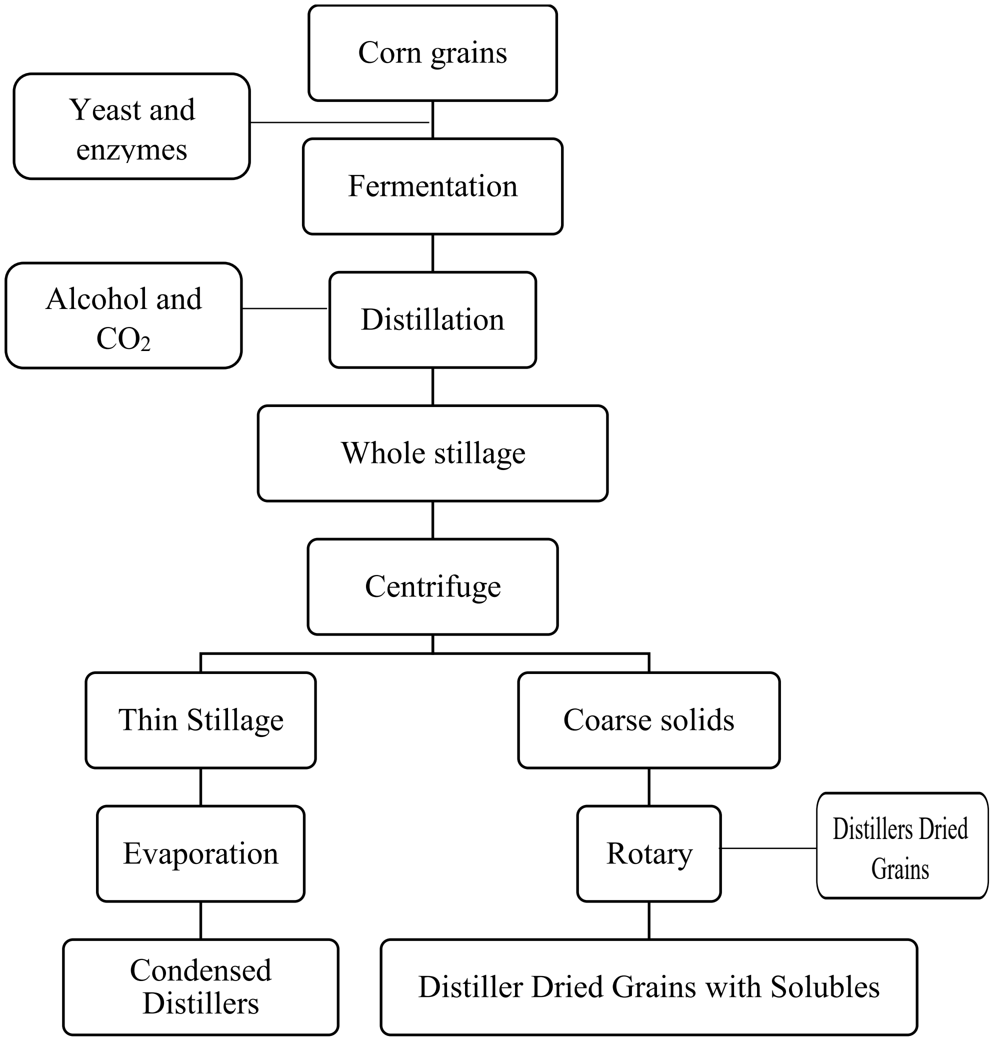

Figure 1.2. Dry grind ethanol production process and by-products. Adapted from Erickson et al (2005).

2. NUTRITIONAL VALUE OF YEAST-BASED HIGH PROTEIN PRODUCTS FED TO GROWING PIGS IN COMPARISON TO COMMONLY USED PROTEIN SOURCES IN SWINE DIETS

ABSTRACT

Two experiments were conducted to determine the standard ileal digestibility (SID) of amino acids (AA) and the concentration of metabolizable energy (ME) in 2 yeast-based high protein DDGS products (HP-GDDY) fed to growing pigs. In Exp. 1, 7 barrows (25 ± 0.8 kg BW) were fitted with a simple T-cannula at the distal ileum to allow for digesta collection. Experimental diets were Nitrogen free (N-free) diet and 6 cornstarch-based diets containing 6 ingredients as the sole source of AA: spray dried HP-GDDY, ring dried HP-GDDY, DDGS, soybean meal (SBM), enzymatically treated soybean meal (ESBM), and fishmeal (FM) provided at 4% of BW. The experiment was conducted as a 7 × 7 Latin square design with 7 collection periods of 7d (5d adaptation and 2d ileal digesta collection). Diets and ileal digesta were analyzed for AA. In Exp. 2, a total of 28 barrows (28.8 ± 1.4 kg BW) were used in a cross-over design where each pig received one of 7 experimental diets in each of 2 experimental periods (n=8 reps/diet). Experimental diets were a corn-based basal diet and 6 corn–based diets containing spray dried HP-GDDY, ring dried HP-GDDY, DDGS, SBM, ESBM and FM. Fecal and urine samples were collected using the marker-to-marker approach for 5d after 7d of adaptation to determine ME content. Overall, SID values were within the mean +/- SD of NRC (2012) values for all ingredients evaluated. The SID of AA was greater ($P < 0.05$) in ESBM than the other protein feedstuffs (90.09% vs. 78.71 – 81.51%). The SID of AA were similar ($P > 0.05$) in SBM, FM, and HP-GDDY (81.49, 78.71, 81.52, and 79.20 %). With respect to the most common first limiting AA for swine, the SID of Lys was greater ($P < 0.05$) in spray HP-GDDY than ring HP-GDDY and DDGS (83.56 vs. 77.33 and 68.53 %, respectively). The ME in SBM (3824

Kcal/Kg), ESBM (3883 Kcal/Kg), and FM (3823 Kcal/Kg) was similar ($P > 0.05$) to spray GDDY and ring GDDY. Collectively, this study demonstrates that HP-GDDY products are potential alternative ingredients for swine diets, particularly SBM and corn and the obtained data allows its use in practical diet formulation.

Keywords: amino acids, crude protein, diet formulation, digestibility, metabolizable energy.

2.1 INTRODUCTION

Animal feed represents one of the most expensive items in animal production, whether for meat, milk, or eggs (Shurson, 2017). Within the challenges of feeding livestock animals, pig producers face high variation in prices for main ingredients used in swine diets (Chassé, et al., 2021) due to the global feed supply and demand, along with supply chain and shipping issues (Pork Checkoff, 2021). Soybeans and corn are the main feedstuffs in pig diets in the United States (Schmit et al., 2009). In the past few years, corn prices have increased due to the ongoing demand on the ethanol industry for ethanol production which is driven in part by the combination of high oil prices and subsidies granted for its production. Approximately 94% of the total feedstock used for ethanol production in the US comes from corn grain or high-starch agricultural crops which contain approximately 60% starch (RFA, 2020). The increased ethanol demand consequently results in higher corn prices and thus higher cost of swine diets (Tyner and Taheripour, 2007). Similarly, soybean prices have varied due to several factors including those mentioned above with corn (Zibin, et al., 2009). In addition, the increase in area planted with corn over soybeans, together with the extreme weather abroad has caused soybean shortages among foreign exporters, resulting in price increases, and affecting pork production costs in the United States (Ralph, 2020).

However, the ethanol industry produces some alternative co-products for livestock nutrition. The co-products of the ethanol industry mainly include dry distillery grains (DDG), wet distillery grains (WDG), dry distillery grains with soluble (DDGS), wet distillery grains with soluble (WDGS) and distillery condensed soluble (syrup). As a result of the starch extraction during the ethanol production process, the remaining nutrients in the corn increase in concentration approximately 3 times (Pati, 2008). Therefore, one strategy used to reduce pig production costs is inclusion of alternative protein ingredients such as DDGS in swine diets (Wu and Munkvold, 2008). In addition, DDGS has been used for the benefit of other monogastric and ruminant species, generating around 25% of total income for ethanol production plants (Hill, et al., 2006). Although the physical appearance, chemical composition, and nutrient digestibility of DDGS can vary considerably, depending on source and processing techniques, DDGS products can be included in phase 2 and 3 nursery diets at levels up to 25% without negatively affecting feed intake, growth rate, or feed conversion (Whitney and Shurson, 2004).

These co-products represent added value in the manufacture of ethanol because the commercialization of co-products as livestock feed increases the economic viability of ethanol plants (Liu and Barrows, 2013). More recently, yeast-based high protein DDGS products (HP-DDGY) have been developed through separation of the protein fraction from DDGS. Han and Liu (2010) reported, in the case of DDGS, there is a significant amount of residual yeast after fermentation, which is estimated to contribute around 20% of the protein content. Therefore, HP-GDDY are expected to contain higher concentrations of yeast and thus higher concentration of AA. Although there have been some previous studies on the nutritional value and growth performance of pigs fed DDGS and HP-DDGS (Widmer et al., 2007; Espinosa and Stein, 2018; Cemin, et al., 2021), there is no information on the nutritional value of these new HP-GDDY products for pigs using different drying techniques. In this regard, understanding product value to practical swine feeding begins with product characterization and determination of feeding value (i.e., nutrient digestibility). The objective of this study was to determine the chemical composition, standardized ileal digestibility (SID) of AA and metabolizable energy (ME) in 2 HP-GDDY fed to growing pigs in comparison to common protein and energy feedstuffs used in swine diets.

2.2 MATERIALS AND METHODS

The protocols for these experiments were approved by the Institutional Animal Care and Use Committee at South Dakota State University (IACUC #18-013A and #2105-023).

2.2.1 EXPERIMENT 1: AA DIGESTIBILITY

2.2.1.1 EXPERIMENTAL DIETS

Cornstarch-based diets were formulated (Table 2.1) using 6 ingredients as the sole source of AA: spray dried HP-GDDY, ring dried HP-GDDY, DDGS, soybean meal (SBM), enzymatically treated soybean meal (ESBM) and fishmeal (FM). The choice of ingredients other than GDDY was based on protein feedstuffs commonly used in swine diets and the existence of previous reports of nutritional value to validate the results obtained. An additional N-free diet was used to estimate endogenous AA losses (Stein, et al., 2007). Minerals and vitamins were added to the diets according to NRC (2012) nutrient requirements for growing pigs. Titanium oxide (0.3%) was included in the diets as an indigestible marker to determine nutrient digestibility (Zhang and Adeola, 2017).

2.2.1.2 ANIMALS AND HOUSING

Seven barrows (25 ± 0.8 kg BW), offspring of PIC 1050 sows and Duroc boars, were surgically fitted with a simple T-cannula at the distal ileum. Pigs were adapted to individual housing equipped with slatted floor, a feeder, and a nipple drinker in a temperature-controlled room.

2.2.1.3 EXPERIMENTAL DESIGN

The experiment was conducted according to a 7 × 7 Latin square design so that each pig received each experimental diet. Feeding levels were based on pig BW (4% of BW) and adjusted for individual pigs at the beginning of each collection period. The diets were administered as mash form twice a day (8:00 am and 16:00 pm) and water was provided ad libitum throughout the experimental period. For each 7d period, the first 5d were considered a period of adaptation to the diet followed by 12 h of continuous collection of ileal digesta on days 6 and 7 (from 8:00 am to 20:00 pm) from a plastic bag fixed to the cannulas. Each plastic bag contained 5 ml of 10% formic acid to limit microbial growth (Htoo, et al., 2007; Ricke, et al., 2020), which was replaced when it reached levels of 50 to 70% of its capacity. At the end of each collection period, samples collected for each pig were mixed.

2.2.1.4 CHEMICAL ANALYSIS

At the end of the collection periods, ingredients were analyzed for dry matter (DM), crude protein (CP), and AA. Diets and ileal digesta were analyzed for AA and titanium. Ileal digesta samples were freeze dried. Diet and ileal digesta samples were ground to pass through a 0.5 mm screen using a mill grinder (Retsch zm 200, ring sieve size: 0.75 mm) before chemical analysis. The DM content in the diets was determined by drying samples at 102°C for 24 hours using a drying oven. To determine the CP and AA content samples were analyzed at a commercial laboratory (University of Missouri, Columbia MO) using the method 990.03; AOAC International, 2007 and method 982.30 E; AOAC, 2005, respectively. Titanium content was analyzed following the procedure from Myers et al. (2004), the procedure includes wet-ash digestion of sample (Njaa, 1961), followed by addition of H_2O_2 as described by Titgemeyer et al. (2001) to produce an orange/yellow color and subsequently the absorbance was measured with SpectraMAX 190 plate reader at wavelength 408nm. Digestibility values were calculated according to the method of De Lange et al. (1998). Apparent ileal digestibility (AID) values were calculated from the difference between the dietary intake of AA and the composition of AA in the digesta present in the distal ileum of pigs according to the equation (Stein, et al., 2007):

$$AID, \% = AA\ intake - ileal\ AA\ out\ flowAA\ intakex\ 100$$

Standardized ileal digestibility (SID) values were calculated by the difference between the amount of AA ingested, the amount of AA from the digesta in the ileum, and the endogenous loss of AA, according to the equation (Stein, et al., 2007):

$$SID, \% = AA\ intake - ileal\ AA\ outflow - basal\ IAAend\ AA\ intakex\ 100$$

2.2.2 EXPERIMENT 2: ENERGY DIGESTIBILITY

2.2.2.1 EXPERIMENTAL DIETS

A corn-based basal diet and 6 corn–based diets containing spray dried HP-GDDY, ring dried HP-GDDY, DDGS, SBM, ESBM and FM were formulated (Table 2.2). Minerals and vitamins were added to the diets according to the nutrient NRC (2012) requirements for growing pigs.

2.2.2.2 ANIMALS AND HOUSING

A total of 28 barrows (28.8 ± 1.4 kg BW), offspring of PIC 1050 sows and Duroc boars, were randomly allotted to 3 groups and seven diets based on initial BW. Pigs were adapted to individual housing equipped with slatted floor, a feeder, and a nipple drinker in a temperature-controlled room. A screen and urine pan were placed under the slatted floor to allow for total, but separate, collection of urine and feces.

2.2.2.3 EXPERIMENTAL DESIGN

Pigs were assigned to a crossover design with 7 experimental diets and 2 collection periods, which provided 8 observations for each treatment. Feeding levels were based on pig BW (4% of the BW) adjusted for individual pigs at the beginning of the experimental period. Feed was provided each day in 2 equal meals (8:00 am and 16:00 pm) and pigs had ad libitum access to water. Feed consumption was recorded daily, and diets were fed to pigs in 2 periods of 12d each. Within each period, the initial 7d were considered an adaptation period to the diets; urine and feces were collected during the following 5d according to standard procedures using the marker-to-marker approach (Adeola, 2001).

2.2.2.4 CHEMICAL ANALYSIS

Urine samples were collected in containers with 50 mL of 6N HCl used as preservative. Fecal samples and 20% of collected urine were stored at -20°C immediately after collection. At the conclusion of the collection periods, fecal samples were homogenized, dried at 102°C for 24 hours using a drying oven, and ground to pass through a 0.5 mm screen using a mill grinder (Retsch zm 200, ring sieve size: 0.75 mm). Urine samples were thawed and mixed within animal and diet to obtain homogenized subsamples prior to analysis. Diets and fecal samples were analyzed for DM. Gross energy (GE) content in diets, fecal samples and urine samples was analyzed by bomb calorimetry (Parr 6300 calorimeter, Parr Instruments Co., Moline, IL). The GE in urine was determined in triplicate following the method described by Kim, et al. (2009). Briefly, approximately 10 ml of urine was added to a small cotton ball (0.2 to 0.3 g) placed in a plastic bag (approximately 0.2 g). The weight of the plastic bag, the cotton ball, and the plastic bag containing the cotton ball and urine were recorded. The bag was then lyophilized, the weight was re-recorded, and the GE of the bag containing the cotton and lyophilized urine was measured. Weight and GE of 6 empty plastic bags and 6 virgin cotton balls were also recorded, and the average GE of the 6 bags and 6 cotton balls per gram was assumed to represent the GE of the bags and the average GE of the bags cotton, respectively. These values were then multiplied by the weight of the bag and cotton ball, respectively, that had been placed in the bomb together with the urine, and the GE contributed by the plastic bag and cotton ball was subtracted from the total GE that was measured in the bag containing the cotton ball and the urine to calculate the GE of the urine in the sample (Kim, et al., 2009).

Values for digestible energy (DE) and ME for each experimental unit were calculated by subtracting fecal energy and both fecal and urinary energy, respectively, from energy intake, according to the next equations (Adeola, 2001).

$$DE = 100\ x\ GEintake - GEoutputGEintake$$

$$Me = 100\ xGEintake - GEoutput - GEurineGeintake$$

2.2.2.5 STATISTICAL ANALYSIS

The SAS UNIVARIATE procedure was used to confirm the homogeneity of the variance and analyze for outliers. Data was then analyzed using the SAS MIXED procedure (SAS Inst. Inc., Cary, NC). For Exp. 1, the model contained the fixed effects of diet and the random effects of pig and period. For Exp. 2, the fixed effect of diet (n = 7) and the block effect of group (n = 3) were included in the main model. Tukey's adjusted means test was used to detect differences between ingredients where $P \leq 0.05$ is considered significant. Contrast statements were used to compare AA and energy digestibility among specific ingredients.

2.3 RESULTS

2.3.1 INGREDIENT'S COMPOSITION AND EXPERIMENTAL DIETS

The analyzed chemical composition of dietary protein feedstuffs used in the present study is presented in Table 2.3. The CP content of the major protein feedstuffs, including DDGS, ranged from 30.4% to 64.2%, with ESBM having the highest CP content and DDGS having the lowest content. The CP content in the HP-GDDY ingredients ranged from 50.0% to 55.0%. Crude fat content ranged from 3.5% to 7.9% with spray GDDY having the lowest content and FM having the highest. The ash content among the main protein resources SBM, ESBM, and FM was 6.0%, 6.9%, and 20.6%, respectively, while DDGS, spray GDDY, and Ring GDDY had ash content of 4.0 %, 3.5 %, and 3.7 %, respectively. Neutral detergent fiber (NDF) content for DDGS, spray GDDY, and ring GDDY was 29.7%, 4.7%, and 4.4%, respectively, starch content for DDGS, spray GDDY, and ring GDDY were 4.3%, 3.9%, and 5.2%, respectively. Among the carbohydrates evaluated, fructose content in DDGS, spray GDDY, and ring GDDY was 0.03%, 0.02%, and 0.03%, respectively, glucose content varied between 0.5%, 0.4%, and 0.3%, while maltose content varied between 0.03%, 0.02%, and 0.02%.

Regarding the main limiting AA, Lysine (Lys) content among the main protein feedstuffs, including DDGS, ranged from 0.9% to 4.6%, with DDGS having the lowest

Lys content and FM the highest Lys content; Lys content for ring GDDY and spray GDDY was 2.57% and 2.22%, respectively. Threonine (Thr) content varied between 1.05% and 2.35%, with DDGS having the lowest and FM the highest Thr content, spray GDDY and ring GDDY, Thr content was 2.21% and 2.04%, respectively. The percentage of Methionine (Met) varied between 0.55% and 1.60%, where DDGS had the lowest Met content and FM the highest Met content, for spray GDDY and ring GDDY, the percentage of Met was 1.24% and 1.10%, respectively.

Experimental diet formulation and composition are presented in Table 2.1, 2.2, and 2.4. The analyzed chemical composition of the diets partly corresponded to the formulated composition and were within the tolerance of normal variance, where the mean and variance of the data analyzed for the chemical composition of the diets and the formulated composition of the diets are independent of each other, which confirms a normal approximation (Valero, et al., 2012). The analyzed GE content of experimental diets was 10% above formulated for the energy digestibility trial.

2.3.2 APPARENT ILEAL DIGESTIBILITY

The AID values for indispensable AA were different among ingredients, where AID in spray GDDY was similar to ring GDDY and SBM and greater than in DDGS and FM (Table 2.5). The AID of AA was greater ($P < 0.001$) in ESBM compared to the other protein feedstuffs. With respect to the most common first limiting AA for pigs, the AID of Lys in spray GDDY were higher ($P < 0.001$) compared to ring GDDY and DDGS, and comparable to FM and SBM. The AID of Thr was similar for spray GDDY, ring GDDY, SBM, and FM. The AID of Met in spray DDGS, ring DDGS and DDGS were comparable to those of SBM, and these values were also higher ($P < 0.001$) than those of FM. The results from the contrast analysis support the previous interpretations, where AID of Lys and Thr were significantly greater ($P < 0.05$) in HP-GDDY products than in DDGS.

2.3.4 STANDARD ILEAL DIGESTIBILITY

The SID values for ESBM were greater ($P < 0.001$) compared to the other protein feedstuffs evaluated; spray GDDY, ring GDDY, and SBM were comparable (Table 2.6). The lowest SID values for AA were found in pigs fed with DDGS and the FM feedstuff.

The SID of Lys was higher ($P < 0.001$) in the diet containing spray GDDY, compared to the diet containing ring GDDY and the diet containing DDGS, but SID of Lys in the diet containing ring GDDY was greater ($P < 0.001$) compared to the diet containing DDGS. SID values for Lys were comparable for diets containing, SBM, FM, and spray GDDY. The SID of Thr in the diets containing spray GDDY, ring GDDY and SBM were similar; although SID value for spray GDDY Thr was higher ($P < 0.001$) compared to diet containing DDG. Spray GDDY, ring GDDY and SBM diets were similar in SID of Met, while FM diet had the lowest SID of Met relative to the assessed diets. The results from the contrasts analysis for SID indicated that SID values of Lys were lower ($P < 0.05$) in the diet containing DDGS when compared to each other diets containing the tested feedstuff, SBM, ESBM, FM, spray GDDY and ring GDDY. However, the SID Met values were similar contrasting the spray GDDY, ring GDDY, and DDGS diets. The SID threonine values were significantly greater ($P < 0.05$) in the diets including HP-GDDY products than in DDGS based-diets.

2.3.5 ENERGY DIGESTIBILITY

Fecal output was greater ($P < 0.05$) for pigs fed DDGS than for pigs fed with other protein feedstuffs (Table 2.7). The concentration of GE in feces from pigs fed FM was lower ($P < 0.05$) in comparison with the other evaluated diets except SBM. However, the concentration of GE in feces from pigs fed diets with spray GDDY, ring GDDY were similar in comparison with corn, SBM and ESBM.

The percentage of DE was lower ($P < 0.05$) in DDGS when compared to the other tested feedstuffs. The concentration of DE was greater ($P < 0.05$) in spray GDDY than other feedstuffs except ring GDDY. However, ring GDDY has similar DE values when compared to the tested SBM, ESBM and FM and greater values than corn and DDGS.

There was no difference in urine output or GE concentration among the urine samples. The percentage of ME was lower ($P < 0.05$) in DDGS (66.32%) in comparison with the other tested feedstuffs. The percentage of ME in the spray GDDY and ring GDDY were similar than corn, SBM, ESBM and FM feedstuff. The concentration of ME was similar in spray GDDY and ring GDDY, and those feedstuffs were greater ($P < 0.05$) than DDGS. The concentration of ME was similar in SBM, ESBM and FM ingredients.

The DE in spray GDDY was greater ($P < 0.05$) than DDGS, FM, and corn, but similar than ring GDDY (Table 2.8). The DE was similar in SBM, ESBM, FM, and ring GDDY. The ME in spray GDDY was greater ($P < 0.05$) in comparison to DDGS and corn. Corn, SBM, ESBM, FM and ring GDDY feedstuff had comparable ME values.

2.4 DISCUSSION

Collectivity, data from this study support that HP-GDDY products from the ethanol industry have greater nutritional value for pigs when compared to DDGS and comparable nutritional value to commonly used protein feedstuffs in swine diets.

The chemical composition of the main protein feedstuffs (SBM, ESBM, FM, and DDGS products) were within the ranges of the NRC (2012) although CP content in the

DDGS used in this study was 2.56% above the average percentage reported in NRC (2012), and the CP for the HP-GDDY was considerably higher than conventional DDGS. Espinosa and Stein (2018) reported the protein content of HP-DDGS was 38% to 44% and DDGS was 27% with NDF content in HP-DDGS at 31.87%. Similarly, Yang, et al. (2018) reported 31.07% NDF. These NDF values are between 6 to 23% higher compared to HP-GDDY evaluated in the present study, in terms of NDF content, the values were lower by 25% compared to HP-DDGY. Those values were expected because as the economic value of corn oil has increased different oil extraction procedures are used to obtain a higher proportion of corn oil. resulting in by-products with lesser oil content (i.e. 4% and 12%; (CEPA, 2011), which increases the concentration of other nutrients in these co-products. The CP content in the HP-GDDY may be related to the process used to extract the fermentable components from DDGS and the partial use of yeast in the fermentation process. Similarly, the content of fibrous components is high in conventional DDGS (NRC, 2012), however, as mentioned above, the content of fibrous components in the present study was lower for HP-GDDY. The decrease in the concentration of NDF and other fibrous components is due to the removal techniques of non-fermentable components such as milling, heating and fermentation (Pedersen, et al., 2014; Urriola, et al., 2010).

Processing methods such as milling (Yáñez, et al., 2011), granulation (Zhu, et al., 2010), and extrusion (Oryschak, et al., 2010) have been suggested to improve the nutritional values of feedstuffs, although these processing methods have not been very effective in reducing the content of poorly digestible nutrients in DDGS (De Vries, et al., 2013). Additionally, heating methods are proposed to improve nutrient digestibility (Zangaro, et al., 2018). According to the presented results, the processing technique carried out to obtain HP-GDDY products improved AA digestibility and ME when compared to conventional DDGS. In relation to the drying process and its effects on the final product characteristics, spray dried GDDY had a higher concentration of nutritional components in the final product when compared to ring dried GDDY (i.e., Lys content and digestibility). However, due to the similarity of these products in overall nutritional value, other aspects such as processing costs, logistics at the plant, or even costumer's preferences should be considered in order to design marketing strategies.

As for AA content and digestibility, the content of Lys, Met and Thr were greater in HP-GGDY products compared to DDGS, which may be attributed to the presence of residual yeast cells mass in the HP-DDGS (Stein and Shurson, 2009). The cell mass is the result of the carbohydrate fermentation process by the yeast, this cell mass provides a value of yeast protein, which contributes significantly to the protein and AA content in the DDGS and HP-GDDY (~ 60g/g100 DM) (Belyea, et al., 2004). In addition, the Lys-to-CP ratio is 4.6%. A Lys-to-CP ratio of not less than 2.8% is the recommended value used when evaluating the quality of DDGS for use in swine diets (Stein, 2007). Stein (2007) suggested that greater Lys-to-CP ratio could indicate the product is suffering less heat damaged and has a good AA digestibility, which can be confirmed analyzing the Lys digestibility.

The AID and SID values for HP-GDDY were higher for the main limiting AA in pigs in comparison with previously values reported for HP-DDGS (Espinosa and Stein, 2018). A probable explanation for this observation may be due to the techniques used for the separation of nutrients and fermentation to obtain co-products, however the extraction efficiency to obtain HP-GDDY results in a product with a higher protein content and a lower fiber content with a greater digestibility in comparison with the conventional DDGS. The observation that the AID and SID for AA in HP-GDDY are greater than in DDGS is in agreement with reported values for HP-DDG (Widmer et al., 2007), where it is also concluded that the increase in the digestibility of AA may be related to the lower percentage of NDF present in HP-DDG and HP-GDDY than in DDGS. In addition, as mentioned previously, endogenous AA losses are related to dietary composition factors that also decrease AID and SID of AA (Souffrant, 2001). According to the results obtained in the composition of DDGS and HP-GDDY the endogenous AA losses also will depend due to dietary fiber, which has been shown to increase gastrointestinal production of mucin, which serves as an intestinal barrier, but this mucin is poorly digested for reabsorption and is considered a major contributor to endogenous AA losses (Urriola et al., 2013; Adeola, et al., 2016). Similarly, the inclusion of a high concentration of protein in the diet may increase the specific endogenous loss of AA, because in response to high protein intake, the secretion of digestive enzymes in the digestive tract will be increased (Adedokun, et al., 2008) which, together with the fiber content, can limit a better use of the protein content in HP-GDDY.

The starch content in the ring GDDY, spray GDDY and DDGS feedstuffs evaluated in this document were below the starch levels reported by NRC (2012) for conventional DDGS, which is between 6% and 10%, which suggests that the starch fermentation process used to produce this ingredient was successful in the fermentation of cornstarch. Residual starch content may indicate that there was a fraction of simple carbohydrates that escaped the fermentation process and could therefore dilute protein and lipid concentrations and lower the nutritional value of the ingredient depending on the portion of residual starch (Srichuwong and Jane, 2011).

Generally, N is excreted as urea in the urine, however, due to the fiber content, N may be excreted as microbial N, reducing the DE value relative to the ME value. (Cristobal et al., 2020). In the case of HP-GDDY, the DE:ME ratio will be affected by the negative relationship between fiber content and N loss. Widmer et al. (2007) reported DE and ME values of 4,763 and 4,476 Kcal/Kg of DM for HP-DDG, respectively, while for DDGS the values of DE and ME were 4,140 and 3,897 Kcal/Kg of DM, respectively. These values are similar to the values obtained in the present study. It is important to note that ME values are also an expression of non-starch polysaccharides (NSP) fraction in each ingredient, since the NSP fraction in each ingredient plays an important role during degradation of ingredients and therefore, the NSP fraction will influence the value energy of the ingredient (Jaworski, et al., 2015). The presence of NSP is mainly digested in the large intestine by microbial fermentation, however, pigs cannot hydrolyze NSP efficiently and hindgut fermentation is less efficient for energy utilization than enzymatic hydrolysis in the small intestine (Noblet, et al., 1994). Therefore, the use of enzymes such as carbohydrases alone or in combination with phytase improves the energy digestibility of the ingredients (Woyengo, et al., 2014; Zeng, et al., 2018) when fibrous components such as NDF are reduced by about 30% in the case of HP-GDDY evaluated in the present study.

Overall, the presented results indicated that HP-GDDY had improved Lys, Thr, and Met content and digestibility in comparison with DDGS and can be potential alternative protein feedstuffs in swine diets, in particular as an alternative to SBM due to the similar SID AA content. In the same way, HP-GDDY has 25 – 40% more metabolizable energy per unit than corn suggesting HP-GDDY has superior nutritional value with respect to energy availability in comparison to corn. Collectively, data from this study demonstrate that HP-GDDY products are potential alternative ingredients for swine diets, particularly SBM and corn and the obtained data allows its use in practical diet formulation.

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Table 2.1. Formulation and calculated composition of experimental diets to evaluate SID of AA in yeast-based high protein products fed to growing pigs in comparison to commonly used protein feedstuffs in swine diets - Exp 1

	N-free	SBM	ESBM	Fishmeal	DDGS	Spray GDDY	Ring GDDY
Ingredient							
Cornstarch	66.44	59.11	58.18	63.30	28.80	51.23	51.23
SBM	0.00	27.00	0.00	0.00	0.00	0.00	0.00
Dakota Gold, DDGS[1]	0.00	0.00	0.00	0.00	55.00	0.00	0.00
Spray GDDY[1]	0.00	0.00	0.00	0.00	0.00	34.00	0.00
Ring GDDY[1]	0.00	0.00	0.00	0.00	0.00	0.00	34.00
Fishmeal	0.00	0.00	0.00	25.00	0.00	0.00	0.00
ESBM[2]	0.00	0.00	28.00	0.00	0.00	0.00	0.00
Sucrose/sugar	20.00	10.00	10.00	10.00	10.00	10.00	10.00
Soya oil	4.00	0.80	0.80	0.80	4.00	1.00	1.00
Solka floc	5.00	0.00	0.00	0.00	0.00	0.00	0.00
Mg sulfate	0.00	0.00	0.00	0.18	0.00	0.00	0.00
Potassium carbonate	0.61	0.00	0.00	0.22	0.00	0.29	0.29
Limestone	0.40	0.55	0.50	0.00	1.55	0.50	0.50
Dicalcium phospate	1.20	1.15	1.80	0.00	0.00	1.70	1.70
Salt	0.25	0.24	0.22	0.00	0.05	0.18	0.18
Monocalcium phosphate	1.60	0.65	0.00	0.00	0.10	0.60	0.60
Grower Vitamin premix[3]	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Mineral premix [4]	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Titanium dioxide	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Analyzed Nutrient Composition							
ME, Kcal/kg	3771	3752	3790	3905	3714	3759	3759
Crude Protein, %	0.20	15.93	15.85	16.01	15.38	15.57	15.57
total Lysine, %	0.00	0.99	0.91	1.14	0.50	0.41	0.41
SID Lysine, %	0.00	0.88	0.83	0.98	0.30	0.29	0.29

SID Met, %	0.00	0.20	0.19	0.38	0.27	0.27	0.27
SID, Met + Cys, %	0.00	0.42	0.39	0.47	0.48	0.49	0.49
SID THR, %	0.00	0.56	0.55	0.52	0.43	0.41	0.41
SID TRP, %	0.00	0.22	0.19	0.12	0.10	0.07	0.07
SID ILEU, %	0.00	0.70	0.66	0.53	0.39	0.50	0.50
SID LEU, %	0.00	1.17	1.07	0.93	1.47	1.81	1.81
SID VAL, %	0.00	0.72	0.71	0.63	0.51	0.56	0.56
SID HIS, %	0.00	0.39	0.38	0.30	0.31	0.29	0.29
SID PHE, %	0.00	0.72	0.74	0.51	0.61	0.69	0.69
Calcium, %	0.66	0.66	0.66	1.05	0.66	0.67	0.67
Phosphorus, %	0.56	0.56	0.56	0.73	0.55	0.56	0.56
Dig P., %	0.43	0.34	0.36	0.56	0.36	0.41	0.41
Avail. P	0.55	0.39	0.44	0.69	0.34	0.51	0.51

[1] POET, LLC, 4506 N Lewis Ave, Sioux Falls, SD 57104 – USA.

[2]ESBM, HAMLET PROTEIN Inc., 5289 Hamlet Drive, Findlay, OH 45840 - USA.

[3]J & R Distributing Inc. 518 Main Ave, Lake Norden, SD 57248 - USA. Minimum provided per kg of diet: Calcium 55 mg, Vitamin A 11,000 IU, Vitamin D3 1,650 IU, Vitamin E 55 IU; Vitamin B12 0.044 mg, Menadione 4.4 mg, Biotin 0.165 mg, Folic Acid 1.1 mg, Niacin 55 mg, d-Pantothenic Acid 60.5 mg, Vitamin B16 3.3 mg, Riboflavin mg, 9.9 Thiamine 3.3 mg.

[4]J & R Distributing Inc. 518 Main Ave, Lake Norden, SD 57248 - USA. Minimum provided per kg of diet: Copper 16.5 ppm, Manganese 44.1 ppm, Selenium 0.03 ppm, Zinc 165 ppm.

Table 2.2. Formulation and calculated composition of experimental diets to evaluate ME in yeast-based high protein products fed to growing pigs in comparison to commonly used protein feedstuffs in swine diets – Exp 2.

Ingredient	Corn	SBM	ESBM	Fishmeal	DDGS	Spray GDDY	Ring GDDY
Corn, NRC 2012	96.99	70.67	69.62	74.77	42.98	63.16	63.16
SBM	0.00	27.00	0.00	0.00	0.00	0.00	0.00
ESBM[2]	0.00	0.00	28.00	0.00	0.00	0.00	0.00
Fishmeal	0.00	0.00	0.00	25.00	0.00	0.00	0.00
Dakota Gold, DDGS[1]	0.00	0.00	0.00	0.00	55.00	0.00	0.00
Spray GDDY[1]	0.00	0.00	0.00	0.00	0.00	34.00	0.00
Ring GDDY[1]	0.00	0.00	0.00	0.00	0.00	0.00	34.00
Limestone	1.05	1.08	1.18	0.03	1.82	1.13	1.13
Salt	0.21	0.20	0.20	0.00	0.00	0.15	0.15
Monocalcium phosphate	1.55	0.85	0.8	0.00	0.00	1.36	1.36
Grower Vitamin premix[3]	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Mineral pmx[4]	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Nutrient in mixed feed							
GE, Kcal/kg[5]	3652	3836	3946	3922	4149	4186	4087
DE, Kcal/kg	3346	3511	3520	3568	3451	3551	3551
ME, Kcal/kg	3288	3362	3401	3484	3324	3410	3410
Crude Protein, %	7.99	18.49	21.62	22.21	20.27	23.91	22.21
total Lysine, %	0.24	0.99	1.08	1.33	0.57	1.03	0.91
SID Lysine, %	0.18	0.85	0.96	1.12	0.36	0.72	0.64
SID Met, %	0.14	0.26	0.30	0.46	0.31	0.46	0.42
SID, Met + Cys, %	0.29	0.53	0.60	0.65	0.62	0.83	0.76
SID THR, %	0.21	0.57	0.70	0.64	0.50	0.70	0.66
SID TRP, %	0.05	0.20	0.22	0.16	0.11	0.19	0.18
SID ILEU, %	0.22	0.72	0.82	0.69	0.57	0.85	0.78
SID LEU, %	0.81	1.47	1.65	1.49	1.81	2.27	2.06
SID VAL, %	0.30	0.79	0.93	0.83	0.71	1.07	0.99
SID HIS, %	0.19	0.45	0.51	0.47	0.42	0.54	0.51
SID PHE, %	0.32	0.82	0.98	0.74	0.74	1.06	0.99
Calcium, %	0.66	0.66	0.66	1.08	0.75	0.66	0.66
Phosphorus, %	0.56	0.56	0.56	0.91	0.64	0.56	0.56
Dig P., %	0.35	0.29	0.32	0.63	0.38	0.38	0.38
Avail. P	0.39	0.27	0.32	0.74	0.35	0.40	0.40

[1] POET, LLC, 4506 N Lewis Ave, Sioux Falls, SD 57104 – USA.

[2]ESBM, HAMLET PROTEIN Inc., 5289 Hamlet Drive, Findlay, OH 45840 - USA.

[3]J & R Distributing Inc. 518 Main Ave, Lake Norden, SD 57248 - USA. Minimum provided per kg of diet: Calcium 55 mg, Vitamin A 11,000 IU, Vitamin D3 1,650 IU, Vitamin E 55 IU; Vitamin B12 0.044 mg, Menadione 4.4 mg, Biotin 0.165 mg, Folic Acid 1.1 mg, Niacin 55 mg, d-Pantothenic Acid 60.5 mg, Vitamin B16 3.3 mg, Riboflavin mg, 9.9 Thiamine 3.3 mg.

[4]J & R Distributing Inc. 518 Main Ave, Lake Norden, SD 57248 - USA. Minimum provided per kg of diet: Copper 16.5 ppm, Manganese 44.1 ppm, Selenium 0.03 ppm, Zinc 165 ppm.

[5] Analyzed value.

Table 2.3. Analyzed chemical composition (as-fed basis) of protein feedstuffs

Item	SBM	ESBM	Fishmeal	DDGS	Spray GDDY	Ring GDDY
Crude Protein, %	46.92	56.71	64.20	30.42	55.02	50.01
Moisture, %	10.98	4.79	6.84	10.32	4.74	9.53
Crude Fat, %	0.00	0.00	7.88	3.97	3.48	3.66
Ash, %	6.03	6.87	20.62	5.41	5.47	5.52
Crude Fiber, %	3.095	5.77	0.4	9.07	6.08	4.055
NDF, %	-	-	-	29.73	4.70	4.41
Starch, %	-	-	-	4.34	3.90	5.19
Fructose, %	-	-	-	0.03	0.02	0.03
Glucose, %	-	-	-	0.47	0.35	0.34
Sucrose, %	-	-	-	0.00	0.00	0.00
Lactose, %	-	-	-	0.00	0.00	0.00
Maltose, %	-	-	-	0.03	0.02	0.02
AA Composition						
Indispensable AA						
Arginine	3.34	3.90	3.66	1.15	2.93	2.80
Histidine	1.24	1.47	1.53	0.78	1.55	1.42
Isoleucine	2.27	2.73	2.52	1.12	2.59	2.32
Leucine	3.57	4.27	4.15	3.13	5.97	5.24
Lysine	3.01	3.25	4.57	0.85	2.57	2.22
Methionine	0.64	0.76	1.60	0.55	1.24	1.10
Phenylalanine	2.40	2.91	2.41	1.35	2.97	2.72
Threonine	1.75	2.12	2.35	1.05	2.21	2.04
Tryptophan	0.68	0.76	0.64	0.22	0.58	0.54
Valine	2.33	2.81	2.88	1.39	3.28	2.99
Dispensable AA						
Alanine	1.99	2.36	3.84	1.84	3.73	3.32
Aspartic Acid	5.15	6.05	5.24	1.70	4.02	3.70
Cysteine	0.69	0.80	0.51	0.60	1.03	0.94
Glutamic Acid	8.39	9.73	7.79	3.86	7.75	6.98
Glycine	1.93	2.32	4.63	1.04	2.32	2.17
Proline	2.22	2.72	2.82	2.23	3.70	3.29
Serine	1.87	2.27	1.92	1.13	2.25	2.08
Tyrosine	1.72	2.06	1.87	1.06	2.49	2.22
Total AA	45.41	53.54	56.87	25.26	53.38	48.28

Table 2.4. Analyzed chemical composition (as-fed basis) of experimental diets to valuate SID of AA in yeast-based high protein products fed to growing pigs in comparison to commonly used protein feedstuffs in swine diets – Exp 1

Item	N-FREE	SBM	ESBM	Fishmeal	DDGS	Spray GDDY	Ring GDDY
ME, Kcal/kg	3771	3752	3790	3905	3714	3759	3759
Crude Protein	0.52	11.46	16.32	16.17	15.62	19.56	18.64
Moisture	4.29	6.14	4.84	5.69	7.08	4.73	6.46
Crude fat	2.86	0.00	0.60	2.50	6.23	1.76	1.55
Crude fiber	3.59	1.00	1.43	0.20	4.91	1.23	1.25
Ash	3.04	3.65	4.25	6.02	4.94	4.71	4.59
Indispensable AA							
Arginine	-	0.67	1.05	0.84	0.62	0.95	0.87
Histidine	-	0.26	0.40	0.33	0.43	0.51	0.45
Isoleucine	-	0.48	0.76	0.59	0.61	0.84	0.73
Leucine	-	0.76	1.20	0.98	1.77	2.01	1.71
Lysine	-	0.64	0.90	1.08	0.47	0.86	0.72
Methionine	-	0.13	0.21	0.38	0.31	0.42	0.36
Phenylalanine	-	0.50	0.80	0.56	0.76	0.99	0.85
Threonine	-	0.37	0.59	0.56	0.58	0.76	0.67
Tryptophan	-	0.15	0.23	0.15	0.12	0.21	0.18
Valine	-	0.48	0.77	0.67	0.77	1.07	0.94
Dispensable AA							
Alanine	-	0.42	0.66	0.95	1.05	1.29	1.11
Aspartic Acid	-	1.10	1.70	1.28	0.99	1.43	1.24
Cysteine	-	0.15	0.22	0.12	0.33	0.35	0.29
Glutamic Acid	-	1.81	2.86	1.92	2.41	2.84	2.48
Glycine	-	0.41	0.65	1.17	0.58	0.80	0.72
Proline	-	0.47	0.74	0.69	1.24	1.24	1.06
Serine	-	0.44	0.67	0.45	0.63	0.77	0.68
Tyrosine	-	0.32	0.50	0.37	0.56	0.72	0.61

Table 2.5. Apparent ileal digestibility (%) of AA in yeast-based high protein products fed to growing pigs in comparison to commonly used protein feedstuffs in swine diets

Item	SBM	ESBM	Fishmeal	DDGS	Spray GDDY	Ring GDDY	SEM	*P*-value
Indispensable AA								
Arg	84.42^{b}	92.49^{a}	81.14^{b}	72.13^{c}	84.13^{b}	85.07^{b}	2.228	<.0001
His	84.97^{b}	91.85^{a}	80.03^{c}	78.18^{c}	82.49^{bc}	81.03^{bc}	1.048	<.0001
Ile	84.33^{b}	92.01^{a}	80.64^{c}	80.10^{c}	84.72^{b}	82.92^{bc}	0.761	<.0001
Leu	83.04^{c}	91.70^{a}	81.39^{c}	87.48^{b}	87.11^{b}	86.71^{b}	0.655	<.0001
Lys	85.15^{ab}	87.25^{a}	81.91^{b}	65.00^{d}	81.56^{b}	75.42^{c}	1.191	<.0001
Met	86.22^{bc}	93.02^{a}	84.09^{c}	86.49^{bc}	88.76^{b}	87.80^{b}	0.578	<.0001
Met + Cys	79.97^{b}	86.30^{a}	72.79^{c}	78.25^{b}	79.26^{b}	76.68^{bc}	0.918	<.0001
Phe	83.87^{b}	92.34^{a}	79.85^{c}	84.65^{b}	86.87^{b}	85.66^{b}	0.710	<.0001
Thr	74.50^{b}	85.64^{a}	76.95^{b}	69.54^{c}	76.70^{b}	72.41^{bc}	1.080	<.0001
Trp	84.60^{b}	90.68^{a}	83.15^{b}	71.59^{c}	84.37^{b}	81.04^{b}	1.188	<.0001
Val	78.39^{bc}	88.82^{a}	77.78^{c}	76.55^{c}	81.88^{b}	79.90^{bc}	0.910	<.0001
Mean	81.68^{bc}	90.15^{a}	78.81^{c}	78.36^{c}	82.90^{b}	81.30^{bc}	0.938	<.0001
Dispensable AA								
Ala	74.98^{c}	85.93^{a}	79.90^{bc}	78.44^{bc}	81.83^{ab}	81.70^{ab}	1.654	0.0002
Asp	81.17^{b}	88.59^{a}	72.72^{cd}	70.13^{d}	75.07^{c}	72.26^{cd}	1.006	<.0001
Cys	73.71^{b}	79.58^{a}	61.49^{d}	70.02^{bc}	69.76^{bc}	65.56^{cd}	1.309	<.0001
Glu	85.59^{b}	91.39^{a}	81.50^{c}	83.39^{bc}	82.90^{bc}	84.84^{bc}	0.960	<.0001
Gly	51.44^{bc}	71.47^{a}	72.01^{a}	40.63^{c}	56.58^{b}	56.88^{b}	4.094	<.0001
Pro	10.77	39.49	39.28	37.24	39.37	30.47	7.243	0.1466
Ser	80.75^{b}	89.57^{a}	75.16^{d}	75.85^{cd}	79.54^{bc}	76.28^{cd}	0.972	<.0001
Tyr	81.41^{c}	89.94^{a}	77.30^{d}	83.12^{bc}	85.30^{b}	84.01^{bc}	0.862	<.0001

Mean	69.94[b]	80.23[a]	71.66[b]	70.13[b]	72.59[b]	69.16[b]	1.349	0.0001
Mean Ind/Dis AA	77.41[bc]	86.02[a]	76.54[bc]	74.33[c]	78.93[b]	76.20[bc]	0.922	<.0001
Total AA*	76.76[bc]	86.39[a]	75.15[bc]	71.29[c]	77.91[b]	76.64[bc]	1.782	<.0001

*Including taurine, hydroxyproline, hydroxylysine, and ornithine.

Table 2.6. Standardized ileal digestibility (%) of AA in yeast-based high protein products fed to growing pigs in comparison to commonly used protein feedstuffs in swine diets.

Item	SBM	ESBM	Fishmeal	DDGS	Spray GDDY	Ring GDDY	SEM	*P*-value
Indispensable AA								
Arg	90.78^{b}	99.62^{a}	84.42^{bc}	80.46^{c}	89.05^{b}	91.16^{b}	2.770	<.0001
His	88.57^{b}	95.54^{a}	81.66^{c}	81.30^{c}	84.25^{bc}	83.04^{c}	1.122	<.0001
Ile	87.21^{b}	95.13^{a}	82.11^{c}	83.86^{bc}	86.63^{b}	85.06^{bc}	0.804	<.0001
Leu	85.89^{c}	94.93^{a}	82.94^{d}	90.99^{b}	88.71^{bc}	88.71^{bc}	0.700	<.0001
Lys	88.07^{ab}	89.35^{a}	83.00^{b}	68.53^{d}	83.56^{b}	77.33^{c}	1.253	<.0001
Met	88.93^{b}	95.91^{a}	84.72^{c}	88.92^{b}	89.91^{b}	89.17^{b}	0.622	<.0001
Met + Cys	82.94^{b}	88.99^{a}	74.16^{d}	80.81^{bc}	80.51^{bc}	78.16^{cd}	0.956	<.0001
Phe	86.42^{b}	95.28^{a}	81.25^{c}	88.36^{b}	88.65^{b}	87.74^{b}	0.753	<.0001
Thr	78.53^{bc}	89.53^{a}	79.21^{b}	74.06^{c}	79.12^{b}	74.95^{bc}	1.118	<.0001
Trp	87.92^{b}	93.75^{a}	85.48^{b}	76.25^{c}	87.03^{b}	83.78^{b}	1.294	<.0001
Val	82.02^{bc}	92.62^{a}	79.73^{c}	80.95^{bc}	84.07^{b}	82.36^{bc}	0.960	<.0001
Mean	85.94^{b}	93.69^{a}	81.31^{d}	81.82^{cd}	85.45^{bc}	83.78^{bcd}	0.990	<.0001
Dispensable AA								
Ala	79.83^{b}	90.64^{a}	81.94^{b}	83.28^{b}	84.24^{b}	84.74^{ab}	1.874	0.0007
Asp	83.88^{b}	91.07^{a}	73.95^{c}	74.10^{c}	76.82^{c}	74.26^{c}	1.034	<.0001
Cys	76.95^{ab}	82.06^{a}	63.60^{d}	72.69^{bc}	71.11^{c}	67.14^{cd}	1.350	<.0001
Glu	88.10^{b}	93.71^{a}	82.88^{c}	86.80^{bc}	84.41^{bc}	86.97^{bc}	1.043	<.0001
Gly	58.62^{c}	77.95^{a}	75.33^{ab}	49.06^{c}	61.10^{bc}	62.31^{bc}	4.525	<.0001
Pro	26.38	53.05	50.36	53.48	49.04	41.03	8.668	0.3262
Ser	85.05^{b}	94.05^{a}	77.67^{d}	80.84^{bcd}	82.14^{bc}	79.05^{cd}	1.025	<.0001
Tyr	84.19^{b}	92.82^{a}	78.81^{c}	86.81^{b}	87.08^{b}	86.11^{b}	0.913	<.0001

Mean	74.89[b]	85.05[a]	74.46[b]	75.59[b]	75.79[b]	72.90[b]	1.532	0.0002
Mean, total	81.49[b]	90.09[a]	78.71[b]	78.96[b]	81.51[b]	79.20[b]	1.062	<.0001
Total AA*	82.52[b]	91.79[a]	78.10[b]	77.97[b]	81.50[b]	80.91[b]	2.038	<.0001

*Including taurine, hydroxyproline, hydroxylysine, and ornithin

Table 2.7. Digestible energy (DE) and metabolizable energy (ME) in experimental diets fed to growing pigs – Exp. 2.

Item	Corn	SBM	ESBM	Fishmeal	DDGS	Spray GDDY	Ring GDDY	SEM	P-value
Feed intake, g/d	1.1	1.1	1.2	1.2	1.1	1.1	1.09	0.051	0.7050
GE intake, Kcal/d	4053[b]	4295[ab]	4547[ab]	4691[ab]	4953[a]	4833[ab]	4471[ab]	197.65	0.0349
Feces output, g/d	0.1[b]	0.1[b]	0.1[b]	0.1[b]	0.3[a]	0.1[b]	0.13[b]	0.018	<.0001
GE in feces, kcal, kg	4457[ab]	4363[bc]	4385[b]	4204[c]	4487[ab]	4549[a]	4482[ab]	37.23	<.0001
Digestibility of GE, %	88.53[a]	88.83[a]	86.20[a]	87.44[a]	69.55[b]	87.40[a]	85.11[a]	1.28	<.0001
DE in diet, kcal/kg DM	3232[c]	3409[bc]	3402[bc]	3429[bc]	2885[d]	3659[a]	3478[ab]	51.44	<.0001
Urine output, g/d	2.5	4.0	3.7	4.1	3.3	3.8	3.279	0.531	0.4059
GE in urine, kcal/kg	31.37	36.99	27.46	33.20	49.90	42.03	49.99	6.518	0.1191
Metabolizable of GE, %	87.03[a]	85.54[a]	83.49[a]	84.76[a]	66.32[b]	84.21[a]	81.70[a]	1.399	<.0001
ME in diet, kcal/kg DM	3178[b]	3283[ab]	3295[ab]	3324[ab]	2751[c]	3225[a]	3339[ab]	56.26	<.0001

Table 2.8. Digestible energy (DE) and metabolizable energy (ME) in feedstuff.

Item	Corn	SBM	ESBM	Fishmeal	DDGS	Spray GDDY	Ring GDDY	SEM	P-value
DE, Kcal/kg	3241^{c}	4137^{ab}	4121^{ab}	4064^{b}	2744^{c}	4762^{a}	4228^{ab}	155.28	<.0001
ME, Kcal/kg	3200^{bc}	3824^{b}	3883^{ab}	3822^{ab}	2554^{c}	4480^{a}	3926^{ab}	169.88	<.0001

3. HIGH-PROTEIN YEAST-BASED DDGS (HP-GDDY) CAN BE INCLUDED IN EARLY NURSEY DIETS WITHOUT COMPROMISING PIG GROWTH PERFORMANCE AND HEALTH STATUS

ABSTRACT

Data from our previous study suggest that high protein yeast-based DDGS (HP-GDDY) products have similar amino acid and energy digestibility compared to common ingredients used in commercial pig diets and could potentially be included as an alternative protein source in nursery diets. This study was conducted to evaluate the effects of including HP-GDDY as an alternative protein resource in nursery diets on growth performance and intestinal health of weaned pigs. A total of 594 weaned pigs were allotted to 36 pens in a randomized complete block design at the SDSU Swine Research and Education Facility. Pens were assigned to one of four dietary treatments: Diet 1 (CON): a corn-SBM basal diet; Diet 2 (SBM75): Diet 1 replacing 75% of SBM with HP-GDDY; Diet 3 (FM/ESBM): Diet 1 without FM and ESBM + HP-GDDY inclusion; Diet 4 (HP-GDDY50): Diet 1 replacing 50% of SBM, FM, and ESBM with HP-GDDY. Experimental diets were formulated to meet nutrient requirements of weaned pigs and provided in meal form through 4 phases during the nursery period. Body weight (BW), average daily gain (ADG), average daily feed intake (ADFI), and gain:feed ratio (G: F) were calculated on days 7, 14, 21, 28, 42, and 53. Pen fecal score was assessed daily from d0 to d14, and three times per week from d15 to d35. Intestinal health was assessed based on plasma immunoglobulin A (IgA) concentration and the differential sugar absorption test (DSAT). The digestibility of dry matter (DM), crude protein (CP), gross energy (GE), and phosphorus was also evaluated. During first 14 days, dietary treatment had no effect (*P*<0.05) on BW, ADG, and ADFI. For the rest of the experimental period (d14 to d53), ADG and ADFI was greater (*P*>0.05) in pigs provided CON in comparison with those provided SBM75 and HP-GDDY50 but similar to pigs provided FM/ESBM. Pigs fed SBM75 and HP-GDDY50 had lesser prevalence of loose watery feces (5%) compared to pigs on CON and FM/ESBM. Pigs feeding with dietary treatments tended (*P*=0.082) to have greater serum IgA concentration on d20 in pigs from HP-GDDY50 when compared to SBM75 and FM/ESBM. There were no differences among pigs feeding with dietary treatments for DM, CP, and GE digestibility. There were no differences in phosphorus digestibility between the CON and FM/ESBM. However, P digestibility was higher in FM/ESBM (P>0.05) compared to SBM75 and HP-GDDY50 diets. These results support the idea that HP-DDGY can be incorporated in nursery diets during the first weeks after weaning without affecting growth performance. However, it is necessary to adjust inclusion levels for the late nursery period, where performance can be compromised when inclusion levels exceed 14%. Overall, HP-GDDY is a valuable feedstuff for nursery pig diets.

Keywords: feeding program, growth performance, inclusion level, postweaning period, protein source.

3.1 INTRODUCTION

Weaning phase is one of the most critical periods during pig production due to the combined stressors faced by piglets such as new environment, social interactions, and change of diet (Zheng, et al., 2021). All these stressors lead to increased susceptibility to gut disorders, which, in concert with immature digestive and immune systems, results in increased incidences of diarrhea, poor growth rate, increased mortality and (or) morbidity, and subsequent economic losses for swine producers (Lallés et al., 2004; Smith et al., 2010; Pohl et al., 2017). Therefore, it is important to implement different management strategies to reduce the stress associated with weaning and promote the physiological development of pigs. One of the commonly used strategies during this period is related with diet formulation, especially the use of ingredients with high protein content and digestibility.

In pork production, the formulation of diets to reduce the negative effects during the weaning period include ingredients with high digestibility and palatability, which increase diets cost. Fishmeal (FM), spray-dried plasma, enzymatic treated soybean meal (ESBM), products derived from blood, and even cereals processed with thermal methods are included as high-quality protein sources in diets for nursery pigs. Those diets are generally referred to as “complex” diets (Collins, et al., 2017). Although feeding pigs with diets rich in high quality protein during the weaning phase are considered essential to guarantee optimal growth in the transition and later phases, the high price and low availability of these ingredients increase the overall cost of pork production (Totafurno, et al., 2019). On the other hand, soybean meal (SBM) is the most common protein source used in swine diets because of its high protein and amino acid (AA) content and consistent quality and availability in the market (Chiba, 2013). However, its inclusion as a feed ingredient is limited, particularly in young animal diets, due to relatively high level of anti-nutritional factors (ANFs) and non-starch polysaccharides (NSP). The presence of ANFs such as trypsin inhibitors, protease inhibitors, lectins, phytoestrogens, oligosaccharides, and phytate interfere with digestion, absorption and nutrient utilization, which can lower overall nutritional value of SBM and negatively affect pig growth performance (Liener, 1994; Pettersson and Pontoppidan, 2013, Jeong, et al., 2016). In addition, two major proteins in SBM (glycinin and β-conglycinin) induce allergic reaction and have been associated with inflammatory response in young pigs (Fu et al., 2007). It has been reported that ANFs in SBM can impair growth performance and compromise intestinal health of pigs, especially when included in young pig diets (Song et al., 2010). Thus, the inclusion of SBM in nursery diets is typically restricted (< 20%) and gradually introduced through their subsequent growth periods (Stein et al., 2013). In this context, it is important to find alternative protein sources that are cost-effective in nursery pig diets without compromising pig performance and health.

Yeast-based high protein DDGS products have been developed through separation of the protein fraction from DDGS and are expected to contain greater concentrations of yeast and thus a better AA profile. In our previous study, a novel yeast-based high protein product (HP-GDDY) had similar standardized ileal digestibility of AA when compared to SBM and FM (Garavito et al., 2022). In addition, the metabolizable energy content was comparable to corn, one of the main energy sources used in commercial pig diets in the United States. Therefore, the objective of this study was to evaluate the dietary inclusion of a HP-GDDY as alternative protein resource for nursery pigs and its effects on pig intestinal health and apparent total tract digestibility of dietary nutrients.

3.2 MATERIALS AND METHODS

This study was approved by the Institutional Animal Care and Use Committee at the South Dakota State University and Use Committee (IACUC # 2105-023A).

3.2.1 ANIMALS AND EXPERIMENTAL DESIGN

A total of 594 barrows and gilts were used in a randomized complete block design at the SDSU Swine Research and Education Center. Pigs were weaned into 36 pens (15-18 pigs per pen and sex ratios were maintained within blocks as was body weight distribution) distributed in 2 rooms. All pens contained one dry self-feeder I and 2 cup waterers to allow for ad libitum access to feed and water. Feeder type was balanced within dietary treatments (4 wet and 5 dry feeders for each treatment). The facility operated with mechanical ventilation, with room temperature set at 30, 29, 28, 26.5, 25, 24 ºC during weeks 1-6 of the nursery period.

The experimental diets (Table 3.1 and 3.2) were formulated to meet or exceed nutrient requirements for weaned pigs (NRC, 2012) and provided in meal form through 4 phases during the nursery period (42 days): Phase 1 (d0-d7), Phase 2 (d8-d21), Phase 3 (d22 - d28) and Phase 4 (d29-d53). Pens were assigned to one of 4 dietary treatments resulting in 9 pens per treatment. The dietary treatments were Diet 1 (CON): a corn-SBM basal diet; Diet 2 (SBM75): Diet 1 replacing 75% of SBM by HP-GDDY; Diet 3 (FM/ESBM): Diet 1 without FM and ESBM + HP-GDDY inclusion; Diet 4 (HP-GDDY50): Diet 1 replacing 50% of SBM, FM, and ESBM by HP-GDDY.

3.2.2 EXPERIMENTAL PROCEDURES

Daily animal care observations included pig behavior, daily room temperature recording, check waterers and feeders, and treatment of pigs when necessary. Pigs were treated when they showed clinical signs of disease. Treatment dose, product used, date of administration, identification of pig and pen, and reason for treatment were recorded throughout the experimental period.

3.2.3 GROWTH PERFORMANCE

Pigs were weighed on d0, 7, 14, 21, 28, 42, and 53. Feed disappearance was measured simultaneously with body weight (BW) and average daily gain (ADG), average daily feed intake (ADFI), and gain:feed (G:F) was determined.

3.2.4 FECAL SCORE AND INTESTINAL HEALTH

Daily from d0 to d14 and 3 times a week from d15 to d35, pen fecal score was visually assessed using a 4-category fecal consistency scale (Pedersen and Toft, 2011). The 4 categories were score 1 = firm and shaped, score 2 = soft and shaped, score 3 = loose, and score 4 = watery, where scores of 1 and 2 represented normal stools and scores of 3 and 4 represented diarrhea. For each pen, a single observer assigned the relative proportion of visible feces that fell within each category, as well as an overall pen score. On days 10 and 20, a blood sample was taken from three pigs that were within the average weight of pen for serum IgA analysis (n = 27 / dietary treatment). Plasma was collected by centrifugation (2000 × g, 15 min, 4 ° C), placed in 1.5 ml microcentrifuge tubes and stored at -20 ° C until analysis (CR412, Jouan Inc. 170 Marcel Drive Winchester, VA 22602 - USA). Plasma IgA was analyzed according to the method described by Chaytor et al. (2011), using commercially available kit (Pig IgA ELISA Kit, Bethyl Laboratories®).

The differential sugar absorption test (DSAT) was completed for 3d to coincide with blood collection (d 9-11 and d 19-21) using 1 barrow per pen. The relevant pig was selected from the pigs used for blood sampling. On each day of the DSAT test, the pigs were randomly transferred to one of 9 individual cages (0.56 × 0.64 × 0.89 m^2) with access to feed and water. Pigs were orally administered a bolus containing 5% lactulose (L) and mannitol (M) at 15 ml / kg (Nguyen, et al., 2014) using a syringe plus a liquid feeding tube followed by total urine collection for 6 hours (Perez-Palencia et al., 2021). Thereafter, the pigs were transferred back to their original pen. A subsample of urine was collected after homogenization and stored at -80 ° C for subsequent determination of lactulose: mannitol (L: M) ratio using commercially available kit (EnzyChrom™ Intestinal Permeability Assay Kit Catalog No: EIPM-100) as a marker of intestinal permeability (Hong, et al. 2020).

3.2.5 PHOSPHORUS RETENTION

Apparent total tract digestibility (ATTD) of dry matter (DM), crude protein (CP), gross energy (GE), and phosphorus of experimental diets were calculated according to the indirect evaluation method during Phase IV (d25 – d35) using celite as the indigestible marker (Kiarie, et al., 2016). On d32 to d35, fresh fecal samples were collected once a day from each experimental pen. The DM content in the diets was determined by drying samples at 102℃ for 24 hours using a drying oven and ground to pass through a 0.5 mm screen using a mill grinder (Retsch zm 200, ring sieve size: 0.75 mm). The GE content in diets and fecal samples were analyzed by bomb calorimetry (Parr 6300 calorimeter, Parr Instruments Co., Moline, IL). The CP (method 990.03), crude fiber (CF, AOAC Official Method 978.10, 2006), neutral detergent fiber (NDF, JAOAC 56, 1352-1356, 1973), acid detergent fiber (ADF, AOAC Official Method 973.18 (A-D), 2006) and phosphorus (P, method AOAC Official Method 966.01) were determined at a commercial laboratory (University of Missouri, Columbia MO). To determine ATTD of nutrients and phosphorus, the following equation described by Adeola (2001) was used:

$$\text{Digestibility (\%)} = 100 - 100 \times Mfeed \times CfecesMfeces \times Cfeed$$

Where M_{feed} and M_{feces} represent concentrations of marker compound in feed and feces, respectively; C_{feed} and C_{feces} represent concentrations of marker in feed and feces, respectively.

3.2.6 STATISTICAL ANALYSIS

The SAS UNIVARIATE procedure was used to confirm the homogeneity of the variance and test for outliers. Data were analyzed as a randomized incomplete block design using the PROC MIXED procedure in SAS. In the model, dietary treatment was considered as the main effect and body weight category as the blocking factor with the pen as the experimental unit. Tukey's adjusted mean test was used to detect differences between treatment groups where $P \leq 0.05$ was considered significant. For analysis of fecal scores, data were analyzed using the PROC FREQ procedure in SAS.

3.3 RESULTS

3.3.1 DIETS COMPOSITION

The analyzed chemical composition of experimental diets is presented in Table 3.3 and Table 3.6 for phase 1 and phase 2, and Table 3.4 and Table 3.6 for phase 3 and phase 4. The CP content for the experimental diets was in average 20.5% for phase 1 and phase 3, for phase 2 the CP content for was in average 19.8% for the experimental diets, and 18.5% for phase 4. The GE content was similar across dietary treatments and experimental groups was in average 3915 Kcal/Kg for the phase 1, 3870 Kcal/Kg for phase 2, 3882 Kcal/Kg for phase 3 and 3878 Kcal/Kg for phase 4. The crude fiber (CF) content for the experimental diets was in average 1.93% for phase 1, 1.97% to for phase 2, 2.13% for phase 3 and 2.33%, where CON diet had the lowest values in comparison with diets containing GDDY product. The highest value of CF was for SBM75 and FM/ESBM diet during phases 1 and 2, but SBM75 had the highest values during phases

3 and 4 following GDDY inclusion. The phosphorus content was similar between all diets for each phase, with differences less than 5%.

Regarding one of the most limiting AA, the variation in total Lys content within each diet phase was on average 6%. For the second limiting AA, the variation in Thr content was on average 7% between diet phases, and the variation in Met content was on average 9% between diet phases. Within the analyzed AA values, in the branched chain amino acids (BCAA), the variation in leucine (Leu) content within each diet phase was on average 13%. While the variation isoleucine (Ile) and valine (Val) content was 7% throughout all phases.

3.3.2 GROWTH PERFORMANCE

During the first 14 days postweaning, dietary treatments had no effect on BW, ADG, and ADFI (Table 3.7). From d14 to 21, BW was greater (P<0.05) in pigs provided CON in comparison with those from HP-GDDY50, but it was similar to pigs fed SBM75 and FM/ESBM diets. The ADG was greater (P<0.05) in pigs from CON and FM/ESBM in comparison with the pigs in SBM75 and FM/ESBM, the ADG from pigs in SBM75 and HP-GDDY50 were similar. There were not significant effects (P>0.05) for ADFI for these periods. From d21 to d28, dietary treatments had no significant effects (P>0.05) on BW, ADG and ADFI. Although CON pigs tended (P=0.092) to have a greater BW in comparison with pig fed HP-GDDY50 diets (9.97 kg vs. 9.36 kg). From d28 to d42, BW was greater (P<0.05) in pigs from CON in comparison with pigs from SBM75 and HP-HDDY50, but similar than pigs in FM/ESBM. The ADG was greater (P<0.05) in pigs from CON diet in comparison with pigs from SBM75, FM/ESBM and HP-GDDY, while pigs from SBM75 and FM/ESBM had superior ADG than pigs from HP-GDDY50. The ADFI was greater (P<0.05) in pigs from CON in comparison with pigs from SBM75 and HP-GDDY50, but it was similar than pigs from FM/ESBM. During the last days of the experiment (d42 to d53), pigs from CON and FM/ESBM had a greater performance (BW, ADG, ADFI) in comparison with pigs from SBM75 and HP-GDDY50. Dietary treatments have not significant effects (*P*>0.05) on G:F ratio during the first 14 days, from d21 to 28, and at the end of the nursery period (d42 to 53). However, from d14 to 21, CON had a greater G:F ratio than SBM75 diet, and similar than FM/ESBM and HP-GDDY50. From d28 to 42, G:F ratio was greater in CON, FM/ESBM, and HP-GDDY50 in comparison with SBM75.

Considering the overall period (d0 to 53), ADG and ADFI was greater (*P*<0.05) in pigs from CON diet in comparison with the pigs in SBM and HP-GDDY50, while G:F ratio was greater in CON diet and FM/ESBM when compared to SBM75 diet.

3.3.3 FECAL SCORE

Dietary treatments had no significant effect (X^2>0.05) on pen fecal scores (Fig. 3.1) during week 1; however, during weeks 2 – 5, pigs fed the diets with the greater inclusion of GDDY (SBM75 and HP-GDDY50) had around 5% less incidence of watery and soft feces in comparison with pigs from CON and FM/ESBM.

3.3.4 DSAT AND IGA

The DSAT and IgA data are presented in Table 3.8. On d10 and d20 postweaning, dietary treatments had no significant effects (*P*>0.05) on urinary lactulose and mannitol concentrations or L:M ratio. However, urinary L:M ratio on d20 tended to be greater (*P*<0.099) on SBM75 diet compared to CON and HP-GDDY50.

Dietary treatments had no significant effects (*P*>0.05) on serum IgA concentration on d10 postweaning. However, on d20 pigs from HP-GDDY50 tended to have greater (*P*<0.082) serum IgA concentration when compared to SBM75 and FM/ESBM.

3.3.5 APPARENT TOTAL TRACT DIGESTIBILITY

There were no differences (*P*>0.05) among dietary treatments for DM, CP, and GE digestibility (Table 3.9). The digestibility of crude fat was greater (*P*<0.05) in FM/ESBM in comparison with other dietary treatments, although the SBM75 had the lowest digestibility of crude fat. The digestibility of fiber components in the diets (CF, NDF, and ADF) was greater (*P*<0.05) in CON and FM/ESBM diets in comparison with SBM75 and HP-GDDY50. Phosphorus digestibility was similar in CON and FM/ESBM, while FM/ESBM had greater (*P*<0.05) digestibility values compared to SBM75 and HP-GDDY50.

3.4 DISCUSSION

Data from this study suggest that HP-GDDY can be fed up with 14% in phases 1 and 2 of nursery period without compromising growth performance. Although after phase 2, when the inclusion level exceeded 15% in the diets, performance was negatively affected. It can probably be affected by high levels of BCAAs (leucine, valine and isoleucine), which generate an imbalance in the relationship with other limiting amino acids (Cemin, et al., 2019).

The BCAA are considered essential AA because pigs cannot synthesize them and thus, they must be provided in the diet to avoid possible BCAA deficiency that can affect growth and health (Gloaguen, et al., 2011; Siebert, et al., 2021). In addition, BCAAs share the same enzyme complex (branched-chain amino acids aminotransferase and branched-chain amino acid a-ketoacid dehydrogenase complex) in their degradative pathway, therefore, high levels of one (e.g., Leu) can induce increased catabolism of all BCAA. Increased BCAA catabolism can have a negative impact on pig growth performance (Langer et al., 2000), and consequently, the animals require more Ile and

Val, especially when diets are high in Leu. (Wiltafsky, et al., 2010). The excess of Leu in diets can also affect the expression of the hormone insulin-like growth factor (GH-IGF-1) which negatively affects growth (Sanderson and Naik, 2000). Gloaguen, et al. (2013) determined that pigs can recognize BCAA imbalance in the diet and reported decrease in feed intake as a response to BCAA deficiency. Li and Patience (2017) also concluded that diets with inadequate levels of protein can inhibit feed intake due to AA imbalance, for example, excess BCAA compete with tryptophan (Trp) by crossing the blood-brain barrier and affecting Trp-related appetite-stimulating hormones.

Intestinal and immune development can be negatively affected by the components present in ingredients that make up the diet, as is the case with some components present in SBM (Kim, et al., 2015). According to what was observed in this study, there was an incidence of soft feces in the control diet compared to the diet where the substitution of SBM by HP-GDDY was performed, representing a reduced trend in the incidence of soft feces. Some ingredients such as SBM contain anti-nutritional factors, such as trypsin inhibitors, oligosaccharides and lectins, and corn by-products such as DDGS or HP-GDDY, have NSP which can increase digesta viscosity in the small intestine and thus reduce digestibility and nutrient absorption (Tiwari, et al., 2018). Antinutritional factors and NSPs present in the ingredients alter the microflora and modify the physiological function of the intestine, such as increasing digestion transit times, intestinal mass, and mucosal cell turnover rates (Kiarie, et al., 2013). Post-weaning diarrhea (PWD) can potentially increase due to the immaturity of the immune system and the digestive system to process diets during the initial phase of weaning (Lallès, et al., 2007).

According to Moeser, et al. (2007), the increase in intestinal permeability in weaned piglets begins around 24 h after weaning and the recovery of the increased permeability occurs gradually over time, generally after the first 2 weeks after weaning. In this study, the lactulose: mannitol (LM) test measures the ability of two unmetabolized sugar molecules, lactulose and mannitol, to permeate the intestinal mucosa and in this way evaluate intestinal permeability (Wijtten, et al., 2011). Due to its relatively larger size compared to other molecules such as mannitol, lactulose could enter the bloodstream through the paracellular route or by damage to the intestinal epithelium at the tight junctional barrier that allows greater penetration of large molecules (Vojdani, 2013). Therefore, an increase in the L:M ratio could indicate a decrease in intestinal barrier function. Comparing the L:M ratio for CON and HP-GDDY50 during d10 and d20 postweaning, there is a reduced value on d20, which These results suggest that the use of high proteins ingredients and the addition of HP-GDDY at levels above 15% cause damage to the intestinal permeability but reducing the high protein sources can reduce the impact on the intestinal permeability. There was a tendency, especially at d20 post-weaning, where the urinary L:M ratio in SBM75 tended to be higher, which may indicate that the SBM75 diet has a negative effect on the intestinal barrier. One of the responses of the pig's immune system to protect the intestinal epithelium from pathogens and toxins is intestinal production of IgA which acts as a first line of defense through receptor blockade (Mantis, et al., 2011). Therefore, in the present study, serum IgA was measured and use as a marker, an indirect method since plasma cells in the intestine produce IgA as the main effect of the intestinal mucosal response (Peng, et al., 2021). During the present study, blood IgA for d10 and d20 post-weaning did not present significant differences, although there was a trend on d20, where SBM75 AND FM/ESBM had a lower concentration of IgA in blood. Indicating that there was less challenge for the intestinal barrier reflected in the decrease in serum IgA. Gao, et al. (2013) suggest that increased IgA secretion may result in increased intestinal permeability, which leads to bacterial translocation and ultimately may trigger inflammation to try to limit the growth of intestinal microbes; on the other hand, the reduced IgA levels observed in animals fed probiotics could be attributed to an improved barrier function (Lessard, et al., 2009).

The intestinal barrier is made up of two parts: a structural fraction that includes the vascular endothelium, epithelial cell lining, and mucosal layer, and a functional immunological fraction that secretes digestive components, immune molecules, and cell products like cytokines, inflammatory mediators, and antimicrobial peptides (Bischoff, et al., 2014). The presence of ANFs, NSP and other components in diets can generate disorders such as altered intestinal architecture, intestinal villus atrophy, crypt hyperplasia and increased intestinal permeability (Spreeuwenberg, et al., 2001). In the case of impaired intestinal barrier or greater intestinal permeability, they can promote the translocation of bacteria and increase the entry of allergenic components from the intestine to the body, causing an increase in immune responses and greater vulnerability to infections (Wijtten, et al., 2011).

Despite the fact that there are no published findings of ATTD of GE and P of HP-GDDY to compare the results obtained in this study. The ATTD results might be interpreted by comparing them to values reported in other studies where ingredients from similar sources were analyzed. In comparison to data reported by Widmer et al. (2007) in growing pigs fed HP-DDG, the ATTD of GE in the current study was approximately 70% lower, and the ATTD of phosphorus was lower (56.6%). Furthermore, in two previous studies using Wheat DDGS and Corn DDGS (Avelar et al., 2010; Rho et al., 2018), the ATTD of CP and DM were higher (20% and 17%, respectively) in comparison to the current study. There were no significant differences between dietary treatments in ATTD of CP, which may suggest that there was no negative impact related to digesta transit time and time for proteolytic enzymatic digestion of CP. (Morel, et al., 2006). However, the ATTD of CP could be higher since there were losses caused by the fecal excretion of nitrogen which could increase mainly due to the presence of fibrous components in the diet (Zhang, et al., 2013). Furthermore, the ATTD of P was higher in the experimental diets in comparison with the ATTD of P in DDGS (60%) reported by NRC (2012) and also the values reported by Pederson et al. (2007) reported values of ATTD of P in DDGS between 50.1% and 68.3%, which are lower (15% approximately) than the values obtained in the presented study, an explanation of the results is because the fermentation process to obtain HP-DDGS some of the P in the phytate is hydrolyzed, therefore, more P is available for absorption in the small intestine of the pig (Widmer, et al. 2007). Consequently, the utilization of organic P is increased, and the need for supplemental inorganic P is reduced if ingredients with higher P digestibility are included in formulations at the expense of corn (Pederson et al. 2007). For ATTD of CF, NDF and ADF the values also decreased in SBM75 and HP-GDDY50 diets, which suggest the level of inclusion of HP-GDDY affect the digestibility because the level of fiber is higher in comparison with CON and FM/ESBM diets and confirm the results mentioned above in relation to the other nutrients, and a possible explanation may be the different NSP compositions, which affect the ATTD in others nutrients, especially β-glucans proportions among the ingredients.

Probably, the growth performance is affected because the increased endogenous secretions or decreased hydrolysis and absorption of nutrients in high fiber diets (Zhao, et al., 2018) which can also cause it to affect the substrate available for microbial growth, depending on the type of fiber, and can impact the decrease in diversity, causing a decrease in the resistance of pathogenic organisms or even facilitating the establishment of a dominant microorganism within the intestinal flora (McDonald, et al., 1999)

3.5 CONCLUSIONS

These data support the idea that HP- GDDY can be included up to 14% in phases 1 and 2 of nursey period without compromising growth performance. HP- GDDY can partially replace SBM and completely replace high quality proteins (FM and ESBM) in the first two weeks after weaning. The inclusion of HP-GDDY as a replacement for SBM potentially reduced the incidence of diarrhea which may contribute to reducing post-weaning stress.

It is important consider the level of inclusion of the HP-GDDY (no major than 14%) in order to maintain the balance in AA supply, especially for BCAA. In this study the increasing levels of HP-GDDY resulted in higher levels of Leu, which may have compromised pig growth performance during late nursery phase. Overall, HP-GDDY is a valuable feedstuff to be included in nursery pig diets, especially during the first couple weeks after weaning. Strategies to minimize the effect of high BCAA content in HP- GDDY are necessary to increase its inclusion in pig diets.

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Table 3.1. Experimental diets Formulation for Phase 1(d0-d7), and Phase 2 (d8-d21) to evaluate the impact of yeast-based high protein DDGS (HP-GDDY) inclusion on digestibility and gut health of weaned pigs.

	Phase 1				Phase 2			
Ingredient	**CON**	**SBM75**	**FM/ESBM**	**HP-GDDY50**	**CON**	**SBM75**	**FM/ESBM**	**HP-GDDY50**
Corn	38.31	38.10	33.08	36.99	43.59	42.87	39.64	42.49
Soybean meal, 46.5%	18.00	4.00	18.00	9.00	20.00	5.00	20.00	10.00
Dried Whey	30.00	30.00	30.00	30.00	25.00	25.00	25.00	25.00
Fish meal	5.00	5.00	0.00	2.50	4.00	4.00	0.00	2.00
HP 300[2]	5.00	5.00	0.00	2.50	4.00	4.00	0.00	2.00
Ring GDDY[3]	0.00	13.50	14.00	14.00	0.00	15.00	11.00	14.00
L-Lysine HCl	0.46	0.64	0.60	0.68	0.33	0.51	0.44	0.54
L- Threonine	0.17	0.16	0.16	0.18	0.11	0.09	0.10	0.11
DL-Methionine	0.12	0.07	0.09	0.09	0.09	0.03	0.07	0.05
L-Tryptophan	0.00	0.03	0.00	0.03	0.00	0.02	0.00	0.02
L-Valine	0.07	0.00	0.00	0.02	0.00	0.00	0.00	0.00
Soybean oil	1.00	1.11	1.15	1.29	0.80	0.85	0.88	0.98
Potassium chloride	0.15	0.00	0.42	0.17	0.14	0.00	0.36	0.10
Limestone	0.95	1.10	1.42	1.30	1.03	1.16	1.38	1.32
Potassium carbonate	0.00	0.52	0.24	0.45	0.00	0.56	0.18	0.46
Salt	0.15	0.15	0.22	0.18	0.16	0.16	0.20	0.18
Grower Vitamin premix[4]	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Mineral premix[5]	0.15	0.15	0.15	0.15Z	0.15	0.15	0.15	0.15
Zinc oxide	0.42	0.42	0.42	0.42	0.42	0.42	0.42	0.42
Swine Larvicide	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13

[1] Dietary treatments: CON: Standard nursery diet; SBM75: CON replacing 75% of SBM by GDDY; FM/ESBM: CON without Fishmeal and ESBM + GDDY inclusion; HP-GDDY50: CON replacing 50% of SBM, FM, and ESBM by GDDY.
[2] ESBM, HAMLET PROTEIN Inc., 5289 Hamlet Drive, Findlay, OH 45840 - USA.
[3] POET, LLC, 4506 N Lewis Ave, Sioux Falls, SD 57104 – USA.
[4] J & R Distributing Inc. 518 Main Ave, Lake Norden, SD 57248 - USA. Minimum provided per kg of diet: Calcium 55 mg, Vitamin A 11,000 IU, Vitamin D3 1,650 IU, Vitamin E 55 IU; Vitamin B12 0.044 mg, Menadione 4.4 mg, Biotin 0.165 mg, Folic Acid 1.1 mg, Niacin 55 mg, d-Pantothenic Acid 60.5 mg, Vitamin B16 3.3 mg, Riboflavin mg, 9.9 Thiamine 3.3 mg.
[5] J & R Distributing Inc. 518 Main Ave, Lake Norden, SD 57248 - USA. Minimum provided per kg of diet: Copper 16.5 ppm, Manganese 44.1 ppm, Selenium 0.03 ppm, Zinc 165 ppm.

Table 3.2. Formulation of experimental diets, Phase 3 (d22-d28), and Phase 4 (d29-d53) to evaluate the impact of yeast-based high protein [1] DDGS (HP-GDDY) inclusion on digestibility and gut health of weaned pigs.

	Phase 3				Phase 4			
Ingredient	**CON**	**SBM75**	**FM/ESBM**	**HP-GDDY50**	**CON**	**SBM75**	**FM/ESBM**	**HP-GDDY50**
Corn	53.08	52.32	50.53	52.11	65.80	64.90	65.80	65.18
Soybean meal, 46.5%	28.00	7.00	28.00	14.00	30.00	7.50	30.00	15.00
Dried Whey	12.00	12.00	12.00	12.00	0.00	0.00	0.00	0.00
Fish meal	3.00	3.00	0.00	1.50	0.00	0.00	0.00	0.00
HP 300[2]	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Ring GDDY[3]	0.00	21.00	5.00	15.50	0.00	22.50	0.00	15.00
L-Lysine HCl	0.38	0.63	0.42	0.58	0.40	0.67	0.40	0.58
L- Threonine	0.16	0.13	0.15	0.15	0.18	0.14	0.18	0.16
DL-Methionine	0.09	0.01	0.09	0.04	0.09	0.00	0.09	0.03
L-Tryptophan	0.00	0.03	0.00	0.02	0.00	0.03	0.00	0.02
L-Valine	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Soybean oil	0.50	0.48	0.55	0.63	0.50	0.50	0.50	0.50
Potassium chloride	0.60	0.18	0.82	0.46	0.90	0.45	0.90	0.63
Limestone	1.10	1.40	1.32	1.40	1.20	1.55	1.20	1.42
Potassium carbonate	0.00	0.76	0.01	0.52	0.00	0.83	0.00	0.55
Salt	0.46	0.45	0.50	0.48	0.60	0.60	0.60	0.60
Grower Vitamin premix[4]	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Mineral premix[5]	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Zinc oxide	0.28	0.28	0.28	0.28	0.00	0.00	0.00	0.00
Swine Larvicide	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13

[1] Dietary treatments: CON: Standard nursery diet; SBM75: CON replacing 75% of SBM by GDDY; FM/ESBM: CON without Fishmeal and ESBM + GDDY inclusion; HP-GDDY50: CON replacing 50% of SBM, FM, and ESBM by GDDY.

[2] ESBM, HAMLET PROTEIN Inc., 5289 Hamlet Drive, Findlay, OH 45840 - USA.
[3] POET, LLC, 4506 N Lewis Ave, Sioux Falls, SD 57104 – USA.
[4] J & R Distributing Inc. 518 Main Ave, Lake Norden, SD 57248 - USA. Minimum provided per kg of diet: Calcium 55 mg, Vitamin A 11,000 IU, Vitamin D3 1,650 IU, Vitamin E 55 IU; Vitamin B12 0.044 mg, Menadione 4.4 mg, Biotin 0.165 mg, Folic Acid 1.1 mg, Niacin 55 mg, d-Pantothenic Acid 60.5 mg, Vitamin B16 3.3 mg, Riboflavin mg, 9.9 Thiamine 3.3 mg.
[5] J & R Distributing Inc. 518 Main Ave, Lake Norden, SD 57248 - USA. Minimum provided per kg of diet: Copper 16.5 ppm, Manganese 44.1 ppm, Selenium 0.03 ppm, Zinc 165 ppm.

Table 3.3. Calculated composition of experimental diets (Phase 1 and Phase 2) to evaluate the impact of yeast-based high protein DDGS (HP-GDDY) inclusion on digestibility and gut health of weaned pigs.

	Phase 1				Phase 2			
Ingredient	CON	SBM75	FM/ESBM	HP-GDDY50	CON	SBM75	FM/ESBM	HP-GDDY50
ME, Kcal/kg	3521	3521	3521	3521	3503	3503	3503	3498
Crude Protein, %	21.5	21.5	21.9	21.1	21.0	21.0	21.0	21.0
Lactose	22.0	22.0	22.0	22.0	18.0	18.0	18.0	18.0
SID Lysine, %	1.50	1.50	1.50	1.50	1.35	1.35	1.35	1.35
SID Met, %	0.43	0.43	0.43	0.43	0.39	0.39	0.39	0.39
SID, Met + Cys, %	0.71	0.72	0.74	0.72	0.67	0.68	0.69	0.68
SID THR, %	0.88	0.88	0.88	0.88	0.79	0.79	0.78	0.79
SID TRP, %	0.25	0.25	0.25	0.25	0.24	0.24	0.24	0.24
SID ILEU, %	0.85	0.84	0.89	0.83	0.83	0.82	0.86	0.81
SID LEU, %	1.56	1.76	1.81	1.74	1.54	1.77	1.73	1.73
SID VAL, %	0.95	0.95	0.97	0.95	0.86	0.94	0.92	0.91
SID HIS, %	0.46	0.47	0.49	0.46	0.46	0.47	0.48	0.46
SID PHE, %	0.83	0.86	0.91	0.85	0.82	0.87	0.89	0.85
Calcium, %	0.85	0.85	0.85	0.85	0.80	0.80	0.80	0.80
Phosphorus, %	0.65	0.65	0.64	0.63	0.60	0.61	0.60	0.58
Dig P., %	0.41	0.43	0.41	0.41	0.36	0.39	0.36	0.36
Avail. P	0.45	0.48	0.46	0.46	0.40	0.43	0.41	0.41
Ca/P	1.86	1.78	1.85	1.86	2.0	1.8	2.0	2.0

[1]Dietary treatments: CON: Standard nursery diet; SBM75: CON replacing 75% of SBM by GDDY; FM/ESBM: CON without Fishmeal and ESBM + GDDY inclusion; HP-GDDY50: CON replacing 50% of SBM, FM, and ESBM by GDDY. Experimental diets were formulated in a 4-phase program with Phase 1 from weaning to d7, Phase 2 from d8 to d21, phase 3 from d22 to d28, and phase 4 from d29 to d53.

Table 3.4. Calculated composition of experimental diets (Phase 3 and Phase 4) to evaluate the impact of yeast-based high protein DDGS (HP-GDDY) inclusion on digestibility and gut health of weaned pigs.

	Phase 3				Phase 4			
Ingredient	**CON**	**SBM75**	**FM/ESBM**	**HP-GDDY50**	**CON**	**SBM75**	**FM/ESBM**	**HP-GDDY50**
ME, Kcal/kg	3474	3474	3474	3458	3521	3498	3458	3474
Crude Protein, %	21.2	21.5	21.5	19.9	21.1	21	20.1	21.1
Lactose	9	9	9	0	22	18	0	9
SID Lysine, %	1.35	1.35	1.35	1.23	1.50	1.35	1.23	1.35
SID Met, %	0.39	0.39	0.39	0.36	0.43	0.39	0.36	0.39
SID, Met + Cys, %	0.66	0.68	0.68	0.62	0.72	0.68	0.63	0.68
SID Thr, %	0.79	0.79	0.79	0.73	0.88	0.79	0.73	0.79
SID Trp, %	0.23	0.23	0.24	0.21	0.25	0.24	0.21	0.23
SID Ileu, %	0.81	0.81	0.84	0.75	0.83	0.81	0.74	0.81
SID Leu, %	1.54	1.86	1.64	1.47	1.74	1.73	1.70	1.77
SID Val, %	0.86	0.95	0.89	0.78	0.95	0.91	0.86	0.92
SID His, %	0.48	0.49	0.49	0.46	0.46	0.46	0.47	0.48
SID Phe, %	0.85	0.92	0.91	0.84	0.85	0.85	0.89	0.90
Calcium, %	0.80	0.80	0.80	0.70	0.85	0.80	0.70	0.80
Phosphorus, %	0.63	0.60	0.63	0.56	0.63	0.58	0.55	0.61
Dig P., %	0.36	0.36	0.36	0.29	0.41	0.36	0.29	0.36
Avail. P	0.40	0.40	0.41	0.33	0.46	0.41	0.33	0.40
Ca/P	2.0	2.0	2.0	2.13	1.86	2.0	2.10	2.0

[1]Dietary treatments: CON: Standard nursery diet; SBM75: CON replacing 75% of SBM by GDDY; FM/ESBM: CON without Fishmeal and ESBM + GDDY inclusion; HP-GDDY50: CON replacing 50% of SBM, FM, and ESBM by GDDY. Experimental diets were formulated in a 4-phase program with Phase 1 from weaning to d7, Phase 2 from d8 to d21, phase 3 from d22 to d28, and phase 4 from d29 to d53.

Table 3.5. Analyzed chemical composition (as-fed basis) of experimental diets (Phase 1 and Phase 2) to evaluate the impact of digestibility and [1]gut health on weaned pigs in comparison with common diet.

	Phase 1				Phase 2			
Ingredient	**CON**	**SBM75**	**FM/ESBM**	**HP-GDDY50**	**CON**	**SBM75**	**FM/ESBM**	**HP-GDDY50**
ME, Kcal/kg	3867	3972	3906	3917	3821	3919	3860	3880
Crude fat, %	2.80	3.09	2.69	3.06	2.62	2.70	2.47	2.82
Crude fiber, %	1.53	2.03	2.23	1.95	1.61	2.10	2.09	2.10
Crude Protein, %	21.0	21.3	20.3	19.5	19.8	20.4	19.4	19.7
Total, %								
Lys	1.55	1.70	1.73	1.68	1.49	1.35	1.51	1.69
Met	0.44	0.45	0.47	0.45	0.38	0.42	0.37	0.39
Met + Cys	0.79	0.81	0.88	0.83	0.68	0.80	0.74	0.74
Thr	1.01	1.09	1.03	0.98	0.87	0.91	0.90	0.89
Trp	0.23	0.23	0.24	0.25	0.22	0.22	0.23	0.23
Ileu	0.98	0.97	1.04	0.94	0.90	0.94	0.94	0.91
Leu	1.70	1.87	1.98	1.80	1.69	1.86	1.77	1.77
Val	1.07	1.07	1.13	1.07	0.97	1.06	1.02	0.99
His	0.49	0.51	0.55	0.49	0.48	0.51	0.50	0.49
Phe	0.92	0.92	1.01	0.90	0.87	0.92	0.92	0.89
Ash	7.15	7.47	7.30	7.53	6.45	7.23	6.69	6.86
Phosphorus	0.63	0.67	0.64	0.65	0.59	0.61	0.60	0.61

[1]Dietary treatments: CON: Standard nursery diet; SBM75: CON replacing 75% of SBM by GDDY; FM/ESBM: CON without Fishmeal and ESBM + GDDY inclusion; HP-GDDY50: CON replacing 50% of SBM, FM, and ESBM by GDDY. Experimental diets were formulated in a 4-phase program with Phase 1 from weaning to d7, Phase 2 from d8 to d21, phase 3 from d22 to d28, and phase 4 from d29 to d53.

Table 3.6. Analyzed chemical composition (as-fed basis) of experimental diets (Phase 3 and Phase 4) to evaluate the impact of digestibility and [1]gut health on weaned pigs in comparison with common diet.

	Phase 3				Phase 4			
Ingredient	**CON**	**SBM75**	**FM/ESBM**	**HP-GDDY50**	**CON**	**SBM75**	**FM/ESBM**	**HP-GDDY50**
ME, Kcal/kg	3845	3926	3832	3925	3823	3951	3803	3935
Crude fat, %	2.71	2.95	2.16	2.78	2.17	3.06	1.86	2.55
Crude fiber, %	1.85	2.45	2.02	2.19	2.03	2.87	2.00	2.45
Crude Protein, %	20.2	21.4	20.1	20.1	18.3	18.6	18.4	18.9
Total, %								
Lys	1.41	1.52	1.39	1.51	1.36	1.54	1.30	1.33
Met	0.38	0.39	0.42	0.38	0.35	0.35	0.34	0.37
Met + Cys	0.72	0.75	0.77	0.74	0.66	0.70	0.65	0.73
Thr	0.89	0.89	0.95	0.91	0.84	0.80	0.81	0.86
Trp	0.22	0.21	0.23	0.22	0.19	0.19	0.18	0.19
Ileu	0.89	0.85	0.93	0.91	0.82	0.79	0.81	0.82
Leu	1.60	1.84	1.73	1.86	1.53	1.76	1.55	1.80
Val	0.97	0.99	1.00	1.04	0.88	0.94	0.88	0.96
His	0.48	0.50	0.52	0.53	0.48	0.49	0.48	0.51
Phe	0.91	0.90	0.98	0.95	0.90	0.89	0.90	0.93
Ash	6.36	6.76	6.38	6.59	5.43	6.12	5.60	5.76
Phosphorus	0.61	0.63	0.59	0.62	0.61	0.58	0.58	0.57

Table 3.7. Main effects of dietary yeast-based high protein DDGS (HP-GDDY) inclusion on pig growth performance throughout the nursery period[1]

Item	Dietary treatments				SEM	*P*-value[2]
	CON	SBM75	FM/ESBM	HP-GDDY50		
Initial BW, kg	5.66	5.67	5.68	5.64	0.03	0.851
Period, d0 - 7						
BW d7, kg	6.1	6.1	6.1	6.1	0.06	0.834
ADG, g	68	65	58	62	5.69	0.678
ADFI, g	103	93	100	97	4.25	0.451
F:G	1.56	1.44	1.73	1.60	0.09	0.169
G:F	0.70	0.76	0.65	0.70	0.05	0.423
Period, d7 - 14						
BW d14, kg	6.7	6.5	6.5	6.4	0.10	0.305
ADG, g	140	140	140	140	0.01	0.238
ADFI, g	220[x]	202[xy]	210[xy]	193[y]	7.25	0.075
F:G	1.78	1.96	1.96	2.02	0.20	0.828
G:F	0.61	0.58	0.56	0.57	0.04	0.806
Period, d14 – 21						
BW d21, kg	8.3[a]	7.9[ab]	8.1[ab]	7.7[b]	0.13	0.010
ADG, g	232[a]	190[bc]	230[ab]	179[c]	10.83	0.000
ADFI, g	353	345	355	315	10.51	0.040
F:G	1.63[a]	2.02[b]	1.78[ab]	1.87[ab]	0.08	0.030
G:F	0.65[a]	0.54[b]	0.63[ab]	0.57[ab]	0.03	0.040
Period, d21 – 28						
BW d28, kg	9.9[x]	9.5[xy]	9.8[xy]	9.4[y]	0.18	0.092
ADG, g	239	238	238	241	15.87	0.999
ADFI, g	419	391	394	388	15.21	0.430
F:G	1.75	1.74	1.68	1.67	0.11	0.918

G:F	0.57	0.57	0.57	0.57	0.04	0.806
Period, d28 - 42						
BW d42, kg	16.9^{a}	14.2^{b}	16.1^{a}	14.9^{b}	0.293	<.0001
ADG, g	500^{a}	338^{d}	448^{b}	395^{c}	10.58	<.0001
ADFI, g	749^{a}	749^{c}	749^{ab}	749^{bc}	18.11	<.0001
F:G	1.50^{b}	1.72^{a}	1.53^{b}	1.59^{b}	0.03	<.0001
G:F	0.67^{a}	0.58^{b}	0.66^{a}	0.63^{a}	0.01	<.0001
Period, d42 - 53						
BW d53, kg	24.4^{a}	19.7^{c}	23.3^{ab}	21.4^{bc}	0.52	<.0001
ADG, g	704^{a}	524^{b}	694^{a}	613^{ab}	25.38	<.0001
ADFI, g	1164^{a}	912^{b}	1150^{a}	1022^{ab}	43.16	0.001
F:G	1.66	1.75	1.67	1.69	0.06	0.715
G:F	0.61	0.61	0.61	0.61	0.02	0.887
Period, d0 - 53						
ADG, kg	360.1^{a}	270.5^{c}	339.1^{ab}	302.8^{bc}	9.84	<.0001
ADFI, kg	501.9^{a}	420.5^{c}	482.9^{ab}	440.8^{bc}	11.32	<.0001
F:G	1.41^{b}	1.58^{a}	1.43^{b}	1.49^{ab}	0.03	0.001
G:F	0.72^{a}	0.64^{b}	0.70^{a}	0.69^{ab}	0.01	0.004

[1]Dietary treatments: CON: Standard nursery diet; SBM75: CON replacing 75% of SBM by GDDY; FM/ESBM: CON without Fishmeal and ESBM + GDDY inclusion; HP-GDDY50: CON replacing 50% of SBM, FM, and ESBM by GDDY. Experimental diets were formulated in a 4-phase program with Phase 1 from weaning to d7, Phase 2 from d8 to d21, phase 3 from d22 to d28, and phase 4 from d29 to d53.

[2]Superscripts ^{abcd} indicate significant difference at $P \leq 0.05$ and ^{wxyz} indicate tendency at $0.05 < P \leq 0.10$ using Tukey's means separation test.

Table 3.8. Urinary lactulose and mannitol and serum Immunoglobulin-A in nursery pigs provided diets containing varying proportions of a yeast-based high protein DDGS (HP-[1] GDDY).

	Dietary Treatments[1]					
Item	**CON**	**SBM75**	**FM/ESBM**	**HP-GDDY50**	**SEM**	***P*- value[2]**
Day 10 postweaning						
Lactulose, mM	0.045	0.063	0.054	0.093	0.0171	0.278
Mannitol, mM	0.113	0.158	0.100	0.101	0.0270	0.451
L:M	0.476	0.473	0.568	0.798	0.1244	0.330
IgA, mg/mL	0.162	0.154	0.168	0.171	0.0077	0.422
Day 20 postweaning						
Lactulose, mM	0.035	0.031	0.034	0.042	0.0067	0.683
Mannitol, mM	0.137	0.122	0.119	0.157	0.0334	0.857
L:M	0.274^{z}	0.377^{y}	$0.292^{z\,y}$	0.232^{z}	0.0365	0.099
IgA, mg/mL	0.192^{yz}	0.175^{z}	0.187^{z}	0.209^{y}	0.0094	0.082

[1] Dietary treatments: CON: Standard nursery diet; SBM75: CON replacing 75% of SBM by GDDY; FM/ESBM: CON without Fishmeal and ESBM + GDDY inclusion; HP-GDDY50: CON replacing 50% of SBM, FM, and ESBM by GDDY. Experimental diets were formulated in a 4-phase program with Phase 1 from weaning to d7, Phase 2 from d8 to d21, phase 3 from d22 to d28, and phase 4 from d29 to d53.

[2]abcd – Letters indicate significant differences at $P \leq 0.05$ using Tukey's means separation test.

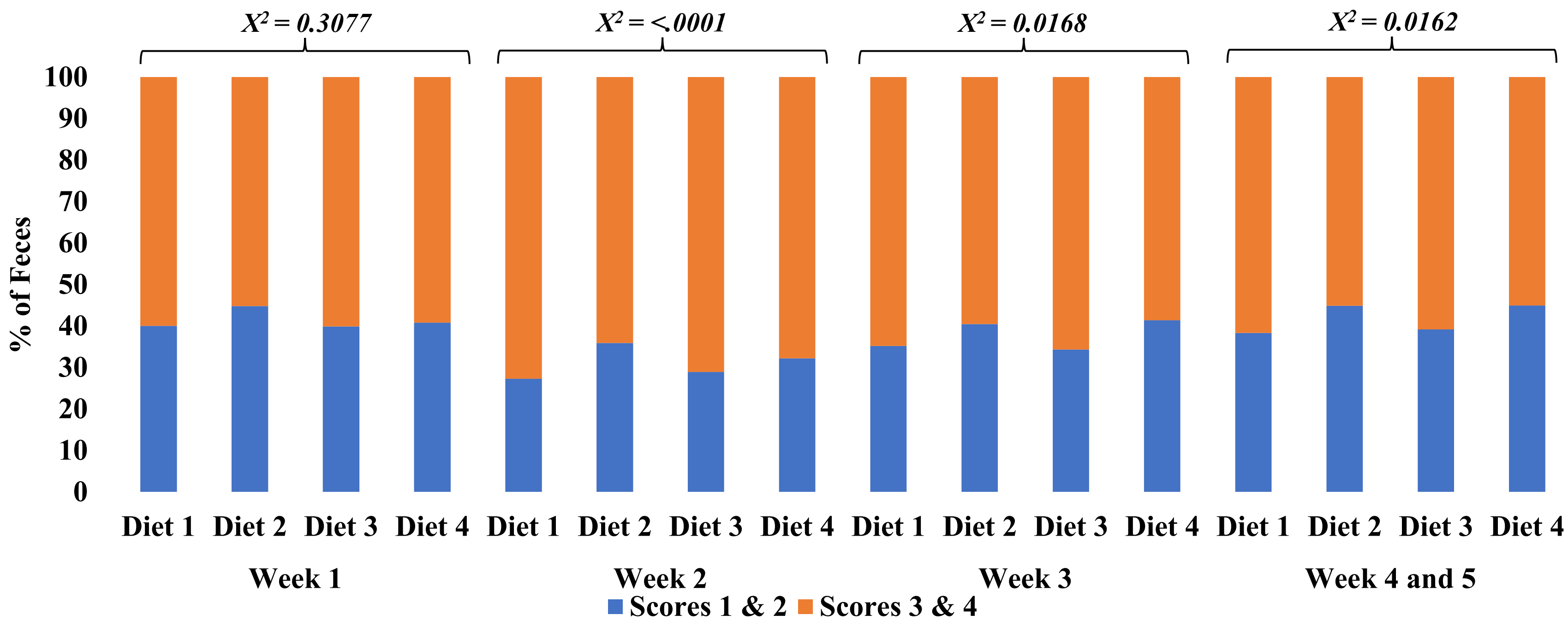
yz – Letters indicate tendencies ($0.05 < P \leq 0.10$) using Tukey's means separation test.

Table 3.9. Apparent total tract digestibility (ATTD) of weaned pig diets containing varying proportions of a yeast-based high protein DDGS (HP-GDDY).

	Dietary Treatments[1]					
Item	CON	SBM75	FM/ESBM	HP-GDDY50	SEM	P-value[2]
DM	71.14	71.01	70.52	70.72	0.623	0.893
CP	50.21	49.83	48.42	51.34	0.895	0.166
C.FIBER	70.74^{a}	52.00^{b}	72.09^{a}	55.97^{b}	1.388	<.0001
NDF	66.66^{a}	59.45^{b}	69.04^{a}	58.02^{b}	1.343	<.0001
ADF	66.50^{a}	53.54^{b}	67.35^{a}	51.83^{b}	1.797	<.0001
GE	18.17	17.69	17.06	16.83	0.497	0.256
PHOSPHORUS	76.64^{ab}	73.29^{c}	78.58^{a}	74.78^{bc}	0.602	<.0001

[1]Dietary treatments:
CON: Standard wean Phase 1 feeding program.
SBM75: Diet 1 replacing 75% of SBM with GDDY.
FM/ESBM: Diet 1with no protein sources (Fish meal and ESBM), adding GDDY.
HP-GDDY50: Diet 1 replacing 50% of each protein sources (SBM, Fish meal, and ESBM), and adding GDDY.
[2]abcd – Letters indicate significant differences at $P \leq 0.05$ using Tukey's means separation test.

Figure 3.1. Relative proportion of pen fecal scores in nursery pigs provided diets containing varying proportions of a yeast-based high protein DDGS (HP-GDDY)[1]



[1]Dietary treatments: CON: Standard nursery diet; SBM75: CON replacing 75% of SBM by GDDY; FM/ESBM: CON without Fishmeal and ESBM + GDDY inclusion; HP-GDDY50: CON replacing 50% of SBM, FM, and ESBM by GDDY. Experimental diets were formulated in a 4-phase program with Phase 1 from weaning to d7, Phase 2 from d8 to d21, phase 3 from d22 to d28, and phase 4 from d29 to d53.

4 GENERAL DISCUSSION

The purpose of this thesis work was to evaluate the suitability of yeast-based high-protein DDGS (HP-DDGY), a by-product of the ethanol industry, as a protein source in swine diets. The work included characterization of the nutritional value and the evaluation of different levels of inclusion to allow the practical incorporation in diets. The HP-GDDY was considered as a viable potential ingredient because it was produced using innovative techniques and technologies for separating the protein portion of DDGS and reducing heat exposure durations during drying. Studies have previously been conducted on the use of co-products of the ethanol industry, such as DDGS or DDG. Woyengo, et al. (2016) concluded that inclusion of 10% wheat DDGS in diet for growing pigs did not affect growth performance but reduced total tract digestibility of GE, N, P and Ca. Rodriguez, et al. (2021) conducted a study that included 15% of cold-fermented or conventional DDGS in diets fed to pigs from 1-week post-weaning and until market and reported no effect on growth performance or carcass characteristics. Other studies have analyzed the nutritional value and growth performance of pigs fed HP-DDG. Cristobal, et al. (2020) concluded that HP-DDG had higher concentrations of DE and ME, but lower standardized total tract digestibility of P compared to conventional DDGS in fed barrows. Widmer, et al. (2008) concluded that the inclusion of HP-DDG in the diet had no effect on the general performance of pigs but has a detrimental effect on belly firmness and iodine levels. However, there is no information on the nutritional profile of HP-GDDY or its incorporation in diets in the first weeks after weaning. In this sense, determining a product's value for practical pig feeding begins with characterization of the nutritional value.

As previously stated, ethanol industry co-products, particularly DDGS, have been used as alternative feed ingredients for monogastric and ruminant species; however, due to the fiber content, nutrient digestibility is negatively affected; thus, DDGS inclusion in swine diets is limited to avoid adverse effects on feed intake, growth rate, and feed conversion. Therefore, the method used to obtain HP-GDDY may be linked to an improvement in nutritional properties, and the inclusion of HP-GDDY can reduce the negative effects of DDGS and could be a ingredient to include as an strategy to reduce the cost of pig production by including this alternative protein ingredient in the pig diets. The characterization of HP-GDDY showed that the CP content is 23% higher compared to DDGS, and even 3% higher than SBM, and was comparable to the CP content of ESBM, although it had lower CP compared to FM. Similarly, it is important to highlight the 25% decrease in NDF in HP-GDDY compared to DDGS, which can be associated with the removal techniques of non-fermentable components such as grinding, heat and fermentation (Pedersen, et al., 2014; Urriola, et al., 2010).

The nutritional values of feedstuffs can be improved by implementing processing methods such as milling, pelleting, and extrusion, and drying methods, have not shown effectiveness in reducing the content of poorly digestible nutrients in DDGS. However, the nutritional value of HP-GDDY produced by two different drying processes, spray-dried and ring-dried, was assessed in chapter two of this thesis. The spray-dried process involves a slurry of product in some type of liquid that is pumped thorough an atomizer at high pressure or atomized with air that creates a fine spray of liquid and wet powder (Febo, 2015; Schappo, et al., 2021). The ring dryer method is an industrial scale, operates by exposing a slurry to superheated air in a circular form that facilitates controlled drying conditions and prevents overheating of the final product (Mouahid, et al., 2020). The results obtained from this work suggests that the processing technique used to obtain HP-GDDY improves the concentration of nutrients, the digestibility of AA and the ME content compared to DDGS. As in the case of lysine, one of the limiting amino acids in cereal-based diets, SID digestibility was improved approximately 15% in HP-GDDY with the spray-dryer method and 8.8% with the ring-dryer method; moreover, the HP-GDDY had similar AA profile and AA digestibility compared to SBM. In the same way the ME content of HP-GDDY with spray-dryer method was 40% ME (4480 Kcal/Kg) higher and 23% ME (3927 Kcal/Kg) higher with ring-dryer method compared to corn. Additionally, the cell mass from the carbohydrate fermentation process by the yeast contributes significantly to the CP content (~ 60g/g100 MS). Although the spray-dryer method increases the concentration and digestibility of several nutrients, the cost of producing HP-GDDY using this drying process is not commercially viable, due in part to the fact that spray drying is an energy intensive process. Even accounting for a significant part of industrial energy use worldwide, the spray drying method can consume 4 to 5 times more energy in evaporation compared to other drying methods (Julklang and Golman, 2015; Baker and McKenzie, 2005); so, when evaluating the potential use of this product, the economic cost of its elaboration must also be considered.

HP-GDDY can be a feedstuff comparable to one of the main energy sources used in commercial pig diets such as corn. The HP-GDDY are also comparable to SBM as a source of protein which makes HP-GDDY a valuable alternative feedstuff to be included in swine diets. However, regardless of the markedly superior nutritional and digestibility characteristics compared to DDGS, the inclusion levels of HP-GDDY must be controlled without compromising growth performance. For example, the inclusion of HP-GDDY can be fed up to 14% in phases 1 and 2 in the nursery period without compromising growth performance, during the later nursery phases, growth performance is affected when HP-GDDY levels are higher than 15%. This negative impact on performance may be due to higher levels of BCAAs in relation to Lys present in HP-GDDY, which may lead to an imbalance of AA in relation to the limiting AA affecting negative growth performance, and feed intake.

However, in order to determine ideal levels of inclusion in the diet, it is necessary to evaluate the chemical composition of HP-GDDY and its relationship with the age of the piglet's immune system. Furthermore, providing suitable levels of an ingredient typically avoids undesirable effects like post-weaning diarrhea and can improve intestinal health development. Furthermore, the presence of yeast in HP-GDDY can improve nutritional digestibility in nursery pigs. The yeast component also promotes the production and function of antibodies and improves intestinal health and microbiota (Long et al., 2021; Lee et al., 2021). In addition, the fiber in HP-GDDY may have potential positive effects on performance and serum immunity, promote intestinal barrier function as well as alter bacterial profiles and metabolites in the cecum of weaned piglets (Wu et al., 2017; Wu et al., 2018). As described in Chapter 3, growth performance was affected during the later phases of weaning, which may be explained by the BCAA content, particularly Leu. However, although the digestibility of DM, CP and GE was not different between the diets during the nursery period, fiber digestibility was lower compared to the control diet, probably due to the fiber composition present in HP-GDDY, because most complex carbohydrates and plant polysaccharides cannot be digested by animal digestive enzymes and, in the case of pigs, the ability to digest dietary fiber is primarily derived from their hindgut microbiota (Niu et al., 2022). In the case of P digestibility in diets, P digestibility was better when protein sources, FM and HP300 were completely replaced by HP-GDDY in the diet. However, the replacement of 50% of the protein ingredients (SBM, FM and HP300) by HP-GDDY in diet and the replacement of 75% of SBM by HP-GDDY in diet, reduced the phosphorus digestibility in weaned pigs. The low digestibility of P implies that undigested P will be excreted in the environment, which represents an additional cost because additional sources of P must be included in diets to meet P requirements.

In general, the chemical and nutritional evaluation of an ingredient is pertinent to the viability of the available resource under the premise of optimizing and reducing production costs in pig production. In the case of HP-GDDY, it is an ingredient with nutritional characteristics similar to other energy resources such as corn and even protein resources such as SBM. However, future research is needed to:

- Evaluate the optimal inclusion level of HP-GDDY in the different phases of weaned pig diets to reduce its impact on intestinal health and improve nutrient digestibility.
- Identify the mechanism to reduce the AA imbalance generated by excess BCAAs using HP-GDDY.
- Evaluate physical, chemical or enzymatic processes to increase P availability in HP-GDDY

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