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#### **REVIEW ARTICLE**



# A strategy of co-fermentation of distillers dried grains with solubles (DDGS) and lignocellulosic feedstocks as swine feed

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#### **ABSTRACT**

To meet the sustainable development of the swine feed industry, it is essential to find alternative feed resources and develop new feed processing technologies. Distillers dried grains with solubles (DDGS) is a by-product from the ethanol industry consisting of adequate nutrients for swine and is an excellent choice for the swine farming industry. Here, a strategy of co-fermentation of DDGS and lignocellulosic feedstocks for production of swine feed was discussed. The potential of the DDGS and lignocellulosic feedstocks as feedstock for fermented pig feed and the complementary relationship between them were described. In order to facilitate the swine feed research in co-fermentation of DDGS and lignocellulosic feedstocks, the relevant studies on strain selection, fermentation conditions, targeted metabolism, product nutrition, as well as the growth and health of swine were collected and critically reviewed. This review proposed an approach for the production of easily digestible and highly nutritious swine feed via co-fermentation of DDGS and lignocellulosic feedstocks, which could provide a guide for cleaner swine farming, relieve stress on the increasing demand of high-value swine feed, and finally support the ever-increasing demand of the pork market.

#### **ARTICLE HISTORY**

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#### **KEYWORDS**

Distillers dried grains with solubles (DDGS); lignocellulose; feedstock; fermentation; swine; recycling resources

#### Introduction

A rapid global growth of population and rising incomes have led to an increasing demand for food, meat, and milk, which are projected to increase by 60, 57, and 48%, respectively between 2005 and 2050 [1]. As animal source food contributed 18% of word's calorie and 25% of protein intake [2], livestock production has been estimated to expand by 21% between 2010 and 2025 [3]. This expected expansion will require an increase in world feed supply from 6.0 to 7.3 billion tons of dry matters [4]. Various feed grains such as corn, wheat and barley are currently the main ingredients used in animal feeds. However, feeding animals with proteins from human-edible crops may be regarded as a direct competition against human food and may not be sustainable [3]. Thus, it is critical to find new feed resources other than food crops such as soybeans and corn, as well as innovation of feed processing technologies [5].

Swine is one of the most important sources of meat and protein for human beings. As shown in Figure 1, annual pig production in most parts of the world exceeds a herd of 798,732 pigs. The production of pigs showed an increasing trend (Figure 2). The second half of the twentieth century witnessed a dramatic increase in world swine production, which currently numbers a herd of 978,332,119 pigs, as showed in Figure 2 [6]. In terms of China, the United States, Canada, Spain, Germany etc., over 12 million swine were produced each year which has become the support of the national economy and the main food source of the residents. Therefore, the demand for swine feed is huge, accounting for more than 20% of all animal feed [3]. The development of nutritious and efficient swine feeds to increase swine growth and production is of strategic importance in addressing global food shortages.

Cereal grains are usually the main dietary energy source for swine. To grow to 100 kg, piglets need to be fed at least 250 kg of grain feeds. Feed costs account for 60–70% of total cost of pork production. Swine are not very efficient at using grain feed. Digestive utilization energy varies from 70–90% for most swine diets, and the rest (10–30%) is excreted in urine, feces, lost as body heat and fermented in the gut and lost as gas [7]. The

traditional way of grain feeding is a huge waste of resources. Traditional grain-fed swine are no longer able to meet the demand for pork. Various agricultural and food industry by-products, providing valuable energy and essential nutrients, are currently being studied as the alternative feed supplements to replace the current supply and meet the increasing demand. In the corn-ethanol dry milling industry, one ton of ethanol can be produced from three tons of corn, with 0.92-0.95 tons of DDGS as the by-product. Annual global production of DDGS is more than 40 million tons, especially in the United States which counts for 58% of total world production [8]. Corn is the predominant source of DDGS in the United States and many other countries. In addition, wheat, sorghum, barley, oats, etc., are also used as feedstocks for fuel ethanol. DDGS production will continue to grow over the next decade as global ethanol production expands [9]. DDGS will help decrease the absolute quantity of feed required and partially alleviate the large shortfall [10]. Substituting DDGS for traditional cereal grain as swine feed can effectively save resources and reduce the cost of raising swine.

DDGS has been used as livestock feed for many years. However, due to differences in nutritional composition and animal digestion, DDGS traditionally has been fed mainly to ruminants. As a monogastric animal, swine has completely different digestive properties from that in ruminants [11]. Ruminants contain a variety of microbial populations in the fore or rumen which could ferment the low-quality and high-fiber feed into microbial proteins, while the digestion of swine is carried out through endogenous enzymes and cannot convert high-fiber feed into nutrients well [12,13]. DDGS is a product left after starch is converted into ethanol and carbon dioxide, therefore it is rich in protein, lipids, fiber, minerals, and vitamins [14]. Utilization of DDGS in swine diets is limited due to variation in nutrient composition such as high content of fiber, presence of phytate and anti-nutritional factors, and unbalanced amino acids [15]. Pedersen et al. demonstrated that the lower gastrointestinal tracts of swine have limited digestibility to the fiber fraction of corn DDGS [16]. Feed composition is the most important factor determining the efficiency of feed utilization by pigs [17]. Therefore, DDGS must be processed and transformed to be suitable for use in swine diets.

In order to provide comprehensive nutrition, it is necessary to find raw materials to make up for lost starch. The annual global production of lignocelluloses is almost  $150-170 \times 10^9$  tons, which is one of the most abundant sustainable resources and renewable energy reservoir [18]. Lignocelluloses may become the most suitable source of sugar supplements due to their high

quantity and low cost. This substantial surplus of lignocelluloses includes straw, maize cobs, etc. [19]. However, the digestion efficiency of cellulose in swine is far from acceptable. Therefore, research is needed to identify practical ways to improve the energy digestibility of DDGS and lignocellulosic substrates for their use as appropriate swine feed. Improving the digestibility of the insoluble fiber fraction and increasing fermentable sugar would be beneficial for swine digesting cellulose for energy [20]. A promising method that can be applied to improve the nutritive quality of these alternative protein sources and the digestibility of lignocellulose is fermentation. Compared with feed additives such as prebiotics, probiotics, synbiotics, plant extracts or enzymes, fermented feed has the advantages of improving feed quality and intestinal ecology.

DDGS alone has sufficient nitrogen source but lacks sufficient fermentable sugar as the carbon source. Cellulose component could be hydrolyzed into glucose by cellulolytic enzymes produced during fermentation. In addition, nutrient complementarity can be formed between DDGS and lignocellulosic feedstocks. However, there are few reports up-to-date on the co-fermentation of DDGS and lignocellulosic feedstocks, their nutritive quality after fermentation, and their application in feeding swine. This review attempts to offer a feasible strategic solution for co-fermentation of DDGS and lignocellulosic feedstocks to produce nutritive swine feed.

#### The potential of DDGS as the main feedstock of fermented swine feed

#### The nutritional advantage of DDGS

DDGS is rich in a variety of nutrients needed by animals, including large amounts of protein, lipids, fiber, minerals, and vitamins. The average corn DDGS composition data calculated from 44 data sets published between 2004 and 2011 and representing 463 samples of corn DDGS showed that the crude protein, crude fat, crude fiber and phosphorus content were 27.9, 10.8, 7.4, and 0.8%, respectively (Table 1) [21]. As more ethanol plants have

Table 1. Variation in chemical composition of corn DDGS from 1997 to 2010 [21].

Analyte	Maximum	Minimum	Average	SD	CV%
Crude protein (%)	34.7	23.3	27.9	2.4	8.5
Crude fat (%)	17.7	3.2	10.8	2.4	22.0
Crude fiber (%)	11.3	6.2	7.4	1.1	15.1
NDF (%)	51.0	27.7	36.6	5.8	15.7
ADF (%)	18.5	8.6	13.6	3.3	24.2
Ash (%)	5.9	3.1	4.5	0.6	13.6
Phosphorus (%)	0.98	0.69	0.80	0.07	8.8

Notice: NDF: neutral detergent fiber; ADF: acid detergent fiber; SD: standard deviation; CV: coefficient of variation.



Table 2. Composition of DDGS of different feedstocks, % dry basis [23].

Feedstock	Crude protein	Crude fat	Crude fiber	
Corn	31.3	11.9	10.2	
Sorghum	30.8	11.4	7.41	
Wheat hard	35.59	7.66	5.56	
Barley (Hulled)	35.3	7.2	8.2	
Barley (Hull-removed)	40.4	8.1	2.7	
Barley (Hull-less)	39.9	8.6	1.6	
Oats	18.8	9.7	25.4	

already removed oil for high energy feed or biodiesel, the oil content in corn DDGS is reduced to 6% or even less [22]. The chemical composition of sorghum DDGS is very similar to that of corn DDGS, while wheat DDGS contains more protein and less fiber than corn DDGS (Table 2) [23]. The amino acid composition of DDGS is diverse, covering the major types needed for pig growth and development, such as arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, valine, etc. The amino acid composition of sorghum DDGS is similar to that of corn DDGS, while that of barley and wheat DDGS are richer than corn DDGS [24]. Although the nutrient composition of different sources of DDGS varies, specific nutrient combinations can be used to produce high-quality animal feed. The greatest coefficient of variation (CV%) of protein in different DDGS was smaller than that in different corn and wheat, which were 5.4, 8.7, and 19.1%, respectively [25]. It indicated that DDGS are more suitable for producing standardized animal feed than pure crops.

Phosphorus is the third most expensive ingredient of swine diets. Unlike other grains and grain by-products, DDGS contains a high concentration of total and digestible phosphorus. Therefore, when adding DDGS to swine diets formulated on a digestible phosphorus basis, significant reductions in inorganic supplementation and diet cost can be achieved. Hanson et al. demonstrated the advantages of formulating DDGS diets on the basis of available phosphorus compared with a total phosphorus basis in diets containing 0, 10 or 20% DDGS [26]. It indicated that increasing diet inclusion rates of DDGS can reduce total dietary phosphorus content and the concentration of fecal phosphorus, but did not affect the excretion, retention and digestibility of phosphorus. Thus, using DDGS as the feedstock to produce fermented swine feed can provide adequate phosphorus requirements for swine without external addition. Furthermore, based on the standardized total tract digestibility values for phosphorus in DDGS for swine, the feed could achieve optimal phosphorus nutrition.

There are also several bioactive compounds in DDGS that provide health benefits, including vitamin E, ferulic acid and carotenoids. These compounds are sometimes described as having functional or nutritional properties, along with others, that may contribute to the antioxidant capacity and potential health benefits [27]. These phytochemical components have beneficial effects on gut health and immune system responses of swine. Studies also have also shown that DDGS contains active substances such as tocopherol, tocotrienol, xanthophylls and ferulic acid, and has two- to three-fold greater concentration than found in corn [28]. Besides, there are small amounts of docohexaenoic acid present, a physiologically important omega-3 fatty acid for neural, retinal and immune functions [29]. Therefore, adding DDGS into swine feed will benefit swine development and health.

#### The energy advantage of DDGS

DDGS is mainly used as an energy source in swine diets because it contains approximately the same amount of metabolizable energy (ME) as corn. Traditionally, DDGS was added to swine diets to primarily replace part of corn or other food crops for providing energy and reducing the cost. Numerous studies have shown that DDGS can account for up to 30% in starter, grower-finisher, and lactation diets, and up to 50% in sow gestation diets, without adverse effects on swine performance. The starch content in DDGS is very low, while crude protein is relatively high compared with other common feed compositions. Nutrient composition of feeding ingredients has a great impact on their energy content [30]. The fatty acid composition of DDGS is important, due to its contribution to ME and net energy (NE) values, affecting pork fat firmness in growing-finishing pigs, and susceptibility to lipid peroxidation during the production process, transport and storage. The main fatty acids present in DDGS corn oil are linoleic acid (54%), oleic acid (26%), and palmitic acid (14%), of which linoleic and oleic acids are unsaturated fatty acids that contribute to the high energy content of DDGS [29]. Furthermore, there was no significant difference in the fatty acid profile between high-oil (greater than 10% crude fat) and reduced-oil (less than 10% crude fat) DDGS sources [29]. Therefore, the fat of different DDGS products all plays an important role in the energy supply characteristics of swine feed.

#### The fermentation necessity of DDGS

The inclusion rates of DDGS in the swine diet are usually restricted due to less fiber digestibility, unbalanced

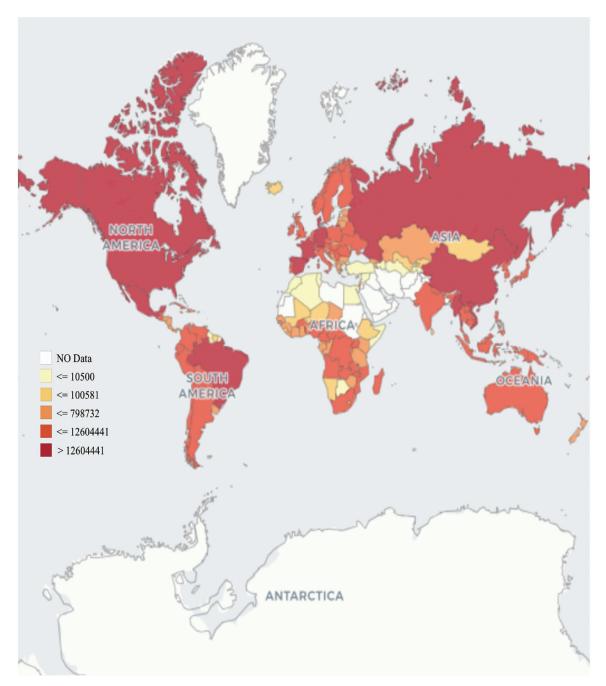


Figure 1. Global distribution of pig production, average 1992–2018, statistics from the 2018 FAO database [6].

amino acids and high variance of DDGS source. Heat treatment during drying of DDGS not only directly reduce the content of lysine, but also increases the proportion of lysine that is less digestible and absorbable [31]. Moreover, lysine can undergo Maillard reactions to produce nutritionally unavailable products [32]. Overall, the low proportion and low quality of bioavailable lysine in DDGS for protein synthesis will lead to reduced growth performance and percentage of carcass lean in pigs [33]. Furthermore, DDGS contains large amounts of protein that ferment in the intestine during feeding.

Fermentation of protein in the pig intestine has potentially harmful effects on gut health and the environment [34]. Therefore, *in vitro* fermentation is essential to improve the amino acid composition and the protein digestibility of DDGS.

In vitro fermentation also has a great potential to break down fiber in DDGS to improve its digestibility. The digestibility of fiber in DDGS is less than 35% in the small intestine and less than 50% over the entire gastrointestinal tract [24,35], indicating the fiber fraction contributes little to the energy value of these

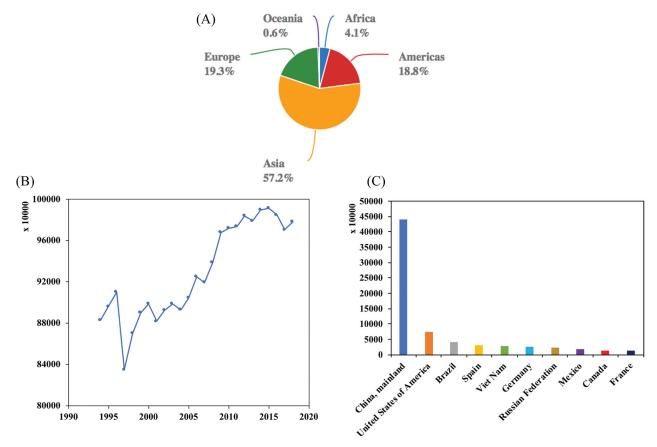


Figure 2. World pig production, statistics from the 2018 FAO database [6]. (A) The proportion of pig production on five continents; (B) The trend of pig production from 1990 to 2018; (C) statistics of pig production among countries.

feeds. In addition, the fiber structure is more complex and variable in corn DDGS compared to wheat DDGS, and therefore, is more difficult to degrade with exogenous enzymes [16]. Therefore, it is necessary for the application of DDGS in swine feed to find an *in vitro* fermentation method that can transform the fiber in DDGS into a substance that can be easily absorbed by swine.

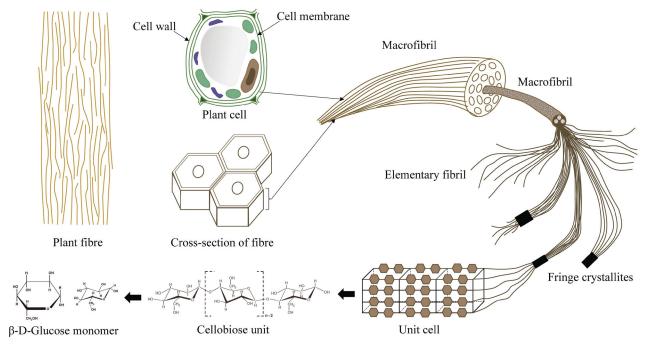
## The potential of lignocellulose as an additive to DDGS for fermented swine feed

DDGS contains high amounts of protein, which has proven to be a good nitrogen source for fermentation. Approximately two-thirds of the raw materials (based on corn starch content) are converted to ethanol and carbon dioxide during the dry milling process. Thus, fermentation of DDGS alone lacks sufficient carbon sources, and it does not have a suitable carbon-to-nitrogen ratio. Adding monosaccharides or polysaccharides has high cost and poor economic benefits. A more economical method is the fermentation of DDGS in the presence of lignocellulosic feedstocks [36,37]. Consumption

of lignocellulosic substrates directly or indirectly produces sugar, making it a sustainable carbon source for DDGS fermentation.

### Lignocellulose as carbon sources during fermentation

Lignocellulose is mainly composed of structural carbohydrates of cellulose, hemicelluloses and lignin. They are entangled together to form lignin-carbohydrate complexes (LCCs). As showed in Figure 3, when LCCs were degraded, the cellulose component would be separated from hemicelluloses and lignin. As the cellulose is further degraded, a large amount of glucose will be produced [39]. As a high-quality carbon source for bacteria, glucose can make up for the deficiency of DDGS carbon source and provide sufficient energy source for strains during fermentation in a bioreactor. The high carbon content ratio of lignocellulose is conducive to adjusting the carbon-nitrogen ratio of the DDGS fermentation process. In addition, some inhibitors derived from lignocellulose would affect the activity of the bacteria during the fermentation process. Multi-strain



**Figure 3.** The degradation process of lignin-carbohydrate complexes, adapted from Wuestenberg, Tanja. (Cellulose: Wuestenberg, Tanja: Cellulose and Cellulose Derivatives in the Food Industry. 104. 2014. Copyright Wiley-VCH GmbH) and reproduced with permission [38]. Lignin-carbohydrate complexes is mainly derived from plant cell walls, the cellulose was separated after pretreatment, and release glucose during degradation.

fermentation can effectively reduce this type of metabolic toxicity [40]. Therefore, this should be considered in the co-fermentation process.

#### Beneficial effects on swine intestinal digestion

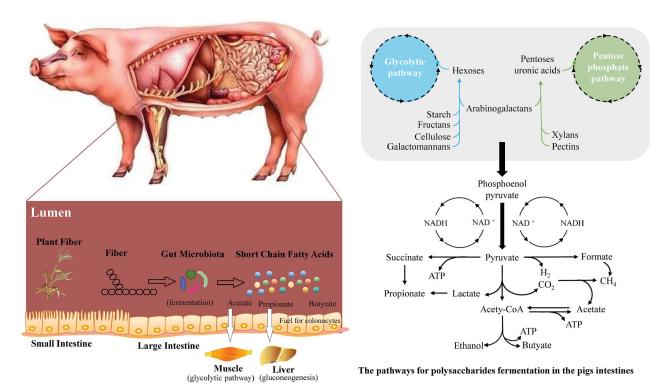
The intake of a certain amount of fermentable fiber in the diet of swine is beneficial to the health, although swine have a poor ability to metabolize fiber [41]. The inclusion of moderately fermentable fiber sources will reduce the production of harmful microbial metabolites in the large intestine as well as the incidence of intestinal disorders in swine. In fact, the ability to digest fiber is poorly predictable from the monomeric composition and are more related to their physico-chemical characteristics, such as physical structure [42]. Some studies showed that low fiber intake often leads to chronic diseases related to nutrient metabolism and intestinal inflammatory disorders [43,44]. Compared with standard weaned pig diets, high fiber intake in the early weaning period has an impact on microbial colonization without reducing enzyme activity or animal performance [45]. Besides, the amount of digesta flow at the terminal ileum is greater in swine fed high-fiber diets than low-fiber diets [46]. Unlike that in the intestines, the retention time of fiber in the stomach will increase, resulting in earlier satiety due to elongation of the stomach wall [47]. Early satiety is important for the welfare of

gestating sows [48]. Digestion was still normal when 30% of tropical tree leaves added in sow diets [49]. Mateos et al. [50] reported that adding up to 4.0% oat hulls to a low-fiber diet (5.5% neutral detergent fiber and 2.2% crude fiber) can reduce post-weaning diarrhea in weaning pigs from 21 to 41 days after weaning.

#### Increasing the energy supply for swine growth

Fiber consumption plays a vital role in maintaining homeostasis of the intestinal ecosystem. Bacterial metabolites and prebiotics from fiber are involved in various physiological processes for modulating health in pigs [51,52]. The production of short chain fatty acid (SCFA) by fiber fermentation plays an important role in regulating host metabolism, immune system and cell proliferation [53,54]. The SCFA can provide up to 15% of the maintenance energy requirement of growing pigs and 30% in gestating sows [46]. In addition, SCFA are capable of inhibiting the growth of some intestinal pathogens such as Escherichia coli, Salmonella sp. and Clostridium sp. [55]. Butyrate, in particular, seems to play a selective antimicrobial role. Therefore, cellulose metabolism plays an important role in the intestinal health of swine.

As the pathways for polysaccharides fermentation in the swine intestines showed in Figure 4, the intestinal bacteria hydrolyze the fiber and metabolize their



**Figure 4.** Schematic representation of the pathways for polysaccharides fermentation in the pigs intestines, adapted from Rajesh Jha et al. and Jérome Bindelle et al. Reproduced with permission [56,57]. The intestinal bacteria hydrolyze the fiber and produces short-chain fatty acids, metabolize their constituent sugars through a series of anaerobic energy-yielding reactions leading to the production of ATP which is used for bacteria basal and growth metabolism.

constituent sugar leads to the production of ATP through a series of anaerobic energy-yielding reactions, which is used for bacteria basal and growth metabolism. A majority of the anaerobes of the large intestine use the glycolysis pathway, that degrades glucose to pyruvate *via* glucose-6-phosphate [58], to ferment the carbohydrates [59]. Finally, the pyruvate would be further converted into nutrition absorbed by muscle and liver for swine growth. Furthermore, polysaccharides made of pentoses and pectins would be first metabolized by the Pentose phosphate pathway and then join the pyruvate metabolism and providing energy [60].

As a summary, lignocellulose has a great potential as a carbon source in the fermentation process, and it also has many positive effects on the intestinal health and the energy supply.

# Potential for co-fermentation of DDGS with lignocellulosic feedstocks as swine feed

# The challenge for co-fermentation of DDGS and lignocellulosic feedstocks

Co-fermentation of DDGS and lignocellulosic feedstocks has become an attractive way to promote their use as non-ruminant feeds. However, to achieve this goal, there are still many challenges.

#### Degradation of fiber

Either from DDGS or lignocellulosic feedstock, indigestible fiber is one of the main factors affecting feed application. The research focus is to convert lignocellulose and fiber in DDGS into carbon sources that can be used by fermentation bacteria and eventually into nutrients that can be digested and absorbed by swine. Pretreatment is usually required for destructing LCCs, but there are still many challenges for pretreatment methods to be economical and efficient, due to the fact of high energy consumption in physical pretreatment, or production of byproducts and the risk to pollute environment in chemical pretreatment. Besides, there are great challenges in the process of fermentation, since cellulose degradation requires efficient production of cellulases, but its induction is not easy [61]. The challenge for degradation of fiber in the process of cofermentation comes from the following aspects.

#### The adverse effects of lignin for co-fermentation.

Lignin cannot be degraded by microbial as a fermentation feedstock, but it has a great impact on degradation efficiency [62]. During the pretreatment, phenolic inhibitors of microbial growth would be generated with the degradation of lignin [63]. Besides, lignin might deposit onto cellulose, making it difficult for cellulases to access

the cellulose surface, or even adsorbing cellulases, which inevitably compromises the effectiveness of the degradation of cellulose [64,65]. Therefore, to degrade lignocellulose and use it as a carbon source for DDGS fermentation, the adverse effects of lignin need to be eliminated first.

#### Transform cellulose into amorphous polymorph.

Cellulose is the most abundant carbohydrate component in lignocellulose [66]. Native cellulose is mainly classified as cellulose  $I_{\alpha}$  and  $I_{\beta}$  [67]. Cellulose  $I_{\alpha}$  seems to prevail in bacterial species and lower plants [68], and cellulose  $I_{\beta}$  is the main component of plant cell walls with higher plants for toughness and strength [69], which is more stable and recalcitrant to degradation. Therefore, harsh reaction conditions are required for transforming cellulose  $I_{\beta}$  in lignocellulosic feedstocks into an amorphous polymorph characterized by a lower crystallinity index for more efficient hydrolysis by cellulases [70].

The directional hydrolysis of hemicellulose. Hemicellulose is the second most abundant carbohydrate in lignocellulosic feedstocks [71]. Xyloglucan and heteroxylan consisting of monomer units such as glucose, xylose, galactose, arabinose for their backbones are major components of hemicelluloses in all plants [72]. Hemicelluloses are branched in nature through their side chains, forming an amorphous structure. Therefore, hemicelluloses can be hydrolyzed into monomer sugars during the pretreatment of lignocellulosic feedstocks [73]. Xylose and arabinose are the most abundant pentose sugar after hemicelluloses hydrolyzed. Pigs can metabolize xylose and arabinose, but with considerably lower efficiency than glucose [74,75]. Therefore, co-fermentation of DDGS and lignocellulosic feedstocks should also be regulated for these two monosaccharides. Besides, acetic acid is a major byproduct of the hydrolysis of hemicelluloses, which inhibits microbial growth [71]. Therefore, understanding the structures of hemicelluloses and the products and byproducts of their hydrolysis is a prerequisite for developing robust strains for cellulose degradation.

#### Improving the composition of amino acids after cofermentation

Digestible amino acids are the second most expensive nutrients in swine feeds, especially lysine, methionine, cysteine, and threonine. However, the digestibility of most amino acids in DDGS is about 10% [76]. The research focus is to convert DDGS protein into microbial protein and improve amino acid composition, as

well as digestibility via co-fermentation. The content of lysine in DDGS is relatively low compared with the requirement from the growth and development of swine, making it the first limiting amino acid in swine diets. Therefore, lysine content and digestibility are one of the main concerns when using co-fermented feed for swine [77]. Excessive leucine, relative to lysine, interferes with the utilization of isoleucine and valine, and may reduce feed intake and growth rate in swine. The microorganisms in the co-fermentation process may synthesize more proteins as they digest the original proteins, but directional accumulation of targeted amino acids and protein by controlling the fermentation conditions and microorganisms is still a challenge in the co-fermentation process.

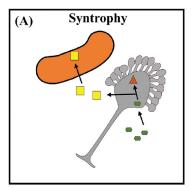
#### Producing standardized fermented feeds

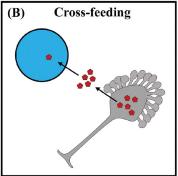
The content of all nutritional components in DDGS and lignocellulosic feedstocks can vary substantially among sources. Due to the disunity of raw material components, the addition ratio of DDGS and lignocellulosic feedstocks and the quality of final products are difficult to be standardized during co-fermentation. Therefore, reasonable fermentation conditions should be selected to adapt to diversified substrates in the co-fermentation process. Also, how to ensure the minimum value of the nutrition, energy and limiting amino acids of the feed can meet the standards of swine diets after fermentation is a very complex problem. Moreover, how to produce standardized feeds for the different nutritional requirement for different types of pigs must be considered.

#### Potential of using constructed microbial consortium to improve performance cofermentation process

Due to the inherent complexity and heterogeneity of DDGS and lignocellulosic feedstocks, efficient biodegradation and transformation requires the actions of different types of hydrolytic enzymes. In nature, complex microbial consortium, that work efficiently and often synergistically, accomplish degradation. Multiple microorganisms composed of bacteria and fungi are a parpromising method to improve ticularly fermentation performance [78]. Interactions between microbial communities is a complex web of interconnected metabolisms [79]. The metabolic potential of isolated microorganisms may not accurately reflect its ecological effects since its full metabolic responses may not be induced without other organisms [80].

There may be several types of co-culture relationships between the two microorganisms [81]. The most





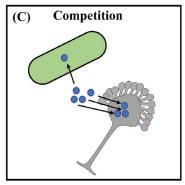


Figure 5. Nutritional interactions between microbes, adapted from Michiko E. Taga et al. and reproduced with permission [82]. (A) one strain consumes an intermediate or end product produced by a partner strain organism allows an otherwise energetically unfavorable reaction; (B) one strain affects the survival of another strain without being affected by it; (C) two strains compete for the same substances (i.e. nutritional divergence), over time, the faster-growing species will dominate.

common three types of nutritional interactions that control the metabolism between bacterial communities were shown in Figure 5. Colony maintenance and stability are essential for any co-culture fermentation application. Different microorganisms in the community must grow in the same environment (temperature, medium, pH, and oxygen), and the growth of one type of organism should not adversely affect others at least for a short period of time. In order to balance the different organisms in the same incubating condition, several methods can be used. First, the inoculation ratio of different microbial cultures must be optimized. Secondly, intermittent supplementation of vulnerable species can prolong co-culture cycle. By regularly adding the required cultures, the optimized microbial composition can sustain the whole fermentation process for a long time. Third, coexisting microbial species often competes for substrates but can avoid substrate competition by taking advantage of nutritional differences or homology (one species depends on the products of another).

These concepts have been widely applied to the utilization of mixed substrates or cascading biodegradation of refractory materials, such as co-fermentation of DDGS and lignocellulosic feedstocks. The degradation of lignocellulose can provide sufficient high-quality carbon source for DDGS fermentation. The co-culture can consume sugar mixtures more rapidly than single-culture methods and is highly resistant to changing sugar concentrations [83]. For cellulosic materials, xylose derived from hemicellulose components cannot be used by model yeast platforms. To solve this problem, co-cultures had been developed for E. coli and beer Saccharomyces cerevisiae fed on xylose, in which E. coli (which can naturally use xylose) produces acetate as a by-product. Saccharomyces cerevisiae consumed acetate and reduces its inhibition on E. coli growth [40].

Similarly, complexes with different engineering Saccharomyces cerevisiae strains can ferment glucosexylose-arabinose mixtures [84]. Trichoderma reesei can effectively degrade lignocellulose to produce a large amount of easy-to-use monosaccharides or oligosaccharides, which were consumed by Lactobacillus plantarum to produce high-quality feed under the condition of rich carbohydrates [85]. Meanwhile, when protein is abundant, Bacillus and Saccharomyces can effectively convert the protein from plant protein to microbial protein which is easily absorbed and digested by animals. Therefore, studying multiple microorganisms in co-fermentation process of DDGS and lignocellulosic feedstocks is fundamental for the establishment of an optimal biological degradation and transformation process.

#### Research progress on co-fermentation of DDGS and lignocellulosic feedstocks

The feasibility to co-ferment DDGS and lignocellulosic feedstocks has been confirmed by some preliminary studies. The result exhibited the advantages of the cofermented feed.

#### Increased digestible high-quality protein

Co-fermentation of DDGS and lignocellulosic feedstocks using Bacillus coagulans [86] increased high quality protein from 16.68% to 19.13%. Likewise, the inoculation with Aspergillus oryzae, Trichoderma reesei, and Phanerochaete chrysosporium showed an increase by 1.3-4.2% in high quality protein content [87]. The increase in crude protein may be attributed to the decline in dry matter content, as well as production of extra microbial protein during fermentation. Wang et al. [88] employed Bacillus subtilis and Lactobacillus

plantarum in the fermentation of the substrates consisting of 80% DDGS and 20% wheat bran. The results showed that the fermented materials contained greater low molecular weight peptides and total amino acids. Bacillus subtilis would also secrete protease to convert the indigestible protein into low molecular weight peptides that are easily digested and absorbed for swine.

#### Improved fat composition in feed

Energy supply is one of the most important functions for swine feed. The fat composition of DDGS is important, due to its contribution to ME and NE values. However, the current ethanol production process mostly implemented oil extraction technology, resulting in a great change in the proportion and type of fat content in DDGS, which greatly reduces the economic value of DDGS [29]. Yang et al. [89] using Mortierella alpina to ferment the DDGS and improve the fat content and composition. The results showed that Mortierella alpina produced total fatty acids of 182.34 mg/g dry substrate under optimal conditions. Co-fermentation of DDGS and lignocellulosic feedstocks effectively improved the fat composition and content of feed.

#### Converted fiber into easily absorbable carbohydrates

The indigestible fiber is one of the main factors affecting DDGS or lignocellulosic feedstocks application. During co-fermentation of DDGS and lignocellulose, cellulose and hemicellulose could be hydrolyzed into glucose, arabinose and xylose. Some of these products can be used as high-quality carbon sources for strains in the fermentation process, and some of them can be used as high-quality carbohydrates for energy supply to swine as co-fermentation feed. A study showed that solid-state fermentation using Bacillus coagulans reduced crude fiber content and increased soluble sugar content [86]. Howdeshell et al. [90] also obtained reducing saccharides from DDGS by concentrating and loosening the cellulose matrix through their activities, using solid-state fermentation. Two agro-industrial byproducts, soybean cotyledon fiber and DDGS, were used as substrates in co-fermentation with three different fungi, Aspergillus oryzae, Trichoderma reesei, and Phanerochaete chrysosporium, and the result showed that the fiber was degraded and converted into oligosaccharide that was easily digested and absorbed. The fiber content of the product was 3.5–15.1% lower after fermentation. Reduction in crude fiber indicated a potential improvement in feed quality [87]. In addition, the fiber content in DDGS significantly declined after

fermentation by Bacillus subtilis and Lactobacillus plantarum [88].

#### Increased other nutrients in the co-fermented feed

The microbes can modify the chemical or physicochemical properties of the DDGS and lignocellulosic feedstocks, as well as degrading them, by producing a wide range of enzymes. Other than enzymes, metabolites such as organic acids and bioactive substances are also produced, promoting the palatability of the feed. A wide variety of microorganisms have been used for the fermentation of DDGS and lignocellulosic feedstocks for nutritional enhancement. Wang et al. [86] fermented DDGS and lignocellulosic feedstocks using *Bacillus* coagulans, resulting in increased production of the total acid from 0.80 to 1.50%, and the decrease of pH from 6.03 to 4.54. The feed after fermentation with Lactobacillus, which increased the content of lactic acid, had improved feed flavor, feed palatability, and the feed intake of pigs. Furthermore, the number of probiotics in the fermented feed increased, which could benefit intestinal health. Aspergillus has the ability to produce enzymes such as cellulase, phytase, amyloglucosidase, hemicellulase, pectinase, protease, amylase, lipase, tannase [91,92] and phytase [93], some of which have the capacity to degrade fiber content during fermentation. Aspergillus oryzae and Aspergillus niger are the two most commonly used fungi, and Aspergillus niger and Aspergillus oryzae secreted oxalic acid and citric acid, respectively, in the fermentation process [94]. Thus, co-fermentation may lead to better nutrient composition in fermented feed.

Altogether, these findings indicated that co-fermentation of DDGS and lignocellulosic feedstocks is a feasible process mainly controlled by microbial enzyme capability, not only increasing protein content and reducing fiber content, but also improving the nutritional quality, creating a path for further research work on co-fermented DDGS and lignocellulosic feedstocks as swine feed.

#### Effects and application of co-fermented feed in swine diets

#### Effects on intestinal microflora of swine

The gastrointestinal tract of pigs is composed of a complex and diverse group of microbes that plays a vital role in the development of the immune system and prevention of the host from infection [95]. It is well known that gastrointestinal tract health is largely affected by the composition of the microbiota and various end-products from bacterial metabolism [96]. The

gut microbiota is a complex system that is increasingly being considered as a factor that shapes host metabolism and health [97].

In pig nutrition, fermented feed improves gut microbial ecosystems by lowering the population of Enterobacteria such as coliform bacteria and salmonella in the gut [98]. Beneficial microbial population also produces short chain fatty acids and thus reduces intestinal pH. A low-pH environment can also mitigate the mold production in DDGS. According to the nature of each organic acid, the specificity of antimicrobial activity is also different. Lactic acid is mainly effective against bacteria, while acetic acid is effective against bacteria, some yeast, and some molds. Propionic acid is primarily effective against molds [99].

Different DDGS fermented feeds have different effects on intestinal microorganisms of pigs. One study showed that fermented DDGS feed inhibited the growth of potential pathogens, including Escherichia coli, Dialister and unclassified Enterobacteriaceae, but promoted the growth of several beneficial bacteria, including Lactobacillus, Bifidobacterium and Roseburia [86]. Pigs fed with fermented DDGS with Bacillus coagulans showed that the counts of enterobacteria fell from  $1.00 \times 10^4$  to  $7.70 \times 10^2$  cfu  $g^{-1}$ , while the mold was completely eliminated from  $3.40 \times 10^2$  cfu  $q^{-1}$  [86]. Importantly, Bacillus coagulans formed spores, so that the probiotics have strong resistance to the technological stresses imposed during production and storage processes, and to gastric and intestinal environments [100]. Another study showed that the feed made up with 80% of DDGS and 20% of wheat bran fermented with Bacillus subtilis and Lactobacillus plantarum could enhance proliferation of all probiotics in swine intestines to 108 cfu  $g^{-1}$ , whereas pathogens such as enterobacterium and molds declined to undetected level [88]. Olstorpe et al. [101] also found that the growth of enterobacteria and molds in swine intestines was inhibited after being fed with fermentation feeds. Feeds fermented with Lactobacillus zeae or Lactobacillus casei reduced the numbers of Salmonella in the spleen in the intestine of Salmonella challenged piglets [102].

Dietary components can also change the composition of swine intestinal microbiota. The porcine intestinal microbiota was formed by dietary carbohydrate composition due to specific substrate preferences of bacteria [103]. Research had shown that pigs fed diets containing wheat middling steeped with xylanase improved villus height. Addition of arabinoxylan and mixed linkage glucans in swine diets had great effect on influencing bacterial community profiles, by reducing those bacteria considered to be detrimental to

human health, while promoting beneficial bacteria [104,105]. During co-fermentation of DDGS and lignocellulosic feedstocks, hydrolysis of hemicellulose can produce arabinose and glucans. From this perspective, cofermentation of DDGS and lignocellulosic feedstocks is a meaningful research. Therefore, nutritional strategies to regulate the intestinal microbial ecosystem appear to be beneficial methods to improve intestinal health.

#### Effects on performance of swine

Many studies have reported the beneficial effects of dietary inclusion of fermented DDGS and lignocellulosic feedstocks on performance of swine [106]. Fermented DDGS and lignocellulosic feedstocks have higher digestibility so that their inclusion rates in swine diets could be enhanced. Feeding trials using DDGS treated with Bacillus coagulans showed increased average daily gain of pigs and significantly decreased average daily feed intake and the ratio of feed/gain [86]. Jakobsen et al. [107] fed pigs with fermented DDGS, which led to significantly increased apparent ileal digestibility of non-starch polysaccharides (NSP), and apparent total tract digestibility (ATTD) of dry matter, crude protein, and phosphorus, along with a tendency to increase ATTD of NSP. In their study, the digestibility of the fermented DDGS in pigs was increased.

During fermentation of DDGS and wheat bran with Bacillus subtilis and Lactobacillus plantarum, all the determined enzymes were significantly improved after inoculation. Remarkably, xylanase and neutral protease were the most secreted enzymes detected [88]. Enzyme activity directly affects the quality of fermented feed and the digestibility of nutrients after feeding animals. The digestibility of dry matter, crude protein and gross energy significantly improved. In addition, the digestibility of 9 animo acids, including 6 essential amino acids and 3 nonessential amino acids, improved greatly. Therefore, co-fermentation feed of DDGS and lignocellulosic feedstocks is beneficial to digestion.

In conclusion, co-fermentation is a low-cost bioprocessing technique for improving the nutrient quality of DDGS and lignocellulosic feedstocks. It is feasible to produce fermented feed for swine with implementing the concept of probiotics and functional aspects of microbial products. The dietary inclusion of the aforementioned fermented feed can improve growth performance and nutrient digestibility of swine.

#### **Future perspectives**

Although previous research has shown positive results of using co-fermentation of DDGS and lignocellulosic feedstocks in swine nutrition, more research and studies on feeding value especially nutrient digestibility, intestinal microflora, and intestinal morphology are needed. The fermentation of feed ingredients is a complex process that requires careful monitoring and optimization. The physical properties of solid substrate such as surface area and texture are important parameters in the process of solid-state fermentation and should be further investigated to improve the physical properties of solid substrate that are favorable for obtaining more digestible fermentation products. The choice of appropriate fermenting microbes is the core factor in affecting fermentation process. The most suitable strain for solid-state fermentation is filamentous fungus. The selection of strains largely depends on the composition of the substrate used in fermentation. For DDGS and lignocellulosic feedstocks, the fungi selected from nature should have an outstanding ability in lignocellulose degradation. In addition, the optimum combination of fermentation conditions is vitally important, such as initial moisture content, pH, incubation time, mixing ratio of fermenting strains, and fermentation temperature. Based on this information, the solid-state fermentation of DDGS and lignocellulosic feedstocks can achieve optimal results.

Development of lignocellulosic bioethanol is hindered by the high cost, and current commercially available bioethanol is mostly produced from corn. It is of utmost importance to establish systems of large-scale co-fermentation process and accurate feed quality evaluation of DDGS and lignocellulosic feedstocks. The realization of stable heat and mass transfer and heat dissipation in the fermentation system is of irreplaceable significance for industrial scale-up. It is notable that if a breakthrough is made in this co-fermentation strategy, 700 million tons of corns currently used as animal feed could be saved for bioethanol production, and its current annual production may be tripled. Co-fermentation of the byproduct DDGS and lignocellulosic feedstocks as feed can make a significant contribution to meet the gap caused by the surge demand in swine feed and renewable transportation fuel. In addition to this, improving feed efficiency by accurately determining animal nutrient requirements, optimizing diet formulation will help decrease the absolute quantity of feed required. Furthermore, how to effectively store, transport, and sell the fermented feed is also an important question. The dried fermented feed may be a good way to supply farmers. Finally, this co-fermentation strategy will contribute to competitiveness for the swine industry in order to meet the globally increasing demand in a sustainable way.

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