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Review Article

Research progress on cottonseed meal as a protein source in pig nutrition: An updated review

An Tao [a, b], Jiahao Wang [a, b], Bin Luo [a, b], Bowen Liu [a, b], Zirui Wang [a, b], Xingping Chen [a, b], Tiande Zou [a, b], Jun Chen [a, b, *], Jinming You [a, b, *]

[a] *Jiangxi Province Key Laboratory of Animal Nutrition, Jiangxi Agricultural University, Nanchang 330045, China*

[b] *Jiangxi Province Key Innovation Center of Integration in Production and Education for High-Quality and Safe Livestock and Poultry, Jiangxi Agricultural University, Nanchang 330045, China*

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ABSTRACT

At a global level, the supply of protein sources is insufficient to support the current magnitude of pig production. Moreover, given the exorbitant expense of conventional protein feed options like soybean meal and fish meal, it becomes imperative to promptly explore alternative sources of protein feed for the sustainable advancement of the pig industry. Cottonseed meal, a by-product from the extraction of cottonseed oil, exhibits significant potential as a protein source for pig feed owing to its high protein content, high yield, low cost, well-balanced amino acid composition, and sufficient accessibility. However, cottonseed meal possesses several anti-nutritional factors, especially gossypol, which adversely affect growth and reproductive performance, resulting in the limited utilization of cottonseed meal in pig feed. To maximize the benefits of cottonseed meal and promote its application in pig production, it is imperative to acquire comprehensive knowledge regarding its nutritional value and current utilization. In this review, we initially presented a summary of the nutritional values of cottonseed meal, primary anti-nutritional factors, and effective approaches for improving its utilization as a protein source feed. Subsequently, we comprehensively summarized the latest research progress of cottonseed meal application in pig nutrition over the past decade. The outcome of this review serves as a theoretical foundation and practical guidance for the research and application of cottonseed meal in pig nutrition and promotes the reduction of soybean meal utilization in the pig industry.

1. Introduction

The amplifying global consumer demand for foods rich in protein is driven by the rise in populations, urbanization, and the aging of societies. The main sources of dietary protein for humans are typically derived from animal-based products, namely meat, eggs, and dairy products (Kumar et al., 2022b). It is estimated that the global population will reach 9.6 billion by the year 2050 (Wu et al., 2014). This will result in a substantial increase in the demand for food, with a particular focus on meat, which is projected to rise by 73%, reaching a total of 455 million tonnes (Kumar et al., 2022b). Importantly, pork is considered to be the most widely consumed type of meat globally, encompassing 36% of the total meat consumption worldwide (Sanchez et al., 2022). More importantly, China is the largest pig-producing and pork-consuming country in the world (Hu et al., 2023). In 2023, the annual pig slaughter accounted for 726.62 million pigs, while the annual pork production reached an impressive 57.94 million tonnes in China (Ministry of Agriculture and Rural Affairs of the People's Republic of China, 2023). Pork is the primary meat consumed by Chinese residents, and the pig industry has progressively emerged as a crucial backbone of the national economy in China (Fan et al., 2023). The increase in meat consumption necessitates larger amounts of protein feed for sustaining animal production levels, particularly considering the growing competition between energy feed for livestock

* Corresponding authors.
E-mail addresses: junchen@jxau.edu.cn (J. Chen), youjinm@jxau.edu.cn (J. You).
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and food supply for humans (Khan et al., 2016). Soybean meal and fish meal are the protein sources most frequently utilized in the conventional feed of pig production (Kim et al., 2019). Nevertheless, the currently prevailing utilization of soybean meal and fishmeal is unsustainable due to environmental factors and escalating costs (Veldkamp and Bosch, 2015). In order to decrease the reliance of farmers on conventional protein sources such as soybean meal and fish meal, as well as to address the scarcity of animal feed and potentially reduce costs, the use of alternative feed sources may serve as a viable and sustainable solution to provide protein sources in pig feed (Mottet et al., 2017). The main alternative protein sources in pig feed currently available include by-products from oil and biofuel production, such as rapeseed meal (He et al., 2023), leguminous plants like fava beans (Grabež et al., 2020), as well as emerging potential protein sources including insect proteins (Yu et al., 2019), microbial proteins (Sobhi et al., 2023), and microalgae (Saadaoui et al., 2021), etc. However, cottonseed meal is often underestimated as a potential alternative protein source compared to other alternatives.

Cottonseed is commonly distributed in many temperate and tropical countries and is recognized as being one of the most abundant sources of oilseeds. Its primary utilization lies in the extraction of oil for edible oil purposes (Zhou et al., 2015). According to Xie et al. (2023), in the 2021/2022 period, global cotton production was projected to reach a significant 43.8 million tonnes, which holds great significance as cottonseed serves as a crucial non-food component. In China, there exists an annual cottonseed production of approximately 10 million tonnes, which has progressively grown over time (Gao et al., 2010; Song et al., 2020). Cottonseed, commonly known as “white gold”, is a type of oilseed that is cultivated on a large scale due to its fibers (Kadam et al., 2021). In addition to fibers, cottonseed also consists of valuable by-products such as cotton husk (22%), linters (7%), cottonseed oil (16%), and cottonseed meal (50%) (Hinze et al., 2015). Cottonseed meal is abundant in crude protein and has balanced amino acids profile, and has been considered as one of the most promising sources for plant proteins (Kumar et al., 2021b). Cottonseed meal has the potential to produce around 10 million metric tons of protein. This amount has the potential to meet the annual protein requirements of over 500 million individuals on a global scale (Wedegaertner and Rathore, 2015). Furthermore, following additional processing, cottonseed meal can acquire protein flour with a protein content of approximately 50%, protein concentrate with a protein content of roughly 70%, and protein isolate with a protein content of about 90% (Bertrand et al., 2005; Martinez et al., 1970). Nevertheless, the inclusion of gossypol in cottonseed meal significantly restricts its utilization in pig feed. Gossypol, classified as an anti-nutritional factor, has the potential to induce detrimental consequences on the growth and reproductive performance of pigs (Haschek et al., 1989; Mudarra et al., 2021).

Fortunately, if the content of free gossypol in cottonseed meal is reduced to a safe point, it is feasible as a protein source in pig feed. The Food and Drug Administration of the United States, along with the World Health Organization, has established a maximum threshold value of 450 mg/kg for free gossypol content in cottonseed meal protein products, deemed safe for usage in non-ruminant animals (Ma et al., 2018b). In pursuit of this objective, researchers have extensively studied the extraction of gossypol from cottonseed meal and developed various methods, including microbial fermentation (Khalaf and Meleigy, 2008), radiation (Bahraini et al., 2017), supercritical CO_2 extraction (Bhattacharjee et al., 2007), and solvent extraction (Pelitire et al., 2014). These efforts have yielded promising outcomes, as gossypol degradation has been achieved alongside an enhancement in protein extraction efficiency. However, there is still a need for further review regarding the utilization of cottonseed meal as a source of protein in pig nutrition. In this review, we will commence by providing an overview of the nutritional values of cottonseed meal, primary antinutritional factors, and efficacious strategies for enhancing its utilization as a protein source feed. Subsequently, the latest research progress in the application of cottonseed meal and its products after removing gossypol in pig nutrition over the past decade (2014 to 2023) will be comprehensively summarized (Fig. 1). This review holds significant implications for facilitating the reduction and substitution of soybean meal in the pig industry, and supporting the sustainable development of pig production.

2. Nutritional value

2.1. Nutritional profiles

The protein content within cottonseed meal varies from 40.67% to 59.67% as feed basis, as indicated in Table 1. According to Kumar et al. (2021b), the cottonseed protein component exhibited the highest content of salt-soluble protein (globulins: 33% to 63.7%), followed by water-soluble protein (albumins: 20.8% to 32.2%) and alkali-soluble protein (glutelins: 9.2% to 28%). The significant variation in nutritional levels of cottonseed meal, particularly in crude protein, arises from disparities in cotton varieties, growth conditions, and processing methods among different sources of cottonseed meal. In a study conducted by Ma et al. (2019a), an analysis was undertaken to assess the chemical composition of 12 different types of cottonseed meal. The findings unveiled that the crude protein content of these 12 types of cottonseed meal varied between 39.12% and 57.52%, exhibiting a coefficient of variation of 10.45% (Ma et al., 2019a). Likewise, Zhuo et al. (2023b) found that the crude protein content in 6 types of cottonseed meal exhibited a range of 40.67% to 59.63%, accompanied by a coefficient of variation exceeding 10%. Additionally, due to progressions in processing technology, the protein content of cottonseed protein derived from different dephenolization techniques of cottonseed meal has experienced noteworthy improvement. For example, degossypolized cottonseed protein is typically produced through the solvent extraction of water-soluble carbohydrates and free gossypol from cottonseed meal (Wang et al., 2019). Its crude protein content reaches as high as 66.26%, which is comparable to that of fish meal.

In terms of amino acid composition, arginine is notably abundant among the essential amino acids within cottonseed meal, whereas glutamic acid stands out as the most abundant nonessential amino acid. Nevertheless, the combination of gossypol with the epsilon amino group of lysine during the process of oil extraction leads to a decrease in the bioavailability and nutritional value of lysine (Nagalakshmi et al., 2007). As a result, it is crucial to appropriately supplement lysine in a diet based on cottonseed meal (Fombad and Bryant, 2004). The crude protein and amino acid content, as well as the apparent ileal digestibility (AID) and apparent standard ileal digestibility (SID), of cottonseed meal and its processed products, are summarized in Table 2.

Furthermore, the crude fiber content of cottonseed meal is comparatively elevated; however, it exhibits a significant reduction following the application of dephenolization treatment. In recent years, there has been a significant focus on crude fiber owing to its functional significance in improving the intestinal health of monogastric animals (Grześkowiak et al., 2022). Improving intestinal health is of great significance in improving feed efficiency, promoting growth performance, and maintaining overall health in monogastric animals (Duarte and Kim, 2022). As shown in Table 1, the ether extract content of cottonseed meal was comparatively lower than that of soybean meal and fish meal, and this disparity could be attributed to the extraction of a greater amount of oil from

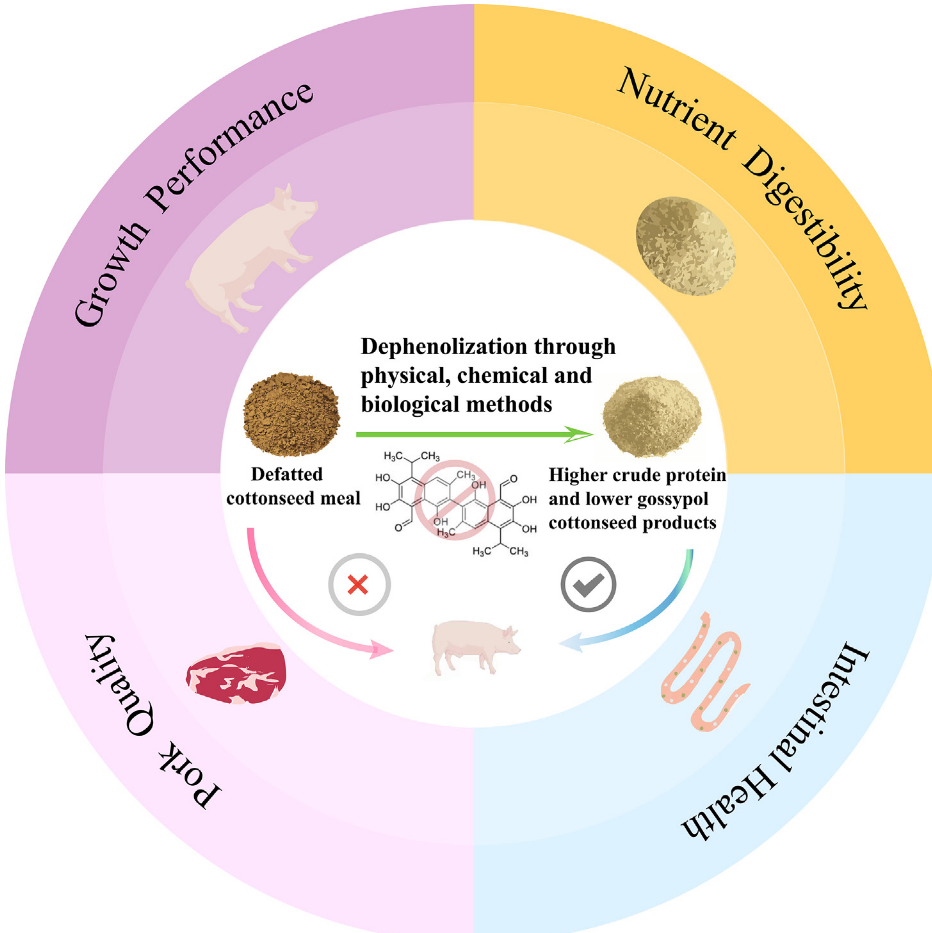

Fig. 1. The overview diagram of the applications of cottonseed meal in pig nutrition. Defatted cottonseed meal is not recommended for direct utilization in pig feed due to its high gossypol levels. The process of degossypolisation of cottonseed meal through physical, chemical, and biological methods effectively reduces the gossypol content and enhances its nutritional value, and thus facilitates the application of cottonseed meal in pig nutrition.

Table 1
Nutritional composition of cottonseed meal in comparison with common protein feed sources (as-fed basis,%).

Item	DM	CP	EE	CF	Ash	NDF	ADF	Starch	Ca	TP	References
Cottonseed meal	87.36 to 95.25	40.67 to 59.63	0.85 to 2.00	3.23 to 15.92	5.81 to 7.09	5.37 to 28.76	1.62 to 17.94	NA	0.20 to 0.24	1.01 to 1.35	Zhuo et al. (2023a)
Fermented cottonseed meal	92.80	49.70	1.40	10.80	6.75	25.40	12.80	NA	0.28	1.00	Ma et al. (2018a)
Concentrated degossypolized cottonseed protein	92.86	59.89	5.03	2.42	7.47	14.67	5.23	0.79	0.31	0.91	Li et al. (2019c)
Degossypolized cottonseed protein	90.25	66.26	2.19	NA	NA	20.48	5.52	NA	NA	NA	Wang et al. (2019)
Fish meal	90.34	66.77	9.07	NA	15.13	NA	NA	NA	3.59	3.30	Li et al. (2019c)
Soybean meal	86.80	42.80	2.10	4.40	5.50	NA	NA	NA	0.23	0.72	Yan et al. (2022)

CF = crude fiber; ADF = acid detergent fiber; Ca = calcium; CF = crude fiber; CP = crude protein; DM = dry matter; EE = ether extract; NDF = neutral detergent fiber; TP = total phosphorus; NA = not available.

cottonseed during the processing phase (Ma et al., 2019a). Additionally, cottonseed meal displays a relatively rich vitamin content, including riboflavin, folate, and vitamin E, while lacking niacin (Chen et al., 2019). It should be noted that cottonseed meal contains less calcium and a higher concentration of phosphorus, specifically phytate phosphorus, resulting in a relatively low digestibility of phosphorus (Thirumalaisamy et al., 2016).

2.2. Available energy value

Cottonseed meal can be used as a plant-based protein source in pig diets, serving as a feasible substitute for both soybean meal and fish meal. It is imperative to comprehend the available energy value in order to formulate feed appropriately (Hu et al., 2023). In recent years, extensive research has been conducted by scholars to assess the available energy value of cottonseed meal and its derivatives when used as feed for pigs. Wang et al. (2019) evaluated the available energy value of degossypolized cottonseed protein for nursery pigs. The authors found that the digestible energy and metabolizable energy values, based on a dry matter basis, were 19.11 and 17.77 MJ/kg, respectively (Wang et al., 2019). In another study, Ma et al. (2019a) assessed the available energy value of 12 types of cottonseed meal for growing pigs. The researchers discovered that the average values of digestible energy and

Table 2
Contents of crude protein and amino acids of cottonseed meal and AID or SID of amino acids for pigs (%).

Item	Concentrated degossypolized cottonseed protein[1]			Fermented cottonseed meal[2]			Cottonseed meal[3,4]			Cottonseed meal[2]		
	Content	AID	SID	Content	AID	SID	Content	AID	SID	Content	AID	SID
Animals												
Crude protein	59.89	79.9	89.8	49.7	84.2	94.0	49.62	79.25	85.38	41.8	NA	NA
Essential amino acids												
Arginine	7.20	93.6	97.5	4.54	91.7	97.6	5.93	91.38	92.94	4.52	76.4	82.6
Histidine	2.76	90.5	94.2	1.41	90.0	93.6	1.34	83.51	86.91	1.10	58.8	64.3
Isoleucine	1.99	85.0	92.5	1.35	83.0	87.9	1.39	74.69	79.87	1.40	40.1	46.9
Leucine	3.44	85.9	93.5	2.61	84.2	90.2	2.75	76.86	82.15	2.52	41.9	48.5
Lysine	3.40	86.4	91.3	1.92	79.5	85.5	2.16	66.45	70.91	1.93	23.9	33.1
Methionine	0.67	92.3	98.6	0.58	84.7	89.6	0.44	77.90	80.88	0.67	41.1	44.6
Phenylalanine	3.44	90.2	93.9	2.33	91.7	94.6	2.60	84.77	88.00	2.18	60.1	66.1
Threonine	2.11	83.6	92.5	1.57	80.4	89.2	1.51	70.18	77.86	1.40	25.6	37.2
Tryptophan	0.59	81.6	86.5	0.52	83.7	90.5	0.50	63.90	74.50	0.30	68.8	77.5
Valine	2.54	84.8	93.2	1.90	81.4	90.2	1.99	77.68	82.31	1.94	34.4	44.4
Non-essential amino acids												
Alanine	2.33	77.8	88.6	1.91	80.5	93.8	1.78	70.88	77.96	1.69	37.8	48.8
Aspartic acid	5.29	89.5	95.9	4.31	84.0	88.8	4.35	80.07	84.25	3.80	50.1	57.7
Cysteine	1.43	90.4	96.5	0.69	81.1	88.7	0.73	76.35	81.88	0.66	40.0	47.0
Glutamic	11.90	93.5	97.3	8.85	89.5	92.0	10.10	88.59	90.68	8.06	69.0	73.1
Glycine	2.38	74.8	100.7	1.87	75.1	106.3	1.92	69.12	82.17	1.77	30.3	53.8
Proline	2.64	71.2	164.7	2.23	68.4	157.6	1.87	68.28	90.08	1.63	26.0	101.0
Serine	2.76	88.2	95.6	1.90	83.6	91.6	2.08	78.17	83.36	1.76	42.9	54.0
Tyrosine	1.97	86.6	92.8	1.20	88.1	93.2	1.40	73.88	80.53	1.15	53.4	62.0
References	Li et al. (2019d)			Ma et al. (2018a)			Zhuo et al. (2023b)			Cotten et al. (2016)		

AID = apparent ileal digestibility; SID = standard ileal digestibility; NA = not available.
[1] Nursery pigs.
[2] Growing pigs.
[3] Average values.
[4] Gestating sows.

metabolizable energy on dry matter basis were 11.62 and 10.68 MJ/kg, respectively (Ma et al., 2019a). However, these values were observed to be lower than what was previously reported by the National Research Council (NRC, 2012). The lower values of digestible energy and metabolizable energy could be attributed to a reduced content of ether extract or fiber in cottonseed meal (Bell, 1993; Ma et al., 2019a). Zhuo et al. (2023a) examined the digestible energy and metabolizable energy values of 6 different types of cottonseed meal in pregnant sows and non-pregnant sows. The variation range of digestible energy and metabolizable energy values based on dry matter was 12.48 to 17.15 MJ/kg and 11.35 to 15.88 MJ/kg, for non-pregnant sows, respectively, and from 12.86 to 16.41 MJ/kg and 12.43 to 14.72 MJ/kg, for gestating sows, respectively (Zhuo et al., 2023a). Furthermore, the author observed that there was a negative correlation between digestible energy and neutral detergent fiber, as well as a negative correlation between metabolizable energy and acid detergent fiber. Conversely, there was a positive correlation between both digestible energy and metabolizable energy and the level of crude protein. This suggests that cottonseed meal with higher crude protein content and lower fiber content exhibits a higher level of digestible energy and metabolizable energy (Zhuo et al., 2023a). Table 3 presents the values for the digestible energy and metabolizable energy of pigs when consuming cottonseed meal and its processed products. These findings suggest that cottonseed meal holds promising potential as an exemplary protein resource for pigs.

3. Anti-nutritional factor

3.1. Gossypol

Gossypol is a polyphenolic compound present in the stems, leaves, and flower buds of cotton, and it is particularly abundant in the seeds of cotton plants. The chemical structural formula of gossypol is 2,2′-bis (8-formyl-1,6,7-trihydroxy-5-isopropyl-3-methylnaphthalene) (Fig. 2) (Liu et al., 2022). It can be categorized into bound gossypol and free gossypol based on its form of existence. Most bound gossypol is not readily absorbed by the gastrointestinal system and is less toxic to animals. Bound gossypol is metabolized via the processes of defecation, urine excretion, and lactic acid conversion. However, the bound gossypol can undergo a transformation from its bound state to a free state within animals. Subsequently, it can infiltrate the bloodstream of the host animals via the mucosa, leading to deleterious effects (Liu et al., 2022; Noftsger et al., 2000). In general, the content of free gossypol found in cottonseed typically ranges from 0.02% to 6.64% (Price et al., 1993). However, this range can be subject to variability due to diverse cotton varieties, climatic factors, and soil conditions (Wang et al., 2021c). The presence of free gossypol poses greater harm and has the potential to induce toxicity. In cases where the concentration of gossypol in the feed surpasses a particular threshold, it can lead to poisoning (Zhu et al., 2019). It has been reported that the long-term feeding of gossypol may lead to loss of electrical rhythm in pigs, consequently resulting in heart failure (Albrecht et al., 1968). A study conducted in Illinois on a pig herd found that supplementing cottonseed meal with a high concentration of free gossypol resulted in a mortality rate of 50% among the 300 growing and finishing pigs. Additionally, within 4 to 6 weeks, 20% of the pigs fell ill (Haschek et al., 1989). Similarly, Basini et al. (2009) also noted that 5 or 25 μg/mL gossypol significantly inhibited pig granulosa cell estradiol 17β and progesterone production. Furthermore, free gossypol affects the function of various enzymes, alters the properties of cell membranes (Dodou et al., 2005), and interferes with the bioavailability of mineral elements (Paim et al., 2016). Taken together, these findings highlight that gossypol is a serious threat to the growth and health of pigs. Consequently, it is imperative to reduce the gossypol content in cottonseed meal to a safe consumable level prior to utilizing it in pig feed.

Table 3
Available energy values of cottonseed meal and its processed products (as dry matter basis).

Item	Animal	Digestible energy, MJ/kg	Metabolizable energy, MJ/kg	References
Cottonseed meal	Pigs	12.18	11.07	NRC (2012)
Degossypolized cottonseed protein	Nursery pigs	19.11	17.77	Wang et al. (2019)
Concentrated degossypolized cottonseed protein	Nursery pigs	15.79	14.68	Li et al. (2019d)
Cottonseed meal[1]	Growing pigs	11.62	10.68	Ma et al. (2019a)
Fermented cottonseed meal	Growing pigs	12.72	12.24	Ma et al. (2018a)
Cottonseed protein (50% crude protein)	Growing pigs	13.19	12.86	Ma et al. (2018a)
Cottonseed protein (55% crude protein)	Growing pigs	15.49	14.83	Ma et al. (2018a)
Cottonseed meal[1]	Gestating sows	14.40	14.24	Zhuo et al. (2023a)
Cottonseed meal[1]	Non-pregnant sows	14.52	13.19	Zhuo et al. (2023a)

[1] Average values.

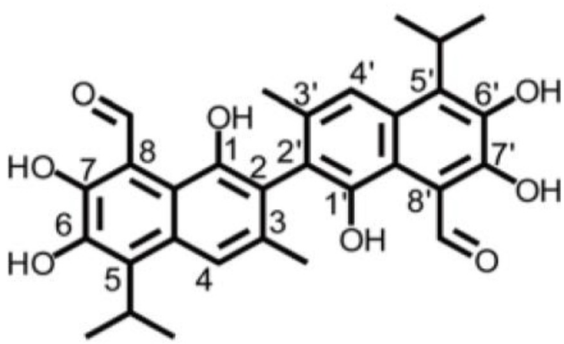

Fig. 2. The chemical structure of gossypol (Liu et al., 2022).

3.2. Cyclopropenoid fatty acids

There are three major cyclopropenoid fatty acids in cotton, namely dihydrosterculic, malvalic, and sterculic acids (Obert et al., 2007). The presence of cyclopropenoid fatty acids in cotton seeds has been demonstrated to have deleterious effects on the well-being of susceptible animals. For instance, Swiss Webster mice fed a diet containing 0.5% cyclopropenoid fatty acids for 6 weeks experienced reduced growth rate, hypercholesterolemia, and elevated concentrations of serum phospholipid and free cholesterol (Matlock et al., 1985). It has been also found that dietary supplementation with 4% cottonseed oil (contains cyclopropenoid fatty acids) increased the concentrations of hepatic cholesterol, serum cholesterol, and low-density lipoprotein-cholesterol in Hy-Line Brown laying hens (Yang et al., 2021). However, there is no literature available regarding the effects of cyclopropenoid fatty acids on pigs, and further researches are warranted to evaluate the potential effects.

3.3. Phytic acid

The typical phytic acid content found in cottonseed meal ranges from 3% to 5%. Although its exact impact animal nutrition remains uncertain, it is widely acknowledged as an anti-nutritional factor (Khajali and Slominski, 2012). Phytic acid has the ability to form insoluble phytate by chelating with divalent or multivalent metal ions (such as zinc, calcium, copper, magnesium, manganese, and iron), thereby reducing the bioavailability of some essential mineral elements. Due to its chelation properties, phytic acid is regarded as the main factor leading to the scarcity of mineral ions in animal nutrition (Samtiya et al., 2020). Phytic acid also could decrease nutrient digestion and absorption in animals through its impact on the functionality of digestive enzymes, such as protease, amylase, and lipase. In addition, it can combine with proteins to form insoluble complexes, which greatly reduces the bioavailability and digestibility of proteins (Ravindran et al., 2000). Phytic acid has relatively stable chemical properties and cannot be degraded by physical methods such as heating. Animals lack an enzyme capable of both digesting and breaking it down. Consequently, the current practice entails utilizing exogenous phytase to facilitate the decomposition of phytic acid. Overall, the presence of anti-nutritional factors greatly restricts the utilization of cottonseed meal in pig diets.

4. Improvement approaches for cottonseed meal utilization as a protein source

4.1. Physical methods

Physical methods such as the liquid cyclone process and the air fractionation process can be employed to eliminate glands containing gossypol from cottonseeds (Decossas et al., 1982; Smith, 1971). Extensive research was conducted in the late 1970s and early 1980s on the physical separation of gossypol from pigmented glands. Several patents have been acquired through physical and mechanical processing for the production of edible cottonseed meal (Rathore et al., 2020). The liquid cyclone process can generate edible cottonseed powder which contains a maximum of 0.04% free gossypol content and a protein content exceeding 65% (Gardner et al., 1976). On July 13, 1972, the Food and Drug Administration approved the usage of liquid cyclone process for the manufacturing of cottonseed meal as a food additive. This was succeeded by the production of dehaired, high-protein edible cottonseed meal at an oil plant located in LaBurke, Texas. The air fractionation process was developed as an advancement of the liquid cyclone process technology (Kumar et al., 2021b). Decossas et al. (1982) found that the air fractionation process is considerably more advanced than liquid cyclone process technology and has obvious advantages, consequently rendering it financially viable. Nevertheless, as a consequence of the inadequacy of business practices of the liquid cyclone process, the cottonseed industry has come under intense scrutiny, leading to the reluctance of the air fractionation process to undertake commercial endeavors on a substantial level in the United States (Decossas et al., 1982). Duodu et al. (2018) found that the utilization of soaking and autoclaving techniques resulted in a reduction of gossypol levels by 34.48% and 27.59%, respectively, while also significantly decreasing the content of phytic acid. Recently, owing to advancements in science and technology, the utilization of gamma rays and electron beams has emerged as notable aspects of observation in society. Moreover, they have found practical applications in the degradation of anti-nutritional factors in plant protein feed (Kadam et al., 2021). Dabbour et al. (2023) showed that the implementation of a response surface methodology proved effective in optimizing the ultrasonic-aided procedure. This optimization was achieved under the conditions of 53 °C, 320 W, 21 min, and a concentration of 4.6 g NaOH/L. As a result of this optimization, the removal rates of free gossypol, total gossypol, and the extractability of cottonseed protein were recorded at 93.21%, 87.56%, and 58.23%, respectively (Dabbour et al., 2023). Bahraini et al. (2017) investigated the effects of electron beam and gamma-ray irradiation with doses of 10, 20, and 30 kGy on the chemical composition, protein quality, and protein digestibility of cottonseed meal. The findings indicated that the protein digestibility of cottonseed meal experienced a significant increase when exposed to gamma ray irradiation at levels below or equal to 30 kGy. Furthermore, the irradiation methods of electron beam and gamma ray resulted in a decrease in the levels of gossypol and crude fiber, while simultaneously enhancing the protein digestibility of cottonseed meal. However, no discernible impact on the protein quality of cottonseed meal was observed (Bahraini et al., 2017).

4.2. Chemical methods

The chemical method is a more appropriate approach compared to the physical method when it comes to eliminating gossypol from cottonseed meal for use as feed for non-ruminants and as a nutritional supplement for humans (Rathore et al., 2020). Since gossypol has solubility in polar solvents, solvent extraction is the most frequently utilized method to remove gossypol (Pelitire et al., 2014). Given the assortment of solvents available, the extraction process presents a wider range of options regarding solvent safety, volatility, cost, and other factors (Byrne et al., 2016). The solvents acetone and ethanol are deemed to be ideal for the extraction of gossypol. These solvents are widely acknowledged as safe, and classified as generally recognized as safe (GRAS) solvents (Kumar et al., 2019, 2021b). Hron et al. (1996) utilized a 95% ethanol solution as a solvent to significantly reduce the total gossypol content present in cottonseed meal, achieving a reduction of approximately 70%. Pelitire et al. (2014) employed either an acetone or ethanol solution in the presence of phosphoric acid to extract gossypol from cottonseed meal. Both solvents effectively reduced the total gossypol level in the meal by 90% to 95%, and increased protein levels. However, it was observed that the extraction process with ethanol exhibited a higher speed compared to acetone (Pelitire et al., 2014). Additionally, gossypol can form strong bonds with iron ions, thus resulting in a significant decline in its bioavailability upon binding (Rathore et al., 2020). Supplementing ferrous sulfate to diets containing cottonseed meal can effectively eliminate the negative effects associated with gossypol (Gaber et al., 2012). Nagalakshmi et al. (2002) found that the addition of 0.5%, 1%, and 2% calcium hydroxide to cottonseed meal resulted in a reduction of free gossypol by 21.25%, 28.15%, and 40.52%, respectively. However, the utilization of calcium hydroxide frequently reduces both the biological activity and detoxification efficiency of vitamins (Barraza et al., 1991).

The utilization of cottonseed meal in the diets of monogastric animals necessitates the reduction of free gossypol content to a permissible level, alongside a requisite high protein content. The aqua alkali-based solvent systems for protein extraction have garnered significant interest due to their superior cottonseed protein recovery and diminished gossypol content, in comparison to both water and enzyme solvent systems (He et al., 2018). Due to the presence of hydrophobic groups and disulfide bonds within the protein molecules of plant cells, the solubility of plant cell proteins in water becomes challenging. Moreover, the utilization of an alkali-assisted extraction technique resulted in an elevation in the surface charge of protein, thereby enhancing the solubility of protein (Cui et al., 2017). Salt is commonly employed to segregate peripheral proteins. Consequently, the optimal protein yield can be attained through the utilization of alkali in conjunction with salt-assisted extraction of proteins (Kumar et al., 2022a). The protein content of cottonseed protein isolate exceeded 96% through the process of acid precipitation in water alkali system (Dowd and Hojilla-Evangelista, 2013). Kumar et al. (2021a) found that employing an aqua-alkali-salt-based system, consisting of alkali: sample ratio (33:1), NaCl (0.15 mol/L), Na_2SO_3 (0.27%), resulted in a reduction in the concentration of free gossypol to 29.0 mg/kg, while achieving a protein recovery rate of 88.5%. In another study, Kadam et al. (2023) subjected cottonseed meal to pretreatment using a microwave, followed by the extraction of cottonseed protein aided by alkali-salt. The crude protein content of cottonseed protein acquired through this methodology was recorded as 92.79%. Furthermore, the detoxification efficiency pertaining to free gossypol amounted to 99.95%, whereas the total gossypol content was measured at 1.14% (Kadam et al., 2023). In another study based on the aqua-alkali system, Ma et al. (2018b) investigated the effects of hot-pressed solvent extraction, cold-pressed solvent extraction, and subcritical fluid extraction on the physicochemical and functional properties of isolated protein from cottonseed meal. The results showed that cottonseed meal obtained through cold-pressed solvent extraction and subcritical fluid extraction exhibited elevated levels of water/oil absorption capacity, emulsifying abilities, surface hydrophobicity, and fluorescence intensity (Ma et al., 2018b). In addition, various studies have demonstrated that the cottonseed protein extracted from cottonseed or cottonseed meal has multiple bioactive functions, such as antibacterial, antioxidant, and immune effects (Song et al., 2020; Wang et al., 2022a).

4.3. Biological methods

Researchers have developed various physical and chemical techniques to eliminate gossypol, yet these approaches are not without their limitations. These methods lead to reduced active vitamin content, diminish protein quality, and impair feed palatability while also incurring substantial energy wastage (Zhang et al., 2018). Compared with physical and chemical methods, the microbial fermentation degradation of anti-nutritional factors appears to be a promising environmentally friendly approach (Garrido-Galand et al., 2021). Since the fermentation process can secrete various enzymes, including protease, cellulase, vitamins, and growth factors, many strains such as *Aspergillus*, *Yeast*, and *Bacillu* have been employed for the microbial fermentation of cottonseed meal (Olukomaiya et al., 2019). Table 4 provides a concise summary of the effect of microbial fermentation on the nutritional characteristics of cottonseed meal.

The detoxification process of microbial fermentation on cottonseed meal can potentially be explained as follows: Firstly, the utilization of gossypol as a carbon source results in a decrease in the total gossypol content in cottonseed meal. Secondly, as a consequence of the involvement of microorganisms during the fermentation procedure, the conversion of free gossypol to bound gossypol reduces the overall toxicity of cottonseed meal (Kumar et al., 2021b). Li et al. (2022) conducted a screening process to identify strains with a high activity of digesting free gossypol from a pool of 7 high-yield protease strains. Those strains demonstrating both gossypol-degrading and protease-producing activities were preferentially selected. *Bacillus subtilis* M15 showed the most favorable fermentation effect among these strains and was subsequently identified as the most suitable candidate (Li et al., 2022). Following inoculation at a rate of 1×10^9 CFU/kg for anaerobic solid-state fermentation over 14 d at room temperature, the gossypol content in cottonseed meal degraded at a rate of 93.46%, while the acid-soluble protein content rose to 13.26% (Li et al., 2022). Another study investigated the solid-state fermentation parameters for

Table 4
Effect of microbial fermentation on the nutritional characteristics of cottonseed meal.

Microorganism	Conditions	Main results	References
Bacillus coagulans S17	1:1 of the material-to-water ratio, 15% (v/w) seed inoculation, 2% expanded corn flour, 1% bran, and 0.3% to 0.8% metal irons at 40 °C for 52 h	• The detoxification efficiency of FG at 81.83%; • The crude proteins increased by 4.83%.	Zhang et al. (2022c)
Bacillus subtilis M-15	The strain was inoculated with 10^9 CFU/kg and fermented at room temperature for 14 d	• The detoxification efficiency of FG at 93.46%; • The content of acid crude protein was 13.26%.	Li et al. (2022)
Candida tropicalis ZD-3	Basal substrate with minerals solution was autoclaved at 112.6 °C for 20 min, then inoculated with 5% seed inoculation, 50% moisture, pH in nature and add minerals solution at 30 °C for 48 h	• The detoxification efficiency of gossypol at 96.67%; • The crude proteins increased by 12.36%.	Zhang et al. (2006)
Candida tropicalis ZD-3	1:1 of the material-to-water ratio and fermented at 30 °C for 48 h	• The detoxification efficiency of FG at 94.6%; • The crude proteins increased by 10.76%.	Zhang et al. (2007)
Lactobacillus agilis WWK129	Anaerobic fermentation by 5% seed inoculation and 50% moisture at 39 °C for 5 d	• The detoxification efficiency of gossypol at 80%; • The contents of NDF and ADF decreased by approximately 4% and 5%, respectively; • The crude protein content has increased by approximately 4%, and the content of most essential amino acids has significantly increased.	Wang et al. (2021b)
Rumen *Bacillus subtilis*	pH 6.5, moisture 50%, and inoculum level 10^7 cells/g at 39 °C for 72 h	• The FG and TG contents of cottonseed meal decreased by 78.86% and 49% respectively; • The content of crude protein and the essential amino acids were increased.	Zhang et al. (2018)
B. subtilis ST-141 and *Saccharomycetes* N5	1:0.5 of the material-to-water ratio, *B. subtilis* ST-141 (10^9 CFU/mL, 2:10, v/w) and *Saccharomycetes* N5 (0.5:100, w/w) at 30 °C for 48 h	• The detoxification efficiency of FG is approximately 58%; • The content of acid crude protein has significantly increased, and the content of CF and pH has decreased.	Wang et al. (2017)
Bacillus subtilis BJ-1	Soaking the cottonseed meal with 1:1 of the material-to-water ratio, 1% (v/w) seed inoculation at 30 °C for 48 h	• The detoxification efficiency of FG is 74.39%; • The content of crude protein and ash has increased, and the content of CF has decreased.	Tang et al. (2012)

ADF = acid detergent fiber; CF = crude fiber; NDF = neutral detergent fiber; FG = free gossypol; TG = total gossypol.

Bacillus coagulans strain S17 using cottonseed meal as the substrate. The parameters included a 1:1 ratio of material to water, a 15% (v/w) seed inoculum, 2% expanded corn flour, 1% bran, and metal irons ranging from 0.3% to 0.8%. The fermentation process was conducted at a temperature of 40 °C for 52 h (Zhang et al., 2022c). The authors found that there was an increase in the crude protein content from 47.98% to 52.82%. However, there was a decrease in the free gossypol content from 923.80 to 167.90 mg/kg, indicating a degradation efficiency of 81.83% (Zhang et al., 2022c). Moreover, when compared to other plant-derived protein feeds, cottonseed meal is susceptible to contamination by aflatoxin B1 (AFB1) (Feizy et al., 2012). The inclusion of feeds contaminated with AFB1 in animal diets can pose significant health risks (Rushing and Selim, 2019). Liu et al. (2017) conducted a solid-state fermentation process using a strain of *Cellulosimicrobium funkei* on cottonseed meal with a material-to-water ratio of 1:0.5. The authors performed a 10% (v/w) seed inoculation at a temperature of 35 °C for 144 h. This process successfully achieved an 83.4% biodegradation of AFB1 (Liu et al., 2017). Furthermore, the author also demonstrated the safety and nutritional benefits of incorporating fermented cottonseed meal into the feeding regimens of ducklings. This was evidenced by the enhanced growth performance and reduction in organ damage observed in ducklings that were fed diets contaminated with AFB1 (Liu et al., 2017). It is noticeable that microbial fermentation cannot only degrade gossypol content, improve its nutritional value, and reduce mycotoxin contamination, but also is economically feasible (Zhang et al., 2022c). However, exist certain challenges such as the need for intricate control over fermentation conditions and the involvement of a complex and time-consuming process (Kumar et al., 2021b). Enzymes capable of degrading gossypol were isolated from the culture supernatants of mixed fungi combinations *Pleurotus sajor-caju* + *Saccharomyces cerevisiae* and *Candida tropicalis* + *S. cerevisiae*, cultured in a medium containing gossypol. The gossypol degrading enzyme belongs to the laccase group of enzymes, exhibiting a molecular weight ranging between 45 and 66 kDa as measured by sodium dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE) (Mageshwaran et al., 2018). The residual (biodegradable) gossypol present in the supernatant was characterized and it was found that the functional aldehyde extension was reduced, and the single isotope mass of biodegradable gossypol was determined to be 474 g/mol (Mageshwaran et al., 2018). In another study, Zhang et al. (2022a) successfully expressed a carboxylesterase (CarE) derived from *Helicoverpa* armigera, known as *CCE001a*, within *Pichia pastoris* GS115. Subsequently, the authors detected the activity of recombinant CarE towards free gossypol (Zhang et al., 2022a). Following the treatment of cottonseed meal with recombinant CarE, the total gossypol degradation rate reached 90%, whereas the free gossypol degradation rate reached 89% (Zhang et al., 2022a). Moreover, the authors have observed that the degradation mechanism of gossypol involves the formation of azide compounds through the action of free radicals and hydroxyl groups, or via binding to amino acids of CarE (Zhang et al., 2022a). Furthermore, it has been discovered that enzymatic hydrolysis of cottonseed protein can yield a range of bioactive peptides, including antioxidant peptides and antimicrobial peptides (Refstie et al., 2004). The peptides generated through Alcalase hydrolysis and *Aspergillus niger* fermentation, as prepared by Wang et al. (2021a), exhibit the capability to scavenge free radicals and chelate Fe^{2+} ions. In addition, a large number of studies have demonstrated that the addition of enzymatic cottonseed protein products to the diet can reduce the digestive burden of animals, increase feed intake and feed utilization (Qiu et al., 2023), promote the development of intestinal structure (Tanumtuen et al., 2020) and enhance immunity (Zhang et al., 2022b).

To sum up, the gossypol present in cottonseed meal can be removed using the aforementioned methods. Nevertheless, it should be noted that those processing methods have their distinct advantages and disadvantages. Traditional physical methods, such as heat treatment, can effectively reduce protein degradability by inducing denaturation or facilitating the formation of protein-carbohydrate and protein–protein cross-links (known as the Maillard reaction) (Ghanbari et al., 2012). In addition, certain emerging technologies, such as gamma rays and irradiation, offer multiple benefits in terms of preserving the integrity of proteins and essential nutrients, eliminating microbial and fungal contamination of feed, and having no residual effects after irradiation (Ghanbari et al., 2012). Other processing techniques, such as pulsed ultrasonic treatment and microwave radiations, offer distinct advantages including reduced energy consumption, enhanced efficiency, and minimized chemical reactions (Fu et al., 2022; Kadam et al., 2023). However, these emerging technologies are currently in the early stages of small-scale application, featuring with relatively high input costs and associated radiation complications that require further extensive investigation (Kumar et al., 2021b). Solvent extraction is a highly effective method for achieving a high extraction rate of protein and gossypol, while minimizing economic costs. However, this method is accompanied by potential challenges, including the potential for reagent residue, environmental pollution, and reduced vitamin content (Nagalakshmi et al., 2002). Biological methods, such as microbial fermentation, offer the potential for enhancing the nutritional value of cottonseed meal, degrading gossypol, and extending its shelf life for preservation (Niu et al., 2020). However, it is necessary to evaluate the safety of certain metabolites produced during fermentation, including fungi and mycotoxins, as they may pose toxicity risks to pigs (Yang et al., 2020). The enzymatic methods are characterized by their safety and efficiency, with reaction conditions maintained within a mild pH and temperature range. However, a notable drawback resides in the considerable associated costs (Kumar et al., 2022a). All in all, further research is imperative to identify cost-effective, healthy-promoting, and environmentally sustainable methods for enhancing protein yield and quality, while minimizing gossypol content and costs. Future research directions in this field could potentially focus on the synergistic utilization of these methods (Kumar et al., 2021b).

5. Application of cottonseed meal in pig nutrition (2014 to 2023)

The investigation of cottonseed meal and its processed product in pig nutrition has mainly focused on weaned piglets, growing pigs, and finishing pigs, as evidenced in Table 5. In light of those findings in Table 5, the recommended inclusion rates of cottonseed meal and its related products are 1% to 6% for weaned piglets and 7.69% to 27.99% for growing pigs, with no detrimental impact on production performance. In this chapter, we will provide a comprehensive overview of the applications research progress of cottonseed meal and its processed products in pig nutrition, mainly focusing on the effects on the growth performance, meat quality, intestinal health, and nutrient digestibility of pigs.

5.1. Growth performance

Recently, various studies tested the effects of cottonseed meal or its processed product on the growth performance of pigs. Tanumtuen et al. (2020) investigated the impact of dietary enzymatic hydrolysate of cottonseed protein at different concentrations (0%, 1%, and 1.5%) on nursery pigs, and found that the inclusion of 1% enzymatic hydrolysate of cottonseed protein led to a numerical increase in average daily feed intake (ADFI) and average daily gain (ADG) while decreasing diarrhea incidence. In a 14-day feeding trial conducted by Gu et al. (2021), the inclusion of 6% fermented cottonseed meal as a replacement for soybean meal remarkably increased the ADG, FCR, and final body weight (BW), and reduced diarrhea incidence of weaned piglets. It is possible that in the fermentation process of cottonseed meal, there was an effective reduction in the level of free gossypol and an increase in the content of acid-soluble protein. This led to an improvement in the digestive enzyme activity and nutrient digestion of weaned piglets, ultimately resulting in improved production performance (Sun et al., 2014; Wang et al., 2017). Another study discovered that the addition of 6% degossypolized cottonseed protein, which served as a substitute for soybean protein concentrate or fish meal, did not exhibit any discernible impact on the growth performance and diarrhea rate in weaned piglets (Wang et al., 2022b). Yet, it resulted in a reduction in ADFI during the period between 14 and 28 d (Wang et al., 2022b). However, Wang et al. (2019) reported that the substitution of 10% degossypolized cottonseed protein for soybean protein concentrate in the diet decreased BW (14 to 28 d), ADG (0 to 14 d) and nutrients digestibility (14 to 28 d), but increased diarrhea frequency (0 to 14 d) of nursery pigs. The decrease in growth performance can potentially be attributed to the elevated presence of free gossypol and the fiber content present in degossypolized cottonseed protein (González-Vega and Stein, 2012; Stein et al., 2016). Palhares et al. (2019) demonstrated that the inclusion of cottonseed cake at varying percentages (0, 20%, 40%, and 60%) replacement of soybean meal in the diet of growing pigs, along with supplementation of an enzymatic complex did not adversely affect on growth performance and carcass traits of pigs. Furthermore, Qin et al. (2015) explored the effects of dietary protein sources (cottonseed meal or soybean meal) and crude protein levels (12% or 14%) on finishing gilts. The findings demonstrated that the inclusion of cottonseed meal in the diet did not have any negative impact on growth performance (Qin et al., 2015). Additionally, supplementing cottonseed meal with low protein diet increased the average daily feed intake and nitrogen efficiency, and a decrease in nitrogen intake, nitrogen excretion, and serum urea nitrogen content of pigs (Qin et al., 2015). The aforementioned findings suggest that the inclusion of moderate levels of cottonseed meal or processed products in the diets will not have a negative impact on the growth performance of pigs.

5.2. Pork quality

According to a recent study, there were no observed effects on the morphometric characteristics of body length, thoracic perimeter, abdominal circumference, rump width, rump height, withers height, and body weight of finishing pigs when a dietary supplement of enzyme complex was combined with varying percentages of cottonseed meal (0%, 5%, 10%, and 15%) as a replacement for soybean meal (De Andrade et al., 2023). However, there is a limited number of reports regarding the impact of cottonseed meal and its processed product on pork quality. Furthermore, there has been a report indicating that low-level protein diets and amino acids have the potential to facilitate the accumulation of fat in subcutaneous, visceral, and intramuscular tissues, resulting in a decrease in the proportion of lean meat (Yan et al., 2023). These findings primarily stem from dietary lysine limitations and the imbalances observed in essential amino acids (Madeira et al., 2013; Xu et al., 2020). As reported by Qin et al. (2015), the dietary inclusion of two protein sources, namely soybean meal and cottonseed meal, with varying levels of crude protein (12% or 14%) did not exhibit any adverse effects on carcass traits or meat quality characteristics in finishing gilts. Specifically, varying protein sources and levels of crude

Table 5
The summary of cottonseed meal as a protein source for pigs during the past decade (2014 to 2023).

Types of materials	Study design	Main findings	References
Enzymatic hydrolysate of cottonseed protein	• Experimental subject: nursery pigs (Yorkshire × Landrace × Duroc) • Feed inclusion level: 0%, 1%, and 1.5% • Duration: 35 d	• Increased ADFI (d 28 to 49: 1% EHCP, d 50 to 63: 1.5% EHCP), ADG (d 28 to 49: 1% EHCP, d 50 to 63: 1.5% EHCP), and decreased FCR (d 28 to 49: 1% EHCP, d 50 to 63: 1.5% EHCP) and diarrhea incidence (d 28 to 49: 1%, 1.5% EHCP); • Decreased crypt depth and decreased villi height to crypt depth ratio in the jejunum and ileum in the jejunum and ileum (1% EHCP); • Increased GSH and SOD in intestinal (1%, 1.5% EHCP).	Tanumtuen et al. (2020)
Fermented cottonseed meal	• Experimental subject: weaned pigs (Duroc × Landrace × Yorkshire) • Feed inclusion level: 6% replacement of soybean meal • Duration: 14 d	• Increased the final BW, ADG, and G:F ratio and decreased diarrhea incidence; • Increased the digestibility of DM, OM, CP, GE, amino acids, and total N; • Increased IgG and decreased MDA in the serum; increased SOD and GSH-Px in serum, jejunum, and ileum and T-AOC in the jejunal and GSH-Px in the liver; • Increased total VFAs in the in ileal digesta and cecal digesta; • Increased beneficial bacteria and reduced harmful bacteria.	Gu et al. (2021)
Concentrated degossypolized cottonseed protein	• Experimental subject: piglets (Landrace × Yorkshire) • Feed inclusion level: 29% (sole protein source) • Duration: 10 d	• No effect on growth performance; • Decreased the protein flows into the hindgut and increased the amount of mixed neutral-acidic mucins in the colon.	Li et al. (2019a)
Degossypolized cottonseed protein	• Experimental subject: nursery pigs (Yorkshire × Landrace × Duroc) • Feed inclusion level: 6% DCP replacement of SPC or fish meal • Duration: 21 d	• No effects on growth performance and diarrhea rate, but decreased ADFI (d 14 to 28); • Weaken the jejunum morphology and decrease the nutrient digestibility (6% DCP replacement of fish meal, d 0 to 14); • Increased beneficial bacteria and reduced harmful bacteria.	Wang et al. (2022b)
Concentrated degossypolized cottonseed protein	• Experimental subject: nursery pigs (Yorkshire × Landrace) • Feed inclusion level: 4% • Duration: 28 d	• No effects on growth performance and nutrient digestibility and decreased fecal N excretion per weight gain compared to fish meal diet; • Increased the pepsin activity in the stomach and decreased pH in the proximal colon.	Li et al. (2019c)
Degossypolized cottonseed protein	• Experimental subject: nursery pigs (Yorkshire × Landrace) • Feed inclusion level: 5% or 10% DCP replacement of SPC or 4% DCP replacement of fish meal • Duration: 28 d	• Decreased BW (d 14 to 28: 10% DCP), ADG (d 0 to 14:10% DCP), nutrients digestibility (d 14 to 28: 10% DCP), and increased diarrhea frequency (d 0 to 14:10% DCP); • No effects on growth performance and nutrient digestibility (d 0 to 14: 4%, 5% DCP, d 0 to 28: 4%, 5% DCP).	Wang et al. (2019)
Concentrated degossypolized cottonseed protein	• Experimental subject: nursery pigs (Yorkshire × Landrace) • Feed inclusion level: 4% • Duration: 28 d	• Decreased DAO, IL-1α and endotoxin in the serum; • Upregulated the occludin mRNA expression in the colon; • Increased beneficial bacteria and reduced harmful bacteria.	Li et al. (2019b)
Cottonseed meal	• Experimental subject: finishing pigs (Yorkshire × Landrace) • Feed inclusion level: 0, 5%, 10%, and 15% with 0.005% enzyme complex • Duration: 42 d	• No effect on growth performance; • Decreased the digestibility coefficients of DM, OM, CP, and increased digestibility coefficients of EE.	Da Silva et al. (2021)
Cottonseed cake	• Experimental subject: growing pigs (large white × Duroc × Landrace) • Feed inclusion level: 9.33%, 18.66%, and 27.99% with 0.030% enzyme complex • Duration: NA	• No effects on growth performance and carcass characteristics; • Linearly decreased total serum protein, no effects on glucose, phosphorus, urea, uric acid, and creatinine in the serum.	Palhares et al. (2019)
Cottonseed meal	• Experimental subject: finishing gilts (Duroc × Landrace × Yorkshire) • Feed inclusion level: 8.15% (12% CP), 13.72% (14% CP) • Duration: 28 d	• No effects on growth performance, meat quality, and carcass characteristics; • Decreased N intake, N excretion, and serum urea nitrogen and improved N efficiency (8.15% cottonseed meal); • Decreased phenylalanine, tryptophan, cysteine, and tyrosin of the longissimus muscle and increased muscle intracellular free valine.	Qin et al. (2015)
Cottonseed meal	• Experimental subject: growing pigs (Duroc × Landrace × Yorkshire) • Feed inclusion level: 7.69% • Duration: 24 d	• No effects on growth performance, serum free amino acid concentrations, and apparent nutrient digestibility; • No effect on the diversity of the fecal microbiota.	He et al. (2023)

ADFI = average daily feed intake; ADG = average daily gain; AID = apparent ileal digestibility; BW = body weight; Ca = calcium; CAT = catalase; CF = crude fiber; CP = crude protein; DAO = diamine oxidase; DCP = degossypolized cottonseed protein; DM = dry matter; EE = ether extract; EHCP = enzymatic hydrolysate of cottonseed protein; FCR = feed conversion ratio; GE = gross energy; G:F = ADG:ADFI; GSH = glutathione; GSH-Px = glutathione peroxidase; IgG = immunoglobulin G; IL-1α = interleukin-1α; MDA = malondialdehyde; N = nitrogen; NA = not available; OM = organic matter; P = phosphorus; SOD = superoxide dismutase; SPC = soybean protein concentrate; T-AOC = total antioxidant capability; VFAs = volatile fatty acids.

protein did not have any impact on carcass weight, dressing percentage, back fat depth, longissimus muscle area, meat color, pH_{24h} value, shear force, drip loss, cooking loss, marbling score, and intramuscular fat content (Qin et al., 2015). However, the $pH_{45\ min}$ of fresh pork from diets with a 12% CP level was slightly lower compared to diets with a 14% CP level in finishing gilts. Furthermore, the utilization of cottonseed meal resulted in a reduction in the levels of phenylalanine, tryptophan, cysteine, and tyrosine within the muscle, while enhancing the levels of intracellular free valine in the muscle (Qin et al., 2015). The authors finally concluded that cottonseed meal as the main protein source is feasible to completely substitute soybean meal, with regards to the growth performance, carcass characteristics, meat quality, and dietary nitrogen efficiency of pigs (Qin et al., 2015). However, this substitution should only be done while ensuring that the essential amino acids and metabolizable energy are maintained to meet the nutritional requirements of finishing gilts (Qin et al., 2015). Likewise, Paiano et al. (2014) noticed that dietary addition of 5%, 10%, or 15% cottonseed meal did not affect carcass traits and plasma urea nitrogen levels in finishing pigs. Further investigation is warranted to evaluate the impact of substituting soybean meal with cottonseed meal, an alternative protein source, on carcass and meat quality traits in pig diets. Additionally, special consideration should be given to gossypol residues.

5.3. Intestinal health

Intestinal health holds utmost importance in facilitating the growth and development of weaned piglets with an immature digestive system (Wang et al., 2020). The gastrointestinal tract also carries significance in terms of digestion, absorption, and immunity of animals (Song et al., 2021). Weaning stress is typically accompanied by intestinal atrophy, which further limits the intestinal digestion and absorption capacity of weaned piglets (Deng et al., 2023a). As a result, the utilization of readily digestible and superior protein supplements becomes particularly imperative in order to mitigate the adverse effects of the weaning stress of piglets. Fish meal and soybean meal have high crude protein-derived content and balanced amino acids, which can meet the nutritional requirements of early-weaned piglets while also enhancing their feed intake and intestinal health (Deng et al., 2023b; Kim and Easter, 2001). However, the utilization of animal protein in nursery diets might be impeded due to factors such as price fluctuation, limited availability, and potential safety concerns (Kim et al., 2019). In this section, we discussed the effects of cottonseed meal processed products on intestinal integrity, gut microbiota and its metabolites in weaned piglets.

5.3.1. Intestinal integrity

The morphological and structural integrity of the intestine plays a crucial role in maintaining intestinal physiological functions (Yu et al., 2020). Concentrated degossypolized cottonseed protein has been reported to have certain advantages over fish meal in maintaining colon integrity (Li et al., 2019b). Specifically, the inclusion of 4% concentrated degossypolized cottonseed protein in the low protein diets with amino acids supplementation resulted in a decrease in serum diamine oxidase level in weaned piglets. This decrease can be attributed to the up-regulation of occludin mRNA expression. It has been also found that a low-protein diet supplemented with 4% concentrated degossypolized cottonseed protein in nursery pigs did not affect the morphological structure of the duodenum and ileum, as well as the short-chain fatty acids profile in the colon. However, there was an increase in pepsin levels in the stomachs of pigs that were fed a concentrated degossypolized cottonseed protein diet (Li et al., 2019c). Besides, Gu et al. (2021) observed that the inclusion of 6% fermented cottonseed meal resulted in elevations of superoxide dismutase (SOD), total antioxidant capability (T-AOC), and glutathione peroxidase (GSH-Px) activities in the jejunum and SOD and GSH-Px activities in the ileum of weaned piglets. This suggests that feeding fermented cottonseed meal enhances intestinal antioxidant capacity. Another study found that the supplementation of 1% enzymatic hydrolysate of cottonseed protein resulted in a noteworthy reduction in the depth of crypt in the jejunum and ileum of nursery pigs (Tanumtuen et al., 2020). Moreover, an increase in the level of enzymatic hydrolysate of cottonseed protein supplementation was found to significantly enhance the villus height/crypt depth of both the jejunum and ileum of pigs (Tanumtuen et al., 2020). However, at 63 d of age, no significant differences were observed among treatments regarding villus height, villus width, villus surface area, duodenal crypt depth, and duodenal villus height/crypt depth in the duodenum, jejunum, and ileum of nursery pigs (Tanumtuen et al., 2020). The addition of 1% enzymatic hydrolysate of cottonseed protein increased SOD activity and glutathione (GSH) levels in the intestine of piglets (Tanumtuen et al., 2020). A recent study showed that the incorporation of degossypolized cottonseed protein as an alternative to fish meal or soybean protein concentrate at a 6% level in weaned pigs was found to adversely affect the intestinal morphology and the ratio of villus height to crypt depth of jejunum. The researchers suggest that this negative impact may be attributed to the elevated level of free gossypol in degossypolized cottonseed protein (Wang et al., 2022b. These findings suggest that cottonseed meal or its derived products have the potential to enhance intestinal integrity and intestinal health in pigs. However, the presence of gossypol should also be taken into consideration when utilizing cottonseed meal or its derived products.

5.3.2. Intestinal microbiota and its metabolites

It has been demonstrated that dietary protein sources have the potential to regulate the intestinal microbiota and its associated metabolites (Deng et al., 2023a; Wang et al., 2023). Plant proteins have been found to have a stronger potential to promote the proliferation of beneficial bacteria when compared to fish meal and animal-derived proteins (Cao et al., 2016). Recent research has additionally corroborated this perspective (Ma et al., 2019b). Cao et al. (2016) investigated the effects of four different dietary protein sources on microflora present in the small intestine of weaned piglets. The results showed that the microbiota in the small intestine of piglets was notably influenced by the type of dietary protein sources. Moreover, the pigs fed cottonseed meal displayed a higher proportion of lactic acid bacteria and clostridium in their small intestine compared to the other treatment groups (Cao et al., 2016). As reported by Li et al. (2019a), the dietary addition of 4% concentrated degossypolized cottonseed protein decreased the protein flows to the hindgut and increased the abundance of *Lactobacillus* in the colon of piglets. Gu et al. (2021) discovered that the inclusion of 6% fermented cottonseed meal resulted in improved relative abundance of *Lactobacillus* and *[Ruminococcus]_torques_group* in the ileum of weaned pigs. Similarly, it also enhanced the concentrations of acetic acid, propionic acid, butyric acid, isobutyric acid, isovalerate acid, and total volatile fatty acids (VFAs) in the ileal digesta. Furthermore, concentrations of acetic acid, propionic acid, valerate acid, isobutyric acid, isovalerate acid, and total VFA were also increased in the cecal digesta of weaned pigs (Gu et al., 2021). It is of note that the inclusion of 6% degossypolized cottonseed protein in the diet resulted in an improvement in the composition of cecum microbiota, thereby alleviating the adverse consequences observed, while not compromising the growth performance of weaned piglets (Wang et al., 2022b). Furthermore, the authors finally concluded that the utilization of degossypolized cottonseed protein presents a viable and economical alternative protein source to substitute fish meal and soybean protein concentrate (Wang et al., 2022b). Herein, the regulation of gut microbiota and microbiota-derived metabolites should be prioritized when assessing the additive effects of cottonseed meal or its derived products.

5.4. Nutrition digestibility

The nutrient digestibility is indicative of animals' ability to effectively digest and assimilate the nutrients. Gu et al. (2021) reported that the inclusion of 6% fermented cottonseed meal in the diet of weaned pigs improved the apparent total tract digestibility and ileal digestibility of various nutrients, including dry matter (DM), organic matter (OM), crude protein (CP), and gross energy (GE). Additionally, the inclusion of 6% fermented cottonseed meal increased the AID of total nitrogen and essential amino acids such as histidine, isoleucine, leucine, phenylalanine, and valine, as well as non-essential amino acids like asparagine, when compared to the group fed with soybean meal (Gu et al., 2021). It has been reported that the inclusion of 6% degossypolized cottonseed protein concentrate in place of fish meal and soybean protein concentrate resulted in a decrease in apparent total tract digestibility of CP, GE, DM, and OM in weaned pigs (Wang et al., 2022b). There are two potential reasons for this phenomenon. Firstly, animal proteins are more easily digested compared to plant proteins due to the presence of plant cell walls or fibers that obstruct the binding of digestive enzymes to nutrients. Secondly, the increased fiber content in degossypolized cottonseed protein may induce rapid flow of chyme in the intestine and reduce the digestibility of nutrients (Lentle and Janssen, 2008). Similar findings were recorded by Wang et al. (2019), that the dietary supplementation with degossypolized cottonseed protein at 10% decreased the apparent total tract digestibility of GE, DM, and CP compared to the control group (d 14 to 28). Interestingly, dietary supplementation with degossypolized cottonseed protein by replacing 5% soybean protein concentrate or 4% fish meal had no effects on apparent total tract digestibility of GE, DM, and CP in nursery pigs compared to that in the control group (0 to 14 d; 0 to 28 d) (Wang et al., 2019). Another study revealed that the addition of 4% concentrated degossypolized cottonseed protein to a low-protein diet for weaned pigs had no effects on apparent total tract digestibility of DM, GE, CP, and EE, but reduced fecal nitrogen excretion when compared to the fish meal diet (Li et al., 2019c). The inclusion of exogenous enzymes in pig diets is generally deemed a crucial means of enhancing nutrient absorption and digestion efficiency (Torres-Pitarch et al., 2019). However, Da Silva et al. (2021) showed that dietary addition of 0%, 5%, 10%, and 15% cottonseed meal in the diet, along with supplementation of the enzyme complex at a concentration of 0.005%, led to a decrease in the digestibility of OM, DM, and CP. Conversely, the elevated inclusion of cottonseed meal resulted in an improved EE content. The inconsistent findings in these reports could potentially be attributed to the levels of free gossypol and fiber content present in cottonseed products.

6. Research gap and future perspectives

In recent years, the rapid development of the pig industry, along with the limited availability and rising prices of conventional protein ingredients, has generated renewed interest in utilizing cottonseed meal in pig diets. However, the use of cottonseed meal in pig feed is still in its early stages, and there are several aspects that necessitate further investigation. Firstly, it is imperative to determine the optimal inclusion level of cottonseed meal or its derivatives for various physiological phases in pigs, with regard to maximizing productivity, nutrient digestibility, intestinal health, and pork quality. For instance, it is widely recognized that gossypol negatively impacts sow reproductive performance, therefore, the inclusion of cottonseed meal in sow diets is generally discouraged. Secondly, the underlying mechanism behind the adverse effects of excessive cottonseed meal or gossypol on pigs remains unclear. Previous research has demonstrated that the growth inhibition and intestinal damage caused by cottonseed meal or its derivative diets can be alleviated through chemical detoxification methods, such as $FeSO_4$, or by supplementing functional additives, such as probiotics, in poultry (Boling et al., 1998) and largemouth bass (Xie et al., 2022). These findings provide valuable insights into the understanding of the underlying mechanism of the adverse effects of excessive cottonseed meal or gossypol. This understanding can greatly enhance our ability to screen potential nutritional strategies aimed at promoting high cottonseed meal inclusion in pig diets. Lastly, future research should prioritize the advancement of sustainable detoxification methodologies for cottonseed meal in order to enhance its safety and suitability for inclusion in pig diets.

7. The limitations to the use of cottonseed meal in pig nutrition

While cottonseed meal is widely regarded as a viable alternative, its utilization as a protein source in pig nutrition continues to encounter limitations and challenges. The main limitations associated with the utilization of cottonseed meal in pig nutrition are primarily attributed to the presence of anti-nutritional factors, notably gossypol, alongside high crude fiber content and low utilization of essential amino acids like lysine (Świątkiewicz et al., 2016). Another challenge encountered in utilizing cottonseed meal in pig nutrition pertains to the issue of mycotoxin contamination. Cottonseed meal was reported as the feed ingredient contaminated with a high level of AFB1 (Liu et al., 2016). The consumption of AFB1-contaminated feed by animals can cause severe health and productivity issues, resulting in huge economic losses (Yang et al., 2020). Despite the limitations, the utilization of cottonseed meal as a cost-effective alternative protein source for pig diets is economically justified, particularly in developing countries (Khajali and Slominski, 2012). Importantly, future research directions in this field should prioritize the resolution of the aforementioned limitations by employing a synergistic integration of physical, chemical, and/or microbial fermentation methodologies.

8. Conclusions

Cottonseed meal exhibits wide-ranging potential for application as a valuable source of protein. Solvent extraction and microbial fermentation methods present a viable approach for achieving economic viability in the current circumstances, with regard to the extraction of gossypol and the production of protein. Once the free gossypol level has been decreased to 450 mg/kg, it becomes suitable for the provision of feed to nonruminant animals. This holds immense importance in terms of diminishing breeding expenditures and guaranteeing the sustainable progress of animal husbandry, as well as mitigating the global insufficiency of feed protein resources. In light of current understanding, it is possible to substitute 6% of soybean meal, soybean protein concentrate, and fish meal in the diet of weaned piglets with cottonseed meal processed products. In growing pigs, cottonseed cake can replace up to 60% of soybean meal. In the forthcoming period, it is imperative to emphasize the optimum supplemental dosage of cottonseed meal and its processed derivatives for swine at varying feeding phases, along with their consequences on pig production performance, meat quality, and intestinal health.

Author contributions

Jun Chen and **Jinming You:** Conceptualization. **Tiande Zou, Jiahao Wang** and **Bowen Liu:** Validation. **An Tao:** Writing-original draft preparation. **Xingping Chen, Bin Luo** and **Zirui Wang:** Writing-review and editing. **Jun Chen** and **Jinming You:** Funding acquisition.

Declaration of competing interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, and there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

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