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Cottonseed peptides produced by dual degradation improved growth performance, nutrient digestibility, and health status of growing-finishing pigs

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ABSTRACT - The objective of this study was to investigate the effect of cottonseed peptides from *in vitro* degradation with protease, *Enterococcus faecalis* (*E. faecalis*), or both on the growth performance, nutrient digestibility, and health status of pigs. We used four diets containing non-degraded, protease-degraded, *E. faecalis*-degraded, or both-degraded cottonseed meal at 20% for growing phase (6 wk) and 16% for finishing phase (7 wk), corresponding to barrows, with six replicates of six pigs each. Results showed that the *in vitro* degradation of cottonseed meal increased proportions of peptides and organic acids and decreased nitrogen-free extract and phytate phosphorus. The degraded cottonseed meal increased feed intake, body weight gain, feed efficiency, apparent ileal digestibility of dry matter, energy, crude protein, Ile, Leu, Lys, Met, Cys, Ser, and Gly, and blood profiles of superoxide dismutase, glutathione peroxidase, catalase, IgA, and IgG. The diet containing cottonseed meal by dual degradation showed more pronounced effects on nutritional compositions, growth performance, nutrient digestibility, and serum parameters than the other diets. The data suggest that cottonseed meal dually degraded *in vitro* with enzymes and probiotics can improve its nutritional properties and application in animal feed.

Keywords: amino acid, antioxidation, *Enterococcus faecalis*, protease

1. Introduction

With the development of animal production, miscellaneous meals are becoming an important supplement for soybean meal; however, the relatively poor quality of miscellaneous meals limits their use in practice. This drives some methods, such as enzymatic hydrolysis and fermentation, to enrich peptides and improve utilization of miscellaneous meals (Wang et al., 2011; Luz et al., 2018; Feng et al., 2020). The degradation of protein-rich materials is mainly involved in proteases and probiotics for enzymatic hydrolysis and fermentation. In contrast to raw materials, these treatment methods can significantly improve digestibility and confer more dietary nitrogen assimilation into body protein (Gu et al., 2021; Niu et al., 2021). Also, with these treatments for protein-sourced feedstuffs, more nitrogen and phosphorus are assimilated in the body, while fewer parts are excreted into the environment (Liu et al., 2018; Tavares-Samay et al., 2019). Additionally, the fermentation can cover bitterness generated from enzymatic processing (Idowu and Benjakul, 2019). Furthermore, the dual degradation can yield more peptides than a single one. Therefore, the dual degradation of dietary ingredients by enzymes and probiotics has recently become a hot topic in the food and feed industry.

Cottonseed meal is a high-protein solid residue derived from oil production, containing approximately 45% of crude protein (CP), 10.5% of crude fiber (CF), and 1% of gossypol (Gadelha et al., 2014). Significantly, this leftover gossypol restricts its utilization in the feed due to its toxicity and hazard for animals and humans (Câmara et al., 2015). With the development of extraction technologies, these concerns of cottonseed meal have been recently resolved by processes including decortication, extraction at a low temperature, and dephenolization. The dephenolized and dehulled cottonseed meal contains approximately 51.1% of CP, 6.9% of CF, and a fine safe content of gossypol. It is reported that fermented cottonseed meal improved production performance and lipid metabolism in broilers chickens (Nie et al., 2020; Niu et al., 2021). Silva et al. (2021) reported that cottonseed meal plus enzymes had no effects on the performance and financial profits of finishing pigs. Abdallh et al. (2020) found that cottonseed meal with enzyme supplementation can substitute up to 90% soybean meal in broiler chicken diets without compromising performance and feed nutritive values. However, literature is unavailable about the application of cottonseed meal dually degraded *in vitro* by proteases and probiotics in meat-type animals.

Hypothetically, the *in vitro* dual degradation by enzymatic hydrolysis and probiotic fermentation can improve the quality of cottonseed meal and its use in feed formulations for animals. The objective of the present study was to compare the compositions of cottonseed meal non-degraded or degraded by protease, *E. faecalis*, or both and their effects on the growth performance, nutrient digestibility, and health status of growing-finishing pigs.

2. Material and Methods

Research on animals was conducted according to the institutional committee on animal use (2021002).

2.1. Cottonseed peptide preparation

Cottonseed kernel meal (dephenolized) is the byproduct obtained after oil extraction from cottonseed. Commercial acid protease (PROMAX® Protease, pH 1.5-6.0) was used for the enzymatic hydrolysis of cottonseed kernel meal. The *E. faecalis* strain CGMCC 18916 was obtained from the China General Microbiological Culture Collection Center for the fermentation of cottonseed kernel meal.

The cottonseed meal was ground (16 mesh size), mixed with corn flour (5%), and sterilized at 121 °C for 20 min. The sterilized cottonseed kernel meal and deionized water (100/35, weight/weight) were mixed as a fermented substrate for solid-state fermentation. The protease was incubated with the fermented substrate at 3,000 U/kg, at 30 °C for 48 h, for preparing enzymatic hydrolysis of cottonseed meal. The *E. faecalis* strain was incubated with the fermented substrate at 1.0×10^9 CFU/kg, 30 °C for 48 h, to prepare fermented cottonseed kernel meal. The same protease activity and *E. faecalis* amount were dually incubated with fermented substrates at 30 °C for 48 h for preparing a double treated cottonseed kernel meal. The statistical unit was from three independent and parallel preparations for the raw, protease-degraded, *E. faecalis*-degraded, and dually degraded cottonseed meal. The products obtained by enzymolysis, fermentation, or both, were freeze-dried and ground (24 mesh size) for the preparation of the four diets.

2.2. Diets, animals, and samples

Four diets contained non-degraded (control), enzymatic (PCM), fermented (ECM), or dually degraded (DCM) dephenolized cottonseed kernel meal at an amount of 20% in the growing phase and 16% in the finishing phase. The diets were formulated referring to the Nutritional Requirement of Chinese Growing-Finishing Pigs (Standards in Agricultural Industries in China, NY/T 65-2004; Table 1). A total of 144 (Duroc × Landrace × Large White) barrows with initial body weight (BW) of 28±2.4 kg (BW±SD) were randomly distributed into four groups with six replicates (pens; size, 4.0 m × 3.0 m) of six pigs each. The feeding trial after a three-day adjustment period lasted for 13 weeks (growing/finishing phase, 6/7 wk). The pig house was controlled at 22 °C of room temperature and automatic

Table 1 - Ingredients and nutrient levels of diets (air-dry basis)

Item	Growing phase (6 wk)				Finishing phase (7 wk)			
	Control	PCM	ECM	DCM	Control	PCM	ECM	DCM
Ingredient (%)								
Cottonseed meal	20.0	20.0	20.0	20.0	16.0	16.0	16.0	16.0
Corn gluten feed	10.0	10.0	10.0	10.0	8.0	12.0	12.0	12.0
Corn	61.8	61.9	62.1	62.4	69.8	65.1	65.1	65.3
Mixed oil	4.0	4.0	3.9	3.8	2.5	3.3	3.3	3.2
Limestone	1.0	1.0	1.0	1.0	0.9	0.9	1.0	1.0
Di-calcium phosphate	0.6	0.5	0.4	0.2	0.4	0.3	0.2	0.1
Premix[1]	2.6	2.6	2.6	2.6	2.4	2.4	2.4	2.4
Nutrient level[2] (%)								
Metabolizable energy (MJ kg^{-1})	12.88	12.89	12.88	12.89	12.87	12.88	12.89	12.87
Crude protein	17.21	16.91	17.19	17.23	15.52	15.63	15.62	15.65
Ca	0.59	0.56	0.57	0.56	0.49	0.47	0.49	0.47
Total P	0.52	0.50	0.51	0.52	0.45	0.47	0.45	0.42
Non-phytate P	0.23	0.25	0.24	0.24	0.19	0.20	0.20	0.21
Digestible Lys	0.75	0.77	0.77	0.75	0.66	0.70	0.70	0.70
Digestible Met	0.28	0.28	0.30	0.28	0.25	0.25	0.25	0.25
Digestible Thr	0.65	0.65	0.65	0.66	0.45	0.44	0.44	0.45
Digestible Trp	0.14	0.14	0.14	0.15	0.14	0.14	0.13	0.14

Control - containing non-degraded cottonseed meal; PCM - cottonseed meal degraded with PROMAX® Protease; ECM - cottonseed meal degraded with *E. faecalis* strain CGMCC 18915; DCM - dual-degraded cottonseed meal with the protease and *E. faecalis*.
[1] Supplied per kilogram of diet for growing/finishing phase: lysine 200/200 mg; threonine 200/0 mg; choline chloride 300/300 mg; vitamin A, 1,400/1,300 IU; vitamin D_3, 160/150 IU, vitamin E, 11/11 IU; vitamin K, 0.50/0.50 mg, thiamin, 1.00/1.00 mg; riboflavin, 2.00/2.00 mg; niacin, 8.50/7.50 mg; pantothenic acid, 7.50/7.00 mg; pyridoxine, 1.00/1.00 mg; biotin, 0.05/0.05 mg; folic acid, 0.30/0.30 mg; vitamin B_{12}, 8.00/6.00 µg; K, 2.1/1.8 mg; Mg, 0.4/0.4 mg; Fe, 60/50 mg; Cu, 4.00/3.50 mg; Mn, 2.00/2.00 mg; Zn, 60/50 mg; I, 0.14/0.14 mg; Se, 0.25/0.25 mg; NaCl, 400/400 mg/kg; TiO_2, 500/500 mg.
[2] Metabolizable energy, calculated according to China Feed Database (Xiong et al., 2020); crude protein, Ca, total P, and non-phytate P, determined; and digestible aminoacids, calculated from determined total aminoacids.

mechanical ventilation. All pigs were given *ad libitum* access to diets and water throughout the feeding trial. Pigs and feed in each pen were weighed weekly, and feed efficiency was adjusted for mortality on a pen basis. The general health of pigs was monitored twice a day.

On the last day of the 6th and 13th weeks, four pigs per pen were randomly selected and fasted. Blood samples were collected from left-ear veins and prepared to quantify serum immunoglobulins and whole blood lymphocytes, and the average of each pen was a statistical unit (Wang et al., 2011). On the last day of the 13th week, after blood sampling, the four pigs were fed, and three hours later were euthanized by electric stunning, and then dissected. Ileal digesta was collected and pooled by replicate to detect the apparent ileal digestibility of nutrients.

2.3. Chemical analysis

The contents of nutrients in the feed and digesta were determined according to AOAC (1990) for total P (964.06), phytate P (927.02), Ca (935.13), and CP (976.05), crude fat (EE, 920.39), CF (962.09), and ash (942.05). Gross energy was measured in an Oxygen Bomb Calorimeter (Model 6300, PARR, Moline, IL, USA). Dry matter (DM) was determined by drying a 2-g sample at 65 °C to a constant mass. Aminoacids were measured using an AA analyzer (Beckman 6300; Beckman Coulter, Inc., Fullerton, CA). Titanium dioxide was used as an exogenous marker for the calculation of digestibility (Liu et al., 2018).

Peptide (≤1 000 Da) was quantified using electrophoresis in sodium dodecyl sulfate-polyacrylamide gels (Fling and Gregerson, 1986). For organic acids estimation, the sample slurry was centrifuged at 1×10^4 g for 20 min. The supernatant was analyzed using HPLC (Model 1100, Agilent Technologies, Inc, Santa Clara, CA, USA) using 10 mM perchloric acid as mobile phase at 40 °C. Protease activity was analyzed according to China National Standard GB/T 28715-2012. A protease activity unit was the enzyme amount that hydrolyzes casein to produce color equivalent to 1.0 µmole of tyrosine per minute

at pH 7.5 at 37 °C. Gossypol contents were detected according to methods for determination of free-gossypol in feeds (China National Standard, GB/T 13086-2020). Briefly, free-gossypol was extracted using a mixed solvent of 3-Amino-1-isopropyl alcohol and isopropyl alcohol-hexane, then reacted with aniline, and the absorbance of aniline-gossypol was measured at 440 nm.

2.4. Biochemical analysis and bacterium enumeration

Concentrations of immunoglobulin A (IgA), immunoglobulin G (IgG), and immunoglobulin M (IgM) in the serum were measured using an automatic biochemistry analyzer (Model 7600, Hitachi High-Tech, Tokyo, Japan). Commercial kits from Nanjing Jiancheng Biological Institutes (Nanjing, China) were used for detecting superoxide dismutase (SOD, A001-0302), glutathione peroxidase (GSH-Px, A005-102), catalase (CAT, A007-1-1).

Enterococcus faecalis was enumerated according to China National Standard GB/T 34224-2017. Briefly, 1 g of the strain powder was diluted with 9 mL of 1% Tryptic Soy Broth medium (TSB, Hopebio Co., Ltd, Qingdao, China) and then homogenized. Viable counts of bacteria were conducted by plating serial 10-fold dilutions onto TSB agar plates after being cultured at anaerobically 37 °C, pH 6.2, for 24 h.

2.5. Statistical analysis

Variables were analyzed according to the following mathematical model:

$$Y_{ij} = \mu + \beta_i + \varepsilon_{ij}, \quad (1)$$

in which Y_{ij} = observation *j* of experimental unit subjected to treatments *i*, μ = general constant, β_i = effects of PCM, ECM, and DCM, and ε_{ij} = random error associated to each observation. All data were expressed as mean and SEM using one-way ANOVA program of IBM SPSS Statistics 23. Differences in variables were separated using Tukey's b test at a significance level of $P<0.05$.

3. Results

Cottonseed meal degraded with protease, *E. faecalis*, or both did not affect the contents of gross energy, DM, CP, crude fat, and ash (some unaffected data not shown), increased ($P<0.001$) peptides and organic acids, and decreased ($P<0.001$) phytate P, nitrogen-free extract, and arginine (Table 2). Compared wiith PCM and ECM, the effect of DCM on peptides, organic acids, and phytate phosphorus was more pronounced ($P<0.05$). Furthermore, the toxic residue, free gossypol, disappeared with the fermentation.

Table 2 - Compositions of cottonseed peptides produced by protease, *E. faecalis*, and both

Item	Control	PCM	ECM	DCM	SEM	P-value
Dry matter (%)	90.7	89.5	89.5	89.5	0.410	0.163
Crude protein (%)	50.0	49.6	49.5	49.6	0.430	0.851
Peptides (≤ 1 kDa, %)	5.42d	40.4b	35.6c	55.6a	0.451	<0.001
Phytate P (%)	0.66a	0.51b	0.42c	0.25d	0.023	<0.001
Organic acids (%)	0.58d	0.82c	3.73b	4.45a	0.043	<0.001
Nitrogen-free extract (%)	27.3a	25.6a	9.51b	5.22c	0.418	0.031
Total Arg (%)	5.79a	5.69a	4.26b	4.04b	0.055	<0.001
Total Lys (%)	2.25b	2.47b	2.47b	2.51a	0.021	0.002
Total Val (%)	2.37b	2.35b	2.48a	2.49a	0.023	0.007
Protease (U kg^{-1})	–	155	–	142	10.15	0.851
E. faecalis (log10 CFU g^{-1})	–	–	6.27	5.46	0.352	0.428
Free gossypol (mg kg^{-1})	55.3a	31.1b	–	–	4.271	0.025

– Undetectable; SEM - standard error of the mean.
Control - containing non-degraded cottonseed meal; PCM - cottonseed meal degraded with PROMAX® Protease; ECM - cottonseed meal degraded with *E. faecalis* strain CGMCC 18915; DCM - dual-degraded cottonseed meal with the protease and *E. faecalis*.
a-d - Means within a row with different letters were significantly different ($P<0.05$, Turkey's b test).

During the growing, finishing, and whole phase, PCM, ECM, and DCM beneficially affected (P≤0.025) average daily feed intake (ADFI), average daily body weight gain (ADG), and feed:gain (Table 3). The DCM treatment was more pronounced (P<0.05) on ADFI than PCM and ECM during the growing and whole phase. For the ADG, during the growing phase, DCM had a better effect (P<0.05) than PCM, but had a similar impact to that of ECM, whereas during the finishing and whole phase, there were no differences among the three degraded groups. For feed:gain, there were no differences among PCM, ECM, and DCM. Additionally, one pig died during the feeding trial, which was irrelevant to the dietary treatment (data not shown).

During the finishing phase, the dietary treatment influenced (P≤0.027) the apparent ileal digestibility of dry matter, energy, crude protein, Arg, Thr, Ile, Leu, Lys, Met, Cys, Ser, and Gly (Table 4). In contrast with the control, all degraded groups increased (P<0.05) the digestibility of DM, energy, CP, Ser, and Cys. Among the degraded groups, DCM showed the most excellent (P<0.05) digestibility on Lys and Gly.

During the growing phase, the dietary treatment increased (P≤0.047) the activities of SOD, GSH-Px, and CAT and the concentrations of IgG, IgA, and IgM in the serum of pigs (Table 5). In contrast with the control, PCM, ECM, and DCM increased (P<0.05) SOD, CAT, IgG, and IgM. The DCM treatment had a more pronounced (P<0.05) effect on IgA, IgG, IgM, and SOD than PCM or ECM.

Table 3 - Effect of cottonseed peptides on the growth performance of pigs

Item	Control	PCM	ECM	DCM	SEM	P-value
Growing phase (6 wk)						
Average daily feed intake (kg pig^{-1})	1.40c	1.61b	1.60b	1.66a	0.013	<0.001
Average daily gain (kg pig^{-1})	0.58c	0.70b	0.74ab	0.80a	0.019	<0.001
Feed:gain	2.43a	2.29ab	2.18ab	2.08b	0.070	0.025
Finishing phase (7 wk)						
Average daily feed intake (kg pig^{-1})	2.69b	2.76a	2.76a	2.77a	0.011	<0.001
Average daily gain (kg pig^{-1})	0.90b	1.03a	1.07a	1.04a	0.030	0.005
Feed:gain	3.01a	2.69b	2.60b	2.68b	0.074	0.006
Whole phase						
Average daily feed intake (kg pig^{-1})	2.05c	2.18b	2.18b	2.22a	0.009	<0.001
Average daily gain (kg pig^{-1})	0.74b	0.87a	0.90a	0.92a	0.013	<0.001
Feed:gain	2.77a	2.52b	2.42b	2.42b	0.034	<0.001

SEM - standard error of the mean.
Control - containing non-degraded cottonseed meal; PCM - cottonseed meal degraded with PROMAX® Protease; ECM - cottonseed meal degraded with *E. faecalis* strain CGMCC 18915; DCM - dual-degraded cottonseed meal with the protease and *E. faecalis*.
a-c - Means within a row with different letters were significantly different (P<0.05, Turkey's b test).

Table 4 - Effect of cottonseed peptides on the apparent ileal digestibility of nutrients of finishing pigs

Item	Control	PCM	ECM	DCM	SEM	P-value
Dry matter (%)	73.7b	81.4a	82.2a	85.3a	4.551	<0.001
Energy (%)	66.3c	71.5b	74.0ab	74.9a	2.851	<0.001
Crude protein (%)	73.2b	80.6a	83.1a	83.9a	4.750	<0.001
Ile (%)	76.5b	83.1a	78.0b	85.3a	1.023	<0.001
Leu (%)	77.6b	83.0a	82.2ab	85.7a	1.318	0.003
Lys (%)	80.3b	83.5b	83.7b	87.8a	1.017	0.001
Met (%)	68.8ab	67.1b	69.4ab	71.4a	0.808	0.018
Cys (%)	80.9b	84.0ab	86.0ab	87.0a	1.376	0.027
Ser (%)	74.0b	80.2a	80.7a	83.4a	1.405	0.002
Gly (%)	70.5c	75.5b	76.0b	81.7a	0.995	<0.001

SEM - standard error of the mean.
Control - containing non-degraded cottonseed meal; PCM - cottonseed meal degraded with PROMAX® Protease; ECM - cottonseed meal degraded with *E. faecalis* strain CGMCC 18915; DCM - dual-degraded cottonseed meal with the protease and *E. faecalis*.
a-c - Means within a row with different letters were significantly different (P<0.05, Turkey's b test).

Table 5 - Effect of cottonseed peptides on the serum parameters of antioxidation and immunity of pigs

Item	Control	PCM	ECM	DCM	SEM	P-value
Growing phase						
Superoxide dismutase (U mL^{-1})	76.7b	81.1a	81.1a	82.9a	1.108	0.008
Glutathione peroxidase (U mL^{-1})	3.31b	3.77ab	3.79ab	4.05a	0.174	0.047
Catalase (U mL^{-1})	101b	119a	119a	123a	2.525	<0.001
IgG (g L^{-1})	7.54c	8.90b	9.12ab	9.88a	0.201	<0.001
IgA (g L^{-1})	1.02b	1.12ab	1.15ab	1.33a	0.031	<0.001
IgM (g L^{-1})	0.73c	0.95b	0.93b	1.12a	0.033	<0.001
Finishing phase						
Superoxide dismutase (U mL^{-1})	77.4c	103b	111a	108ab	2.127	<0.001
Glutathione peroxidase (U mL^{-1})	3.55b	4.33a	4.19a	4.39a	0.154	0.004
Catalase (U mL^{-1})	92.2b	125a	135a	133a	2.549	<0.001
IgG (g L^{-1})	7.91c	9.46b	11.1a	11.9a	0.323	<0.001
IgA (g L^{-1})	1.02c	1.18b	1.28ab	1.39a	0.044	<0.001
IgM (g L^{-1})	0.77b	0.95a	0.95a	1.02a	0.030	<0.001

SEM - standard error of the mean.
Control - containing non-degraded cottonseed meal; PCM - cottonseed meal degraded with PROMAX® Protease; ECM - cottonseed meal degraded with *E. faecalis* strain CGMCC 18915; DCM - dual-degraded cottonseed meal with the protease and *E. faecalis*.
a-c - Means within a row with different letters were significantly different (P<0.05, Turkey's b test).

4. Discussion

Compared to soybean meal, cottonseed meal has a similar protein level, but toxic free gossypol limits its use in feed formulation (Gadelha et al., 2014). Recently, with the global protein shortage and the oil extracting development, modifying the nutritional values of miscellaneous meals by the technologies of fermentation and enzymolysis has been a hot topic. As known, fermentation is a conventional and natural method for food quality modification and detoxification (Liu et al., 2017, 2018). The fermented cottonseed meal and its application has been well documented, whereas information about the *in vitro* dual degradation with enzymes and probiotics is unavailable. Niu et al. (2021) reported that fermented cottonseed meal increased CP (40.2% versus 44.4%), but decreased free gossypol (150 versus 36 mg/kg). Also, Jazi et al. (2017) compared the significant changes between none versus fermented cottonseed meal for DM (92.6% versus 90.1%), pH (5.91 versus 4.15), and CP (36.3% versus 39.2%). Additionally, Wang et al. (2021) observed that hydrolyzed cottonseed meal was rich in acidic/basic and aromatic aminoacids, and identified 19 peptides. The present study did not identify and compare functional peptides of cottonseed meals *in vitro* degraded, which deserves further study.

The present study showed that dual degradation with the enzyme and the probiotic was more effective on producing peptides. However, more studies are needed on the property of cottonseed meal treated by dual degradation, especially with multi-enzymes and multi-probiotics with the focus on peptides, antinutritional factors, and toxins. Additionally, in the present study, the content of free gossypol in protease-degraded cottonseed meal was reduced from 55.3 to 31.1 mg/kg, whereas it disappeared with the fermentation. This is consistent with the finding that fermentation decreased free gossypol from 584 to 68 mg/kg (Jazi et al., 2017). The Food and Drug Administration of America limited free gossypol to 45 mg/kg in 1974 and has recently approved ultra-low gossypol cottonseed for human and animal consumption in 2019. Currently, in China, the content of free gossypol is limited to 60 mg/kg in the feed of growing/finishing pigs (GB 13078-2001). Obviously, the dephenolization, ultra-low gossypol, dehulling, and *in vitro* degradation will incredibly increase the availability of cottonseed meal in feed formulation.

In the present study, the *in vitro* enzymolytic, fermented, or dually degraded cottonseed meal significantly improved the ADFI, ADG, and feed efficiency of growing-finishing pigs. Similar results were found that fermented cottonseed meal improved the growth performance and decreased the diarrhea rate of broilers and weaned piglets (Jazi et al., 2017; Gu et al., 2021). Diets containing 5–10% cottonseed meal plus enzyme complex did not negatively affect the growth performance of finishing pigs

(Silva et al., 2021). Literature is unavailable about the property of cottonseed meal *in vitro* treated with enzymes or dually treated with enzymes and probiotics and its application in animal feed. In theory, the *in vitro* degradation of cottonseed meal benefits the digestibility of nutrients: the more peptides, the higher digestibility of CP and aminoacids. Indeed, in the present study, the advanced degradation of cottonseed meal by both protease and *E. faecalis* significantly increased the apparent ileal digestibility of most nutrients, indicating that the degradation considerably improved the digestible properties of cottonseed meal. This is consistent with the finding that the fermented cottonseed meal significantly enhanced the digestibility of DM, CP, energy, and aminoacids of weaned piglets (Gu et al., 2021).

The increased serum anti-oxidase activity and immunoglobulins in the degraded groups of the present study indicate that the *in vitro* degradation of cottonseed meal can improve the antioxidant capacity and immune function of pigs. These benefits are mainly ascribed to the peptides and organic acids of degraded cottonseed meal. Fermented cottonseed meal improved IgG, IgM, anti-oxidases, membrane lipids antioxidation, and gut microbiota of broilers and piglets (Tang et al., 2012; Gu et al., 2021). Cottonseed meal fermented by *Candida tropicalis* enhanced health status by improving fat metabolism and altering cecum bacteria-host metabolic cross-talk of broilers (Niu et al., 2021). Wang et al. (2021) observed that cottonseed peptides prepared by enzymatic hydrolysis and microbial fermentation exhibited excellent radical scavenging activity and ferrous ion chelating activity. Yuan et al. (2020) also found that cottonseed protein hydrolysate enhanced metabolism, antioxidant capacities, and innate immunity of blunt snout bream hepatocytes. The present study first reported the effect of cottonseed meal *in vitro* degraded with proteases, probiotics, or both on the antioxidant capacity and immunity of animals; however, more studies are needed.

Additionally, to simplify the experiment, the present study selected a protease and a probiotic strain as the *in vitro* dietary treatment factors. As known, degradation with multi-enzymes and multi-probiotics will have a better effect than a single enzyme or probiotic strain on the quality improvement of feedstock, and so does the diversity of dietary protein sources. Undoubtedly, these may confer cottonseed meal more useful in practice.

5. Conclusions

Degradation by protease, *E. faecalis*, or both improved the nutritional compositions of cottonseed meal, growth performance, nutrient digestibility, and health status of growing-finishing pigs. The effect of dual degradation on these parameters is more pronounced. It is suggested that using dual degradation can improve the quality and application of miscellaneous meal in the feed.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

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