Quality Changes of Cottonseed Meal Before and After Extrusion and Its Effects on Growth Performance, Serum Biochemical Parameters, and Apparent Nutrient Digestibility in Growing-Finishing Pigs (Postprint)

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Abstract

This experiment aimed to investigate the effects of wet extrusion processing on nutrient composition and free gossypol content in cottonseed meal, as well as the impacts of extruded cottonseed meal on growth performance, serum biochemical indices, and nutrient apparent digestibility in growing-finishing pigs. First, cottonseed meal was extruded using a Muyang 56×2 extruder with previously optimized processing parameters, and the nutrient composition and free gossypol content of cottonseed meal and extruded cottonseed meal were comparatively determined. Subsequently, using cottonseed meal and extruded cottonseed meal as primary test materials, 80 Duroc × Landrace × Large White crossbred pigs with an initial body weight of (28.78±3.09) kg were selected and randomly allocated into 5 groups with 4 replicates per group and 4 pigs per replicate (half barrows and half gilts). The control group was fed a corn-soybean meal basal diet, group 1 was fed a diet supplemented with regular cottonseed meal (5% during the growing period and 10% during the finishing period), and groups 2, 3, and 4 were fed diets supplemented with extruded cottonseed meal (5%, 10%, and 15% during the growing period, and 10%, 15%, and 20% during the finishing period, respectively). All diets were formulated to contain equivalent levels of metabolizable energy and crude protein. The experimental period lasted 13 weeks (6 weeks for the growing period and 7 weeks for the finishing period). The results showed that: 1) Extrusion processing had no significant effect on the nutrient content of cottonseed meal; total amino acid content and each essential amino acid content in extruded cottonseed meal increased slightly, while free gossypol content decreased by 87.85%. 2) During the growing period,

compared with the same inclusion level of regular cottonseed meal, dietary supplementation with 5% extruded cottonseed meal increased average daily feed intake and average daily gain of growing pigs (P>0.05), significantly decreased feed-to-gain ratio (P<0.05), and significantly improved apparent digestibility of crude protein, dry matter, ether extract, and partial amino acids (P<0.05). Dietary supplementation with extruded cottonseed meal compared with cottonseed meal significantly decreased serum malondialdehyde (MDA) content (P<0.05) and significantly increased total antioxidant capacity (T-AOC), superoxide dismutase (SOD) activity, and glutathione peroxidase (GSH-Px) activity (P<0.05). Dietary supplementation with extruded cottonseed meal tended to increase serum immunoglobulin, triiodothyronine, and thyroxine levels in growing pigs compared with cottonseed meal. With increasing extruded cottonseed meal inclusion levels, growth performance and nutrient apparent digestibility of growing pigs showed a decreasing trend, and no significant differences were observed at the 15% inclusion level compared with the regular cottonseed meal group (P>0.05). 3) During the finishing period, final body weight, average daily gain, and average daily feed intake of all extruded cottonseed meal groups showed no significant differences compared with the control and regular cottonseed meal groups (P>0.05), but the feed-to-gain ratio of groups 2 and 3 was significantly lower than that of group 1 throughout the period (P<0.05). Dietary supplementation with extruded cottonseed meal also significantly improved antioxidant capacity and immune function of finishing pigs compared with cottonseed meal (P<0.05), and with increasing extruded cottonseed meal inclusion levels, serum MDA content decreased significantly (P<0.05) while T-AOC, SOD activity, and GSH-Px activity increased gradually (P<0.05). Apparent digestibility of crude protein and dry matter in all treatment groups was significantly lower than that in the control group (P<0.05), with no significant differences in crude protein apparent digestibility between extruded cottonseed meal groups and group 1 (P>0.05), but dry matter apparent digestibility in all extruded cottonseed meal groups was significantly higher than that in group 1 (P<0.05). Ether extract apparent digestibility in groups 3 and 4 was significantly higher than that in the control and group 1 (P<0.05), and increased gradually with increasing extruded cottonseed meal inclusion levels. Dietary supplementation with appropriate levels of extruded cottonseed meal significantly improved amino acid apparent digestibility compared with cottonseed meal (P<0.05). In conclusion, extrusion processing had minimal effects on nutrient content of cottonseed meal while significantly reducing free gossypol content. Dietary supplementation with extruded cottonseed meal in growing-finishing pigs can significantly improve growth performance, antioxidant capacity, immune function, and nutrient apparent digestibility, with inclusion levels up to 15% for growing pigs and up to 20% for finishing pigs.

Full Text

Quality Changes of Cottonseed Meal Before and After Extrusion and Their Effects on Growth Performance, Serum Biochemical Indices, and Nutrient Apparent Digestibility in Growing-Finishing Pigs

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Abstract

This study investigated the effects of wet extrusion processing on nutrient composition and free gossypol content in cottonseed meal (CM) and evaluated the impacts of extruded cottonseed meal (ECM) on growth performance, serum biochemical indices, and nutrient apparent digestibility in growing-finishing pigs. First, a Muyang 56×2 extruder with optimized processing parameters was used to extrude CM, and the nutrient composition and free gossypol content were compared between CM and ECM. Subsequently, eighty Duroc × Landrace × Large White crossbred pigs with an initial body weight of (28.78±3.09) kg were randomly allocated to five groups with four replicates per group and four pigs per replicate (half barrows and half gilts). The control group received a corn-soybean meal basal diet, experimental group 1 received diets supplemented with regular CM (5% during the growing period and 10% during the finishing period), and experimental groups 2, 3, and 4 received diets supplemented with ECM at 5%, 10%, and 15% during the growing period and 10%, 15%, and 20% during the finishing period, respectively. All diets were formulated to contain equivalent levels of metabolizable energy and crude protein. The experiment lasted 13 weeks (6 weeks growing period and 7 weeks finishing period). The results showed that: (1) Extrusion had no significant effect on the nutrient content of CM, though total amino acid and essential amino acid contents increased slightly, while free gossypol content decreased by 87.85%. (2) During the growing period, compared with the same inclusion level of regular CM, dietary supplementation with 5% ECM improved average daily feed intake and average daily gain ($P>0.05$), significantly reduced feed-to-gain ratio ($P<0.05$), and significantly increased apparent digestibility of crude protein, dry matter, ether extract, and some amino acids ($P<0.05$). ECM supplementation also significantly reduced

serum malondialdehyde (MDA) content (P<0.05) and significantly increased total antioxidant capacity (T-AOC), superoxide dismutase (SOD) activity, and glutathione peroxidase (GSH-Px) activity (P<0.05) compared with CM. ECM supplementation tended to increase serum immunoglobulin, triiodothyronine, and thyroxine levels. However, as ECM inclusion increased, growth performance and nutrient apparent digestibility showed a decreasing trend, with no significant difference observed at 15% inclusion compared with the regular CM group (P>0.05). (3) During the finishing period, final body weight, average daily gain, and average daily feed intake did not differ significantly among ECM groups, the control group, and the regular CM group (P>0.05), but the feed-to-gain ratio in experimental groups 2 and 3 was significantly lower than in experimental group 1 (P<0.05). ECM supplementation significantly improved antioxidant capacity and immune function in finishing pigs (P<0.05), with serum MDA content decreasing significantly (P<0.05) and T-AOC, SOD activity, and GSH-Px activity increasing gradually (P<0.05) as ECM inclusion increased. Apparent digestibility of crude protein and dry matter was significantly lower in all treatment groups than in the control group (P<0.05), while crude protein digestibility in ECM groups did not differ significantly from experimental group 1 (P>0.05). Dry matter digestibility in all ECM groups was significantly higher than in experimental group 1 (P<0.05). Ether extract digestibility in experimental groups 3 and 4 was significantly higher than in the control group and experimental group 1 (P<0.05), increasing gradually with ECM inclusion. Appropriate ECM supplementation significantly improved amino acid apparent digestibility compared with CM (P<0.05). In conclusion, extrusion processing has minimal impact on CM nutrient content while significantly reducing free gossypol content. Dietary ECM supplementation can significantly improve growth performance, antioxidant capacity, immune function, and nutrient apparent digestibility in growing-finishing pigs, with recommended inclusion levels up to 15% for growing pigs and 20% for finishing pigs.

Keywords: extruded cottonseed meal; growing-finishing pigs; free gossypol; growth performance; serum biochemical indices; apparent digestibility

Introduction

Cottonseed meal is a high-quality protein source used in livestock and poultry feed production due to its low cost and high crude protein content. However, its utilization is severely limited by high free gossypol content, which restricts its inclusion rate in feed formulations [1-3]. As the most abundantly produced oilseed meal in China, cottonseed meal utilization in animal feed remains below 35%. Therefore, developing processing methods to reduce free gossypol content and improve protein utilization is crucial for alleviating protein feed resource shortages and reducing production costs.

Extrusion is a process that uses screw propulsion to move material forward,

subjecting it to mixing, stirring, friction, and high shear forces that generate high temperature and pressure. Upon exiting the die, the sudden pressure drop causes instantaneous expansion and volume increase, altering product morphology [4]. Extrusion gelatinizes starch, denatures proteins, and modifies other nutrients while degrading some free gossypol and binding another portion to proteins, thereby significantly reducing free gossypol content [6-7] and achieving detoxification. Previous studies have demonstrated that extrusion effectively reduces free gossypol and improves cottonseed meal quality [8-10]. Wei et al. [11] found that temperature, time, and humidity during heat treatment significantly affect total, bound, and free gossypol elimination, with most free gossypol being degraded. However, previous research has focused primarily on detoxification effects and impacts on poultry performance, lacking comprehensive studies on extrusion technology and its effects on growth performance, serum biochemical indices, and nutrient digestibility in growing-finishing pigs. Therefore, this experiment aimed to investigate the effects of extrusion on nutrient composition and free gossypol content in cottonseed meal and evaluate the feasibility of using ECM as a protein source for growing-finishing pigs to improve utilization and inclusion rates while reducing feeding costs.

Materials and Methods

1.1.1 Extrusion Process and Parameters **Extrusion material:** Feed-grade cottonseed meal
Equipment: Muyang 56×2 extruder
Parameters: Conditioning temperature 95°C, material moisture after conditioning 17.6%, screw speed 210 r/min, feed rate 75 kg/h, extrusion temperature 132°C, die opening area 300 $mm^2/(t \cdot h)$

1.2 Experimental Animals and Design Eighty healthy crossbred growing pigs (Duroc × Landrace × Large White) with an average initial body weight of (28.78±3.09) kg were randomly allocated to five groups with four replicates each and four pigs per replicate (half barrows and half gilts). Body weight was consistent across groups and replicates ($P>0.05$).

1.3 Experimental Diets and Design The 13-week experiment consisted of a 6-week growing period and 7-week finishing period. The control group received a corn-soybean meal basal diet. Experimental group 1 received diets supplemented with regular cottonseed meal (5% during growing, 10% during finishing). Experimental groups 2, 3, and 4 received diets supplemented with extruded cottonseed meal at 5%, 10%, and 15% during the growing period and 10%, 15%, and 20% during the finishing period, respectively. All diets were formulated to equivalent metabolizable energy and crude protein levels according to NRC (2012) standards for 30-120 kg pigs. Diet composition and nutrient levels are shown in Table 1 .

Table 1 Diet Composition and Nutrient Levels (Air-Dry Basis)

Item	Control Group	Experimental Group 1	Experimental Group 2	Experimental Group 3	Experimental Group 4
	Growing	Finishing	Growing	Finishing	Growing
Ingredients					
Corn	[values]	[values]	···	···	···
Soybean meal	[values]	[values]	···	···	···
Cottonseed meal	0	0	5%	10%	0
Extruded cotton-seed meal	0	0	0	0	5%
Wheat bran	[values]	[values]	···	···	···
Soybean oil	[values]	[values]	···	···	···
Premix[1]	[values]	[values]	···	···	···
Nutrient Levels[2]					
ME (MJ/kg)	13.39	···	···	···	···
CP	···	···	···	···	···
EE	···	···	···	···	···
TP	···	···	···	···	···
Lys	···	···	···	···	···
Met+Cys	···	···	···	···	···
Free gossypol (mg/kg)	···	···	···	···	···

[1]Premix provided per kg of diet: VA 65,000 IU, VD 37,500 IU, VE 450 mg, VK 15 mg, VB 24.2 mg, VB 73 mg, VB 36.2 mg, VB 0.73 mg, folic acid 7.5 mg, pantothenate 250 mg, biotin 5.0 mg, Fe 2.5 g, Cu 1.5 g, Zn 2.5 g, Mn 0.75 g, I 3.5 mg, Se 5.0 mg.
[2]CP, EE, Lys, and Met+Cys were measured values; others were calculated values.

1.4 Animal Management The experiment was conducted at the Nankou Pilot Base of the Chinese Academy of Agricultural Sciences. Pig houses were

cleaned and disinfected one week before the trial. A 3-day adaptation period preceded the 13-week experimental period. Pigs were housed in mixed-gender pens with ad libitum access to feed and water. Houses were kept clean and ventilated, with controlled temperature and regular disinfection. Body weight was measured after overnight fasting at the end of weeks 6 and 13.

1.5 Sample Collection and Analysis

1.5.1 Nutrient and Free Gossypol Content Crude fat was determined using an M392885 Soxtec2050 automatic Soxhlet extraction system. Amino acids were analyzed using an L-8900 high-speed amino acid auto-analyzer. Crude protein, dry matter, crude ash, and free gossypol were determined according to GB/T 6432–1994, GB/T 10358–1989, GB/T 6438–2007, and GB/T 13086–1991 methods, respectively.

1.5.2 Growth Performance Growth performance was calculated per replicate, including final average body weight, average daily feed intake, average daily gain, and feed-to-gain ratio.

1.5.3 Serum Biochemical Indices At the end of weeks 6 and 13, eight pigs per group (two per replicate, one barrow and one gilt) were randomly selected for jugular blood collection (5-10 mL). Serum was prepared by centrifugation at 3,000 r/min for 5 min. Serum MDA content, T-AOC, and SOD and GSH-Px activities were measured using kits from Nanjing Jiancheng Bioengineering Institute with an L-3180 semi-automatic biochemical analyzer. Serum IgG, IgA, and IgM were measured using a KHB-1280 automatic biochemical analyzer. Serum triiodothyronine (T) and thyroxine (T) were determined by radioimmunoassay using a GC-1200 automatic radioimmunoassay counter.

1.5.4 Nutrient Apparent Digestibility During the last 3 days of weeks 6 and 13, fresh feces were collected daily from each group, mixed, and weighed. Twenty mL of 5% HCl was added per 100 g feces, then samples were dried at 65°C for 72 hours, equilibrated at room temperature for 24 hours, ground through a 40-mesh sieve, and stored for analysis. Nutrient and acid-insoluble ash contents in diets and feces were determined according to national standards to calculate apparent digestibility.

Apparent digestibility (%) = 100 - [(Indicator in diet × Nutrient in feces) / (Indicator in feces × Nutrient in diet)] × 100

1.6 Statistical Analysis Data were initially processed using Excel 2007. One-way ANOVA and covariance analysis were performed using SAS 9.2 software. Duncan' s multiple range test was used for significance analysis. Results are expressed as means ± standard deviation, with significance set at $P<0.05$.

Results

2.1.1 Changes in Nutrient Content Before and After Extrusion As shown in Table 2 , extrusion caused minor changes in major nutrient contents. Dry matter decreased by 2.58%, crude protein decreased by 4.37%, and crude fiber increased by 14.00%. Extrusion had minimal effects on ether extract, crude ash, and amino acid contents. Notably, lysine (a primary limiting amino acid for pigs) increased by 1.53%, methionine decreased by 9.52%, and total amino acid content increased by 0.50%.

Table 2 Changes in Nutrient Content of Cottonseed Meal Before and After Extrusion (DM Basis)

Item	Cottonseed Meal	Extruded Cottonseed Meal
Dry matter	···	···
Crude protein	···	···
Ether extract	···	···
Crude fiber	···	···
Crude ash	···	···
Amino acids		
Asp	···	···
Thr	···	···
Ser	···	···
Glu	···	···
Gly	···	···
Ala	···	···
Cys	···	···
Val	···	···
Met	···	···
Ile	···	···
Leu	···	···
Tyr	···	···
Phe	···	···
Lys	···	···
His	···	···
Arg	···	···
Pro	···	···
Total AA	···	···

2.1.2 Changes in Free Gossypol Content Before and After Extrusion As shown in Table 3 , free gossypol content decreased from 589.47 mg/kg to 71.06 mg/kg after extrusion, a reduction of 87.95%. This demonstrates that extrusion effectively reduces free gossypol content, primarily because some free gossypol is degraded while the remainder binds to proteins [7-8], achieving detoxification.

Table 3 Changes in Free Gossypol Content of Cottonseed Meal Before and After Extrusion (DM Basis)

Item	Cottonseed Meal	Extruded Cottonseed Meal
Free gossypol (mg/kg)	589.47	71.06

2.2 Effects of Extruded Cottonseed Meal on Growth Performance As shown in Table 4 , during the growing period, final body weight and average daily gain in experimental groups 1 and 4 were significantly lower than in the control group (P<0.05), while groups 2 and 3 did not differ significantly from the control (P>0.05). Average daily feed intake did not differ among groups (P>0.05). Feed-to-gain ratio was significantly higher in groups 1 and 4 than in the control (P<0.05), significantly lower in group 2 than all other groups (P<0.05), and not different between group 3 and the control (P>0.05) but significantly lower than group 1 (P<0.05).

During the finishing period, no significant differences were observed among groups for final body weight, average daily gain, average daily feed intake, or feed-to-gain ratio (P>0.05). However, group 1 showed the poorest performance, while groups 2 and 3 had lower feed-to-gain ratios. Notably, 20% ECM inclusion did not adversely affect finishing pig performance.

Over the entire experimental period, average daily feed intake and average daily gain did not differ significantly among groups (P>0.05). Feed-to-gain ratio was significantly higher in groups 1 and 4 than in the control (P<0.05), significantly lower in group 2 than the control (P<0.05), and not different between group 3 and the control (P>0.05).

Table 4 Effects of Extruded Cottonseed Meal on Growth Performance of Growing-Finishing Pigs

Item	Control	Experimental Group 1	Experimental Group 2	Experimental Group 3	Experimental Group 4
Growing period					
Initial BW (kg)	28.89±3.27	29.02±3.82	28.08±2.59	29.06±3.50	28.89±3.54
Final BW (kg)	62.17±6.52	62.17±5.69	59.80±4.50	59.60±8.36	55.63±9.00
ADFI (kg)	1.62±0.11	1.46±0.10	1.60±0.16	1.59±0.21	1.49±0.33

Item	Control	Experimental Group 1	Experimental Group 2	Experimental Group 3	Experimental Group 4
ADG (kg)	0.77±0.10	1.61±0.05	0.74±0.07	0.71±0.13	0.58±0.16
F/G	2.26±0.02	5.43±0.02	2.07±0.11	2.29±0.10	2.54±0.12
Finishing period					
Final BW (kg)	108.05±12.38	103.93±8.39	106.12±7.60	104.11±7.05	101.00±14.16
ADFI (kg)	2.73±0.12	7.67±0.25	2.75±0.12	2.53±0.03	2.59±0.26
ADG (kg)	0.92±0.13	3.92±0.07	0.93±0.12	0.89±0.04	0.91±0.11
F/G	2.86±0.22	0.91±0.06	2.81±0.07	2.85±0.08	2.86±0.12
Whole period					
ADFI (kg)	2.26±0.12	8.15±0.24	2.40±0.24	2.16±0.15	2.24±0.18
ADG (kg)	0.85±0.10	2.77±0.06	0.84±0.09	0.81±0.05	0.77±0.12
F/G	2.59±0.02	2.73±0.04	2.50±0.01	2.61±0.05	2.73±0.08

Values in the same row with different superscripts differ significantly (P<0.05). The same applies below.

2.3.1 Effects of Extruded Cottonseed Meal on Serum Antioxidant Indices As shown in Table 5 , during the growing period, serum MDA content decreased significantly with increasing ECM inclusion (P<0.05), with groups 3 and 4 significantly lower than the control and group 1 (P<0.05). Serum T-AOC and SOD and GSH-Px activities in group 4 were significantly higher than in the control (P<0.05), and all antioxidant indices in groups 3 and 4 were significantly better than in group 1 (P<0.05).

During the finishing period, serum MDA content also decreased significantly with increasing ECM inclusion (P<0.05), with groups 3 and 4 significantly lower than group 1 (P<0.05). Serum SOD activity in group 1 was significantly lower than in the control, group 3, and group 4 (P<0.05), while group 4 was significantly higher than the control (P<0.05). Serum T-AOC in group 1 was significantly lower than the control (P<0.05), while groups 3 and 4 were significantly higher than the control (P<0.05). Serum GSH-Px activity in group 1 was significantly lower than all other groups (P<0.05).

Table 5 Effects of Extruded Cottonseed Meal on Serum Antioxidant Indices of Growing-Finishing Pigs

Item	Control	Experimental Group 1	Experimental Group 2	Experimental Group 3	Experimental Group 4
Growing period					
MDA (nmol/mL)	4.87±0.16	4.04±0.60	5.20±0.13	4.24±0.32	3.58±0.37
SOD (U/mL)	92.00±1.77	96.78±4.00	82.68±6.53	96.31±4.50	102.98±4.78
T-AOC (U/mL)	9.14±0.57	10.17±0.37	8.64±0.49	9.40±0.93	10.96±0.59
GSH-Px (g/L)	3.72±0.43	4.03±0.38	3.46±0.23	4.05±0.19	4.52±0.27
Finishing period					
MDA (nmol/mL)	5.13±0.06	4.03±0.55	5.62±0.30	4.55±0.10	4.28±0.44
SOD (U/mL)	90.60±5.79	95.94±1.68	85.95±4.81	94.82±6.32	99.18±6.15
T-AOC (U/mL)	8.39±0.19	9.80±0.50	8.20±0.19	8.98±0.36	9.70±0.40
GSH-Px (g/L)	4.16±0.53	4.36±0.57	4.05±0.18	4.27±0.35	4.48±0.32

2.3.2 Effects of Extruded Cottonseed Meal on Serum Immune Indices
As shown in Table 6 , during the growing period, serum IgG and IgA in group 4 were significantly higher than in the control and other groups (P<0.05). Other groups showed slightly lower IgG and slightly higher IgA than the control, but differences were not significant (P>0.05). Except for group 3, which did not differ significantly from the control or group 1 in IgM, groups 2 and 4 showed significantly higher IgM than the control and other groups (P<0.05). Serum T in group 2 was significantly higher than in group 1 (P<0.05), and all ECM groups had significantly higher T than group 1 (P<0.05), though not different from the control (P>0.05).

During the finishing period, all treatment groups showed significantly lower IgG than the control (P<0.05). Except for group 2, which did not differ from

the control in IgA, all other groups had significantly lower IgA than the control (P<0.05). Groups 1 and 4 had significantly lower IgM than the control (P<0.05), while groups 2 and 3 did not differ from the control (P>0.05). No significant differences were observed in T or T among treatment groups and the control (P>0.05), though groups 2 and 3 had significantly higher T and T than group 1 (P<0.05).

Table 6 Effects of Extruded Cottonseed Meal on Serum Immune Indices of Growing-Finishing Pigs

Item	Control	Experimental Group 1	Experimental Group 2	Experimental Group 3	Experimental Group 4
Growing period					
IgG (g/L)	9.07±0.48	8.43±0.93	8.04±0.95	8.05±0.39	10.28±0.57
IgA (g/L)	1.03±0.06	0.09±0.10	1.10±0.05	1.05±0.05	1.27±0.05
IgM (g/L)	0.87±0.07	0.75±0.05	1.00±0.09	0.80±0.08	1.04±0.09
T (ng/mL)	1.09±0.10	2.81±0.06	1.41±0.47	1.06±0.21	1.11±0.14
T (ng/mL)	59.30±2.49	8.97±2.38	64.22±6.63	61.06±3.98	57.74±2.01
Finishing period					
IgG (g/L)	11.58±0.79	9.91±0.62	9.83±0.32	9.64±0.57	8.18±0.71
IgA (g/L)	1.32±0.08	1.08±0.12	1.29±0.15	1.07±0.12	1.04±0.09
IgM (g/L)	1.08±0.05	0.74±0.05	0.96±0.10	0.94±0.17	0.79±0.07
T (ng/mL)	1.13±0.14	1.93±0.05	1.44±0.43	1.03±0.16	1.30±0.32
T (ng/mL)	6.74±0.55	2.89±0.88	7.36±1.12	6.99±0.46	6.89±0.89

2.4.1 Effects of Extruded Cottonseed Meal on Nutrient Apparent Digestibility As shown in Table 7 , during the growing period, crude protein and dry matter digestibility in group 1 were significantly lower than in the control (P<0.05), while ECM groups did not differ from the control (P>0.05). All ECM groups showed significantly higher crude protein digestibility than group 1 (P<0.05), and groups 2 and 3 had significantly higher dry matter digestibility

than group 1 (P<0.05). Only group 2 showed significantly higher ether extract digestibility than the control and group 1 (P<0.05). These results indicate that ECM supplementation significantly improved nutrient apparent digestibility in growing pigs, though digestibility tended to decrease as ECM inclusion increased.

During the finishing period, crude protein digestibility was significantly lower in all treatment groups than in the control (P<0.05). Groups 1 and 4 had significantly lower dry matter digestibility than the control (P<0.05). Crude protein digestibility in ECM groups did not differ significantly from group 1 (P>0.05), while dry matter digestibility in all ECM groups was significantly higher than in group 1 (P<0.05). Ether extract digestibility in groups 3 and 4 was significantly higher than in the control and group 1 (P<0.05), increasing gradually with ECM inclusion.

Table 7 Effects of Extruded Cottonseed Meal on Nutrient Apparent Digestibility of Growing-Finishing Pigs

Item	Control	Experimental Group 1	Experimental Group 2	Experimental Group 3	Experimental Group 4
Growing period					
CP	81.65±1.28	78.74±1.45	82.29±1.00	81.55±0.94	80.91±0.63
EE	79.97±0.80	77.97±1.41	82.93±1.04	80.74±1.14	81.29±0.71
DM	84.69±1.82	82.30±1.38	84.94±0.32	84.50±0.25	83.42±0.26
Finishing period					
CP	86.36±1.88	83.55±1.78	83.23±2.36	83.11±1.46	82.93±1.33
EE	74.46±1.74	74.12±1.68	78.45±5.31	80.77±1.83	82.61±0.52
DM	86.51±0.83	84.10±0.46	85.74±1.59	85.81±0.93	84.73±0.82

2.4.2 Effects of Extruded Cottonseed Meal on Amino Acid Apparent Digestibility As shown in Table 8 , during the growing period, no significant differences were observed among groups in apparent digestibility of valine, methionine, lysine, histidine, aspartic acid, tyrosine, glutamic acid, alanine, or total amino acids (P>0.05). Group 1 showed significantly lower digestibility of isoleucine, leucine, threonine, serine, cysteine, and proline than the control (P<0.05), while ECM groups did not differ from the control (P>0.05). All ECM groups had higher amino acid digestibility than group 1, with digestibility increasing as ECM inclusion increased. Extrusion significantly improved apparent digestibility of phenylalanine, arginine, threonine, serine, glycine, and cysteine (P<0.05).

As shown in Table 9 , during the finishing period, groups 1 and 4 showed significantly lower digestibility of valine, isoleucine, leucine, lysine, histidine, threonine, aspartic acid, tyrosine, serine, cysteine, and proline than the control ($P<0.05$). Other amino acid digestibility values in ECM groups were lower than the control but not significantly different ($P>0.05$). All ECM groups had higher amino acid digestibility than group 1, with phenylalanine, arginine, serine, glutamic acid, and glycine digestibility significantly higher than group 1 ($P<0.05$). Amino acid digestibility increased initially then decreased with ECM inclusion, but remained higher than group 1 even at 20% inclusion.

Table 8 Effects of Extruded Cottonseed Meal on Amino Acid Apparent Digestibility of Growing Pigs

Item	Control	Experimental Group 1	Experimental Group 2	Experimental Group 3	Experimental Group 4
Essential AA					
Val	62.04±3.54	40.53±4.37	61.60±4.18	62.60±5.90	61.11±1.39
Met	92.29±2.90	90.87±2.40	91.21±0.29	90.60±1.42	92.12±2.16
Ile	79.28±1.73	65.11±2.64	77.31±2.25	76.84±3.64	75.45±0.77
Leu	87.84±1.24	87.06±1.47	86.25±1.85	86.98±1.98	86.12±0.17
Phe	86.15±1.23	83.80±1.43	86.03±1.25	86.98±1.57	86.95±0.42
Lys	83.30±0.78	68.67±2.28	81.73±2.21	80.41±3.58	79.66±0.05
His	89.49±1.28	88.01±0.81	89.21±0.93	89.97±1.30	90.15±0.79
Arg	92.15±0.77	87.48±0.28	93.65±0.52	94.27±0.77	94.52±0.33
Thr	82.37±1.78	78.93±1.71	80.59±2.08	81.67±2.16	80.76±0.56
Non-essential AA					
Asp	84.90±1.82	82.30±1.35	85.09±1.43	84.31±2.88	83.49±0.25
Tyr	88.93±0.89	86.97±1.48	88.84±1.03	86.94±2.57	88.15±0.16
Ser	88.68±1.35	85.44±0.94	86.96±1.41	88.16±1.30	87.87±0.05
Glu	90.80±0.87	87.47±0.84	91.23±1.18	91.49±1.57	91.02±0.09
Gly	81.74±1.72	72.39±2.05	81.51±1.76	82.44±2.49	82.09±0.57
Ala	82.39±1.52	82.99±1.97	82.47±2.16	82.44±3.62	81.32±0.19
Cys	92.90±0.94	91.69±0.42	93.59±0.35	93.22±1.10	93.10±0.13
Pro	86.59±2.82	82.33±1.47	83.83±2.10	83.49±1.91	83.02±0.92
Total AA	85.78±1.83	84.03±1.43	85.53±1.57	85.86±2.22	85.47±0.21

Table 9 Effects of Extruded Cottonseed Meal on Amino Acid Apparent Digestibility of Finishing Pigs

Item	Control	Experimental Group 1	Experimental Group 2	Experimental Group 3	Experimental Group 4
Essential AA					
Val	85.35±2.72	82.44±1.73	82.61±1.65	83.30±1.69	81.47±2.02
Met	77.09±3.72	74.54±3.25	74.35±2.65	72.53±2.42	72.49±4.15
Ile	84.46±2.57	81.78±1.95	81.84±1.78	81.74±1.88	79.15±2.40
Leu	88.01±2.23	85.54±1.58	85.95±1.63	85.59±1.22	84.71±1.58
Phe	87.99±2.83	86.10±1.38	86.19±1.33	86.73±1.24	86.33±1.37
Lys	86.17±3.79	81.38±1.74	82.20±2.08	83.12±1.75	78.59±2.48
His	91.75±2.48	88.87±1.01	89.66±1.41	89.32±0.94	88.45±0.99
Arg	93.35±1.89	94.98±0.81	92.05±1.00	92.37±0.73	92.24±0.83
Thr	85.08±2.78	80.32±1.93	81.77±1.72	83.48±1.67	81.27±2.23
Non-essential AA					
Asp	87.04±2.82	82.55±1.65	85.12±1.66	85.63±1.41	84.01±1.98
Tyr	87.14±2.28	88.50±2.13	85.19±1.36	84.14±1.63	84.09±1.76
Ser	89.28±1.94	94.44±1.22	87.38±1.40	87.69±0.88	86.85±1.47
Glu	91.02±1.87	87.83±1.25	90.02±1.52	90.54±0.78	90.37±1.41
Gly	84.95±1.78	78.1±1.90	83.14±1.49	83.73±1.45	82.23±2.11
Ala	83.97±2.75	79.35±2.05	81.93±2.19	82.45±1.63	80.71±2.28
Cys	91.12±1.89	91.16±0.53	89.64±0.48	89.76±1.15	89.11±1.24
Pro	90.55±2.26	86.35±1.25	87.38±1.29	86.86±1.04	85.49±1.08
Total AA	88.24±2.83	83.51±1.49	86.25±1.54	86.63±1.12	85.60±1.65

Discussion

3.1.1 Effects of Extrusion on Cottonseed Meal Nutrient Content Extrusion is generally considered to have minimal effect on crude protein content, while amino acid destruction depends on processing conditions. Hu et al. [12] found that extruding cottonseed meal at 120°C did not significantly change crude protein content but reduced crude fiber content. Other studies have reported that extrusion degrades crude fiber [13-14]. However, our study showed a slight increase in crude fiber after extrusion, which contradicts previous findings and warrants further investigation. The slight decrease in crude protein and increase in amino acids observed in our study may be due to protein denaturation and degradation of high-molecular-weight proteins into smaller peptides and amino acids. The absence of amino acid destruction suggests our extrusion parameters were appropriate. Liu et al. [10] reported decreased ether extract after corn extrusion. The reduced ether extract in our ECM may be due to formation of starch-lipid complexes during extrusion [15], though further research

is needed on extrusion' s effects on ether extract.

3.1.2 Effects of Extrusion on Free Gossypol Content Yu et al. [6] reported that under high temperature, pressure, and shear forces, free gossypol partially binds to proteins while another portion degrades, significantly reducing free gossypol content. Wei et al. [11] found that temperature, time, and humidity during heat treatment significantly affect gossypol forms. In our study, extrusion reduced free gossypol from 589.47 mg/kg to 71.06 mg/kg (87.85% reduction), demonstrating effective detoxification through degradation and protein binding [7-8], thereby improving cottonseed meal utilization in animal feed.

3.2 Effects of Extruded Cottonseed Meal on Growth Performance Extrusion effectively removes free gossypol, degrades fiber, denatures proteins, gelatinizes starch, and improves palatability through characteristic flavors [14,16]. Qin et al. [17] found that 10% regular cottonseed meal significantly reduced growing pig average daily gain and increased feed-to-gain ratio. Li [18] reported optimal inclusion rates of 5-15% for degossypolized cottonseed protein in growing-finishing pigs without affecting carcass traits or meat quality.

Our results showed that appropriate ECM supplementation significantly improved growing pig performance, while regular cottonseed meal at 5% inhibited performance compared to ECM, demonstrating that extrusion improves cottonseed meal utilization. This aligns with Qin et al. [17], as free gossypol inhibits gastrin release, causing bloating and reduced feed intake [19]. However, 15% ECM inclusion inhibited growing pig performance, indicating that despite improved digestibility, optimal inclusion levels must be observed for different growth stages. This may be due to accumulation of residual free gossypol and other antinutritional factors reducing feed utilization. In finishing pigs, ECM inclusion up to 20% did not adversely affect performance, and 10-20% ECM groups outperformed the 10% regular cottonseed meal group, with no significant differences from the control. This confirms that extrusion increases cottonseed meal inclusion rates in finishing pig diets, reducing feed costs and improving feed efficiency when appropriate levels are used for different growth stages.

3.3.1 Effects of Extruded Cottonseed Meal on Serum Antioxidant Indices T-AOC reflects overall antioxidant capacity and free radical metabolism status [20-21]. SOD eliminates superoxide anion radicals, protecting cellular function, with activity indicating free radical scavenging ability [22]. SOD and GSH-Px are complementary components of the antioxidant enzyme system. Knych [23] reported that SOD inhibits gossypol-induced vascular endothelial dysfunction. MDA content reflects cellular damage and lipid peroxidation. Yang [24] found no significant effect of dietary free gossypol on SOD activity in laying hens, while Zhang et al. [25] observed no significant differences in serum antioxidant indices between treatment and control groups.

Our results showed that ECM supplementation significantly increased T-AOC,

SOD, and GSH-Px activities while reducing MDA content in growing pigs, indicating improved antioxidant capacity and free radical metabolism. Regular cottonseed meal supplementation significantly reduced antioxidant capacity compared to the control, likely due to free gossypol impairing antioxidant function and lipid metabolism. These findings differ from Yang [24] and Zhang et al. [25], possibly due to variations in cottonseed meal quality, extrusion parameters, detoxification efficacy, and inclusion levels. Limited research exists on ECM' s effects on antioxidant function in pigs, and further investigation is needed on how free gossypol and other antinutritional factors influence these parameters.

3.3.2 Effects of Extruded Cottonseed Meal on Immune Indices Serum IgG, IgA, and IgM reflect humoral immunity. IgG is the primary and most persistent antibody in primary immune responses; IgA has antimicrobial and antiviral activity; IgM comprises 5-10% of serum immunoglobulins and functions mainly during early infection. T and T are primary indicators for diagnosing thyroid function, being elevated in hyperthyroidism and reduced in hypothyroidism.

During the growing period, ECM supplementation tended to increase IgG, IgA, IgM, T , and T , suggesting improved immune function and basal metabolic rate, possibly due to lower free gossypol content. Extrusion may also reduce toxic components and harmful microorganisms, degrade and refine crude fiber, and improve nutrient digestibility, thereby enhancing growth status and mitigating free gossypol' s immunosuppressive effects, though further research is needed.

During the finishing period, ECM groups showed significantly lower IgG and IgA than the control ($P<0.05$), with IgM also reduced in some groups. T and T did not differ significantly from the control ($P>0.05$), though they were higher than in the regular cottonseed meal group. This suggests that while ECM improves immune function in growing pigs, high cottonseed meal diets reduce immune performance, and long-term ECM feeding may moderately suppress immunity without affecting thyroid function, consistent with Zhang et al. [27].

3.4 Effects of Extruded Cottonseed Meal on Nutrient Apparent Digestibility Extrusion destroys protein tertiary structure, exposing hydrophobic groups and causing irreversible denaturation that facilitates enzymatic digestion. Thomas et al. [15] reported that extruded cottonseed meal forms starch-lipid complexes, increasing lipid stability and improving feed appearance and palatability. Our study demonstrated that ECM supplementation significantly improved crude protein, ether extract, dry matter, and amino acid digestibility in growing pigs compared to regular cottonseed meal, with no significant differences from the control. This confirms that extrusion significantly improves cottonseed meal digestibility, making its feeding value comparable to soybean meal, consistent with Liu et al. [26].

Over time, crude protein, ether extract, and dry matter digestibility in ECM

groups tended to decrease, suggesting that prolonged feeding reduces nutrient digestibility. This may result from accumulation of unmetabolized free gossypol, which damages the digestive tract, stimulates mucosal surfaces, causes ulcers and gastroenteritis, or inactivates enzymes by binding to substrates or active sites [19,27]. Studies show that supplemental ferrous sulfate, lysine, or vitamin E can mitigate gossypol's effects [28], as lysine and vitamin E bind free gossypol to form less toxic complexes [29]. Therefore, using these additives in combination with ECM should be considered.

Conclusion

1. Extrusion processing has minimal impact on cottonseed meal nutrient content, slightly increases total and essential amino acid contents, and significantly reduces free gossypol content by up to 87.85%.
2. Compared with regular cottonseed meal at the same inclusion level, ECM significantly improves growth performance in growing pigs. Extrusion increases the maximum inclusion rate of cottonseed meal in growing-finishing pig diets, with recommended levels up to 15% for growing pigs and 20% for finishing pigs. However, further increases in ECM inclusion reduce average daily feed intake and gain while increasing feed-to-gain ratio.
3. Compared with regular cottonseed meal, ECM significantly improves serum antioxidant capacity and immune function in growing pigs.

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Note: Figure translations are in progress. See original paper for figures.