Effects of Cottonseed Meal on Growth Performance, Liver Redox Status, and Serum Biochemical Parameters in Goslings

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Research

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Abstract

Background: Cottonseed meal (CSM), a relatively rich source of protein and amino acids, is used as an inexpensive alternative to soybean meal (SBM) in poultry diets. Two experiments were conducted to investigate the effects of CSM on growth performance, liver redox status, and serum biochemical parameters in goslings. In experiment 1, 300 1-d-old male goslings were randomly divided into 5 groups (10 goslings/replicate, 6 replicates/group) and subjected to a 28-d experiment. In experiment 2, 240 28-d-old male goslings were randomly divided into 5 groups (8 goslings/replicate, 6 replicates/group) and subjected to a 35-d experiment. Five isonitrogenous and isoenergetic diets were formulated to produce diets in which 0% (control), 25% (CSM_{25}), 50% (CSM_{50}), 75% (CSM_{75}), and 100% (CSM_{100}) of protein from SBM was replaced by protein from CSM.

Results: In experiment 1, dietary CSM was associated with linear decreases in body weight, average daily feed intake and average daily gain and linear increases in the feed-to-gain ratio and mortality from 1 to 28 d of age ($P < 0.05$). Increased dietary CSM was associated with linear decreases in the hydroxyl radical scavenging ability and superoxide dismutase, catalase and glutathione peroxidase activities in the liver ($P < 0.05$). Dietary CSM was associated with linear decreases in serum total protein, albumin, and globulin concentrations and linear increases in serum uric acid concentrations ($P < 0.05$). In experiment 2, the growth performance from 29 to 63 d, redox status indicators (except for malondialdehyde [MDA]) in the liver, and serum biochemical parameters at d 63 were not affected by dietary CSM concentrations ($P > 0.05$). The MDA content in the liver in the CSM_{100} group was lower than those in the other four groups ($P < 0.05$).

Conclusion: CSM in gosling diets should not exceed 7.08% in the early growth stage (d 1 to 28), but it can be increased to 22.65% from d 29 to 63. A high concentration of CSM may reduce growth performance in 1- to 28-d-old goslings due to decreases in feed intake, liver metabolism and antioxidant capacity.

Background

Feed cost accounts for approximately 70% of the total cost of poultry production [1], and the protein source accounts for a large proportion of that cost. Therefore, some less-expensive byproducts that can replace soybean meal (SBM) as a protein source are used in goose feed by diet formulators and poultry producers in China [2–4].

Cottonseed meal (CSM), an oil industry byproduct, is an inexpensive alternative to SBM in poultry diets due to its relatively high concentrations of protein (30 to 50%) and amino acids [5]. However, the toxicity of free gossypol (FG) in CSM has been a primary concern, limiting its application as a raw protein source in poultry feed [6]. FG, a polyphenolic compound, is associated with reduced performance and increased mortality in broiler and meat ducks [7–10]. Our previous study in goslings found that low-gossypol CSM can completely replace SBM in the diet, with no adverse effects on the growth performance [4]. However,

the effect of CSM with a relatively high FG concentration on goose growth performance needs further evaluation.

In poultry species, gossypol is considered a hepatic toxin. The development of perivascular lymphatic aggregation, biliary hyperplasia, and hepatic cholestasis are typical syndromes of gossypol toxicity in chickens [7]. A study in meat ducks also showed that the degree of liver damage increased with increasing dietary CSM and FG concentrations [10]. These liver injuries are partly due to the formation of free radicals since gossypol plays a major role in forming reactive oxygen species (ROS) through redox cycling by electron transfer functions [11]. Further research on the redox state of the liver in geese is needed.

Therefore, the present study aimed to evaluate the effects of SBM replacement with CSM on the growth performance, liver redox status, and serum biochemical parameters in goslings.

Materials And Methods

Animals, experimental design, and management

The experiments were carried out in Jiangnan White geese. The Jiangnan White goose is a 3-line-crossed commercial white goose in China and was certified by the National Examination and Approval Committee of Domestic Animal and Poultry Breeds in 2018. They are characterized by an intermediate size, rapid early growth, good meat quality, and a strong tolerance and adaptability to coarse feed.

Two experiments were conducted to evaluate the effects of CSM on growth performance, liver redox status, and serum biochemical parameters in goslings of different ages. Both experiments were performed at Yangzhou University Experimental Farm (Gaoyou, China). Experiment 1 was performed from April to May 2019. A total of 300 1-d-old male goslings were obtained from a commercial hatchery (Changzhou Four Seasons Poultry Industry Co. Ltd., Jintan, China). The goslings were randomly divided into 5 groups, with 6 replicates per group and 10 goslings per replicate; the test period was 28 d. In experiment 2, a total of 240 28-d-old male goslings were obtained from Suqian Lihua Animal Husbandry Co. Ltd. (Shuyang, China). The goslings were randomly divided into 5 groups, with 6 replicates per group and 8 goslings per replicate; the test period was 35 d from October to November 2020.

In both experiments, five isonitrogenous and isoenergetic diets were formulated to meet or exceed the nutrient requirements of geese according to the National Research Council (NRC) [12] and previous studies on medium-sized geese from our laboratory [13–16]. A corn-soybean meal (corn-SBM) basal diet was used as the control; 25, 50, 75 or 100% of dietary protein provided by SBM was replaced with protein provided by CSM in the other 4 diet groups (7.08, 14.15, 21.23 and 28.30% CSM (FG: 780 mg/kg) in experiment 1; 5.66, 11.33, 16.99 and 22.65% CSM (800 mg/kg) in experiment 2), which are referred to as the CSM_{25}, CSM_{50}, CSM_{75}, and CSM_{100} groups, respectively. All energy- and protein-containing ingredients (i.e., corn, SBM, CSM, rice husk, and wheat bran) were analyzed for crude protein, crude fiber,

and calcium concentrations before diet formulation. The compositions of the experimental diets are listed in Tables 1 and 2.

Table 1
Feed ingredient and nutrient composition of experimental diets for d 1 to 28 (air-dry basis)

Items	Control[1]	CSM_{25}	CSM_{50}	CSM_{75}	CSM_{100}
Ingredient, %					
Corn	59.50	61.02	62.55	64.07	65.60
Soybean meal (46.96% CP)	27.60	20.70	13.80	6.90	0.00
Cottonseed meal (46.14% CP)	0.00	7.08	14.15	21.23	28.30
Rice husk	3.90	3.17	2.45	1.72	1.00
Wheat bran	5.40	4.36	3.31	2.27	1.23
Limestone	1.00	1.07	1.15	1.23	1.30
Calcium hydrogen phosphate	1.16	1.12	1.08	1.04	1.00
DL-Methionine	0.14	0.14	0.13	0.13	0.12
L-lysine.HCl	0.00	0.04	0.08	0.11	0.15
Salt	0.30	0.30	0.30	0.30	0.30
Premix[2]	1.00	1.00	1.00	1.00	1.00
Total	100.00	100.00	100.00	100.00	100.00
Nutrient level[3], %					
ME (MJ/kg)	11.39	11.39	11.39	11.39	11.39
CP	19.26	19.26	19.26	19.27	19.28
Crude fiber	5.39	5.39	5.39	5.39	5.39
Calcium	0.87	0.87	0.87	0.87	0.87
Available phosphorus	0.41	0.41	0.41	0.41	0.41

[1]Control group was fed a corn-soybean meal basal diet; CSM, cottonseed meal; CSM_{25}, CSM_{50}, CSM_{75}, and CSM_{100} indicate that 25, 50, 75, and 100% of the protein content provided by soybean meal in the control diet was replaced with CSM respectively

[2]Provided per kilogram of complete diet: Vitamin A, 9000 IU; vitamin D, 3000 IU; vitamin E, 18 IU; vitamin K, 1.5 mg; vitamin B_1, 0.9 mg; vitamin B_2, 8 mg; vitamin B_6, 3.2 mg; vitamin B_{12}, 0.01 mg; nicotinic acid, 45 mg; pantothenic acid, 11 mg; folic acid, 0.65 mg; biotin 0.05 mg; choline, 0.35 g; Fe (as ferrous sulphate), 60 mg; Cu (as copper sulphate), 10 mg; Mn (as manganese sulphate), 95 mg; Zn (as zinc sulphate), 90 mg; I (as potassium iodide), 0.5 mg; Se (as sodium selenite), 0.3 mg

[3]Calculated values

Items	Control[1]	CSM_{25}	CSM_{50}	CSM_{75}	CSM_{100}
Lysine	0.94	0.94	0.94	0.94	0.94
Methionine	0.40	0.40	0.40	0.40	0.40
Free gossypol (mg/kg)	0.00	55.19	110.37	165.56	220.74

[1]Control group was fed a corn-soybean meal basal diet; CSM, cottonseed meal; CSM_{25}, CSM_{50}, CSM_{75}, and CSM_{100} indicate that 25, 50, 75, and 100% of the protein content provided by soybean meal in the control diet was replaced with CSM respectively

[2]Provided per kilogram of complete diet: Vitamin A, 9000 IU; vitamin D, 3000 IU; vitamin E, 18 IU; vitamin K, 1.5 mg; vitamin B_1, 0.9 mg; vitamin B_2, 8 mg; vitamin B_6, 3.2 mg; vitamin B_{12}, 0.01 mg; nicotinic acid, 45 mg; pantothenic acid, 11 mg; folic acid, 0.65 mg; biotin 0.05 mg; choline, 0.35 g; Fe (as ferrous sulphate), 60 mg; Cu (as copper sulphate), 10 mg; Mn (as manganese sulphate), 95 mg; Zn (as zinc sulphate), 90 mg; I (as potassium iodide), 0.5 mg; Se (as sodium selenite), 0.3 mg

[3]Calculated values

Table 2
Feed ingredient and nutrient composition of experimental diets for d 29 to 63 (air-dry basis)

Items	Control[1]	CSM_{25}	CSM_{50}	CSM_{75}	CSM_{100}
Ingredient, %					
Corn	59.10	60.41	61.73	63.04	64.35
Soybean meal (43.00% CP)	25.05	18.79	12.53	6.26	0.00
Cottonseed meal (46.14% CP)	0.00	5.66	11.33	16.99	22.65
Rice husk	6.76	6.48	6.21	5.93	5.65
Wheat bran	5.30	4.80	4.30	3.80	3.30
Limestone	1.00	1.06	1.12	1.17	1.23
Calcium hydrogen phosphate	1.27	1.23	1.19	1.14	1.10
DL-Methionine	0.12	0.12	0.12	0.12	0.12
L-lysine.HCl	0.00	0.05	0.10	0.15	0.20
Choline chloride	0.10	0.10	0.10	0.10	0.10
Salt	0.30	0.30	0.30	0.30	0.30
Premix[2]	1.00	1.00	1.00	1.00	1.00
Total	100.00	100.00	100.00	100.00	100.00
Nutrient level[3], %					
ME (MJ/kg)	11.03	11.03	11.03	11.03	11.03
CP	17.00	17.00	17.00	17.00	17.00
Crude fiber	6.45	6.45	6.45	6.44	6.44
Calcium	0.86	0.86	0.86	0.86	0.86

[1]Control group was fed a corn-soybean meal basal diet; CSM, cottonseed meal; CSM_{25}, CSM_{50}, CSM_{75}, and CSM_{100} indicate that 25, 50, 75, and 100% of the protein content provided by soybean meal in the control diet was replaced with CSM respectively

[2]Provided per kilogram of complete diet: Vitamin A, 9000 IU; vitamin D, 3000 IU; vitamin E, 18 IU; vitamin K, 1.5 mg; vitamin B_1, 0.6 mg; vitamin B_2, 6 mg; vitamin B_6, 2 mg; vitamin B_{12}, 0.01 mg; nicotinic acid, 30 mg; pantothenic acid, 9 mg; folic acid, 0.5 mg; biotin 0.04 mg; Fe (as ferrous sulphate), 60 mg; Cu (as copper sulphate), 10 mg; Mn (as manganese sulphate), 95 mg; Zn (as zinc sulphate), 90 mg; I (as potassium iodide), 0.5 mg; Se (as sodium selenite), 0.2 mg

[3]Calculated values

Items	Control[1]	CSM_{25}	CSM_{50}	CSM_{75}	CSM_{100}
Available phosphorus	0.42	0.42	0.42	0.42	0.42
Lysine	0.87	0.87	0.87	0.87	0.87
Methionine	0.37	0.37	0.37	0.37	0.37
Free gossypol (mg/kg)	0	45.30	90.60	135.90	181.20

[1]Control group was fed a corn-soybean meal basal diet; CSM, cottonseed meal; CSM_{25}, CSM_{50}, CSM_{75}, and CSM_{100} indicate that 25, 50, 75, and 100% of the protein content provided by soybean meal in the control diet was replaced with CSM respectively

[2]Provided per kilogram of complete diet: Vitamin A, 9000 IU; vitamin D, 3000 IU; vitamin E, 18 IU; vitamin K, 1.5 mg; vitamin B_1, 0.6 mg; vitamin B_2, 6 mg; vitamin B_6, 2 mg; vitamin B_{12}, 0.01 mg; nicotinic acid, 30 mg; pantothenic acid, 9 mg; folic acid, 0.5 mg; biotin 0.04 mg; Fe (as ferrous sulphate), 60 mg; Cu (as copper sulphate), 10 mg; Mn (as manganese sulphate), 95 mg; Zn (as zinc sulphate), 90 mg; I (as potassium iodide), 0.5 mg; Se (as sodium selenite), 0.2 mg

[3]Calculated values

The geese were raised in wire-floor pens (1.5 m × 0.9 m in experiment 1; 1.8 m × 0.9 m in experiment 2) and had ad libitum access to feed and water. Each pen was equipped with a separate feeder and 2 automatic nipple drinkers. In experiment 1, the geese were exposed to a 24-h photoperiod from d 1 to 14 and an 18-h photoperiod from d 15 to 28. The house temperature was maintained at 28 to 30℃ for the first week and decreased by 2℃ each week until the house temperature was approximately 22℃ on d 28. In experiment 2, the birds were reared under natural daylight, and the room temperature was maintained at approximately 20℃. The experimental protocol and use of animals were approved by the animal care and use committee of Yangzhou University (Yangzhou, China).

Sample collection and preparations

Birds were individually weighed on their arrival. At the end of each trial (d 28 in experiment 1; d 63 in experiment 2), goslings were weighted after a 6-h feed withdrawal, and feed consumption of each replicate pen was recorded. Average daily feed intake (ADFI), average daily gain (ADG), and the feed to gain ratio (F:G) were calculated. Mortality was recorded daily.

At the end of each trial (d 28 in experiment 1; d 63 in experiment 2), 30 goslings (1 gosling per pen) with average body weight (BW) were selected. Approximately 2 mL of blood was collected via the wing vein and centrifuged at 2,000 × *g* for 10 min at 4℃. Serum was collected and stored at − 20℃ until biochemical parameter analysis. Once the blood was collected, birds were exsanguinated by severing the jugular vein and carotid artery on one side of the neck. Samples of the liver (left lobes, 6 goslings/treatment) were excised, flash-frozen in liquid nitrogen, and stored at − 70℃ until further analysis.

Antioxidant capacity

The liver samples were homogenized according to a previously described method [17]. The protein concentrations of the liver homogenates were determined using a Total Protein Quantitative Assay Kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

The scavenging abilities of the superoxide radical ($O_2^{\bullet -}$) and hydroxyl radical (OH•) and the activities of superoxide dismutase (SOD) and catalase (CAT) were measured using a 725N ultraviolet-visible spectrophotometer (INESA Scientific Instrument Co., Ltd., Shanghai, China) with respective commercial kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). The results were calculated based on the protein concentrations of liver homogenates and are expressed as units per milligram of protein for $O_2^{\bullet -}$ scavenging ability (U/mg protein) and units per gram of protein for OH• scavenging ability (U/g protein). SOD and CAT activities are expressed as units per milligram of protein (U/mg protein).

Changes in glutathione (GSH) concentration and activities of GSH-related enzymes in the liver were measured spectrophotometrically with corresponding commercial kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). The GSH concentration is expressed as milligrams per gram of protein (mg/g protein). The activities of glutathione reductase (GR), glutathione peroxidase (GSH-Px), and glutathione S-transferase (GST) were calculated based on the protein concentration and are expressed as units per milligram of protein (U/mg protein).

Reactive oxygen metabolites, malondialdehyde, and protein carbonyl concentrations

The reactive oxygen metabolite (ROM) concentrations in the liver were measured spectrophotometrically according to the d-ROM test based on Costantini and Dell'Omo [18]. The results are expressed as millimoles of hydrogen peroxide (H_2O_2) per gram of protein in the liver (mmol H_2O_2/g protein). The concentration of malondialdehyde (MDA), an index of lipid peroxidation, was evaluated by the thiobarbituric acid reactive substance reaction. The MDA assay kit was purchased from Nanjing Jiancheng Bioengineering Research Institute (Nanjing, China), and the operation method followed the manufacturer's instructions. The MDA concentration was calculated as nanomoles per milligram of protein (nmol/mg protein). Protein carbonyl (PC) concentrations, an indicator of protein oxidation, were determined by the 2,4-dinitrophenylhydrazine reaction according to the method of Gaona-Gaona et al. [19] and are expressed as nanomoles per milligram of protein (nmol/mg protein) in the liver.

Serum biochemical parameters

Serum concentrations of alanine aminotransferase (ALT), aspartate aminotransferase (AST), total protein (TP), albumin (ALB), globulin (GLOB), and uric acid (UA) were determined using a UniCel DxC 800 Synchron fully automatic biochemical analysis system (Beckman Coulter, Los Angeles, CA, USA).

Statistical analysis

One-way ANOVA followed by linear contrast analysis were used to analyze all data in SPSS 17.0 (SPSS Inc., Chicago, IL, USA). Each replicate pen served as the experimental unit for growth, while individual goslings served as the experimental unit for the other experimental data. Mortality data were arcsin transformed before analysis. Data are expressed as means and standard errors of the means (SEMs). Significant differences among the treatment means were determined at $P < 0.05$ by Duncan's multiple range tests.

Results

Experiment 1

BW, ADFI, ADG, and the F:G of goslings fed diets with increasing dietary CSM concentrations are presented in Table 3. The BW of goslings at 28 d linearly decreased with increasing dietary CSM replacing SBM ($P < 0.05$). Dietary CSM linearly decreased the ADFI and ADG but linearly increased the F:G and mortality at 1 to 28 d of age ($P < 0.05$).

Table 3
Effects of cottonseed meal on growth performance of goslings from 1 to 28 d of age

Item	Control[1]	CSM_{25}	CSM_{50}	CSM_{75}	CSM_{100}	SEM	*P*-value	
							ANOVA	Linear
BW, g								
Day 1	90.33	90.08	90.33	90.50	90.50	0.076	0.426	0.177
Day 28	1379^{a}	1299^{a}	1101^{b}	1126^{b}	861^{c}	36.95	< 0.001	< 0.001
Days 1–28								
ADFI, g/d/bird	99.03^{a}	91.98^{ab}	81.81^{c}	84.08^{bc}	65.12^{d}	2.444	< 0.001	< 0.001
ADG, g/d/bird	46.04^{a}	43.19^{a}	36.11^{b}	36.99^{b}	27.53^{c}	1.317	< 0.001	< 0.001
F:G, g/g	2.15^{b}	2.13^{b}	2.27^{a}	2.27^{a}	2.37^{a}	0.021	< 0.001	< 0.001
Mortality (%)	5.00	8.33	11.67	11.67	23.33	2.510	0.192	0.027

^{a,b,c}Mean values within a row with the same superscript letters were not significantly different ($P < 0.05$)

[1]Control group was fed a corn-soybean meal basal diet; CSM, cottonseed meal; CSM_{25}, CSM_{50}, CSM_{75}, and CSM_{100} indicate that 25, 50, 75, and 100% of the protein content provided by soybean meal in the control diet was replaced with CSM respectively

BW, body weight; ADFI, average daily feed intake; ADG, average daily gain; F:G, feed to gain ratio; SEM, standard errors of the mean

The effects of dietary CSM on the antioxidant capacity of the liver are shown in Table 4. As the dietary CSM concentration increased, the OH• scavenging ability and SOD and CAT activities of the liver linearly decreased ($P < 0.05$). However, no effects on the scavenging ability of $O_2 \cdot^-$ in the liver were observed among the five groups ($P > 0.05$). The activity of GSH-Px decreased linearly in response to increasing dietary CSM ($P < 0.05$). Increasing dietary CSM did not affect the GSH concentration or GR and GST activities in the liver ($P > 0.05$).

Table 4
Effects of cottonseed meal on antioxidant capacity in the liver of goslings at 28 d of age

Item	Control[1]	CSM_{25}	CSM_{50}	CSM_{75}	CSM_{100}	SEM	*P*-value	
							ANOVA	Linear
$O_2 \cdot^-$ (U/mg protein)	1456	1375	1374	1382	1347	24.11	0.709	0.245
OH· (U/g protein)	184.3^a	151.1^{ab}	126.8^b	134.9^b	116.4^b	7.182	0.016	0.002
SOD (U/mg protein)	236.8	231.7	232.2	227.8	213.7	2.990	0.126	0.018
CAT (U/mg protein)	33.54^a	27.91^{ab}	26.82^{ab}	20.23^{bc}	17.75^c	1.548	0.003	< 0.001
GSH (mg/g protein)	67.45	62.98	65.64	69.41	62.22	4.271	0.986	0.902
GR (U/mg protein)	7.31	6.86	5.76	6.61	6.85	0.562	0.945	0.782
GSH-Px (U/mg protein)	205.3^a	177.9^{bc}	186.0^b	186.8^b	165.6^c	3.497	0.002	0.001
GST (U/mg protein)	50.65	50.84	53.84	56.19	52.77	1.089	0.495	0.229

^{a,b,c}Mean values within a row with the same superscript letters were not significantly different ($P < 0.05$)

[1]Control group was fed a corn-soybean meal basal diet; CSM, cottonseed meal; CSM_{25}, CSM_{50}, CSM_{75}, and CSM_{100} indicate that 25, 50, 75, and 100% of the protein content provided by soybean meal in the control diet was replaced with CSM respectively

$O_2 \cdot^-$, superoxide radical; OH·, hydroxyl radical; SOD, superoxide dismutase; CAT, catalase; GSH, reduced glutathione; GR, glutathione reductase; GSH-Px, glutathione peroxidase; GST, glutathione S-transferase; SEM, standard errors of the mean

Dietary CSM did not affect the concentrations of ROM, MDA, or PC in the liver ($P > 0.05$; Table 5).

Table 5
Effects of cottonseed meal on the ROM, MDA, and PC concentrations in the liver of goslings at 28 d of age

Item[1]	Control[2]	CSM_{25}	CSM_{50}	CSM_{75}	CSM_{100}	SEM	*P*-value	
							ANOVA	Linear
ROM(mmol H_2O_2/g protein)	50.14	49.17	55.18	58.99	51.23	1.524	0.222	0.260
MDA(nmol/mg protein)	0.63	0.56	0.76	0.60	0.60	0.048	0.776	0.983
PC(nmol/mg protein)	1.84	2.05	1.83	1.96	1.73	0.124	0.945	0.727
[a,b]Mean values within a row with the same superscript letters were not significantly different ($P < 0.05$)								
[1]Control group was fed a corn-soybean meal basal diet; CSM, cottonseed meal; CSM_{25}, CSM_{50}, CSM_{75}, and CSM_{100} indicate that 25, 50, 75, and 100% of the protein content provided by soybean meal in the control diet was replaced with CSM respectively								
ROM, reactive oxygen metabolites; MDA, malondialdehyde; PC, protein carbonyl; SEM, standard errors of the mean								

As shown in Table 6, increasing dietary CSM proportions were associated with linearly decreasing serum TP, ALB, and GLOB concentrations and linearly increasing serum UA concentrations ($P < 0.05$). However, no effects were observed on serum concentrations of ALT or AST ($P > 0.05$).

Table 6
Effect of cottonseed meal on serum biochemical parameters of goslings at 28 d of age

Item	Control[1]	CSM_{25}	CSM_{50}	CSM_{75}	CSM_{100}	SEM	*P*-value	
							ANOVA	Linear
ALT (U/L)	39.50	27.80	34.50	44.00	41.17	2.868	0.473	0.378
AST (U/L)	91.67	76.50	84.60	89.40	109.00	7.740	0.784	0.435
TP (g/L)	38.80	38.48	33.94	32.25	29.83	1.231	0.065	0.005
ALB (g/L)	12.07^{a}	11.05^{ab}	9.22^{bc}	9.80^{ab}	7.30^{c}	0.450	0.003	< 0.001
GLOB (g/L)	26.73	27.43	24.72	22.45	22.53	0.924	0.278	0.043
UA (mmol/L)	131.8^{b}	147.7^{b}	129.5^{b}	159.2^{ab}	223.3^{a}	11.27	0.041	0.011

^{a,b}Mean values within a row with the same superscript letters were not significantly different ($P < 0.05$)

[1]Control group was fed a corn-soybean meal basal diet; CSM, cottonseed meal; CSM_{25}, CSM_{50}, CSM_{75}, and CSM_{100} indicate that 25, 50, 75, and 100% of the protein content provided by soybean meal in the control diet was replaced with CSM respectively

ALT, Alanine aminotransferase; AST, Aspartate aminotransferase; TP, total protein; ALB, albumin; GLOB, globulin; UA, uric acid; SEM, standard errors of the mean

Experiment 2

The effects of CSM on the growth performance of goslings from d 29 to 63 are shown in Table 7. During the experimental period, no birds died in any group; thus, mortality was not evaluated. Dietary CSM had no significant effect on the growth performance of goslings from d 28 to 63 ($P > 0.05$).

Table 7
Effects of cottonseed meal on growth performance of goslings from 28 to 63 d of age

Item	Control[1]	CSM_{25}	CSM_{50}	CSM_{75}	CSM_{100}	SEM	*P*-value	
							ANOVA	Linear
BW, g								
Day 28	1699	1699	1699	1699	1699	0.454	0.998	0.972
Day 63	3799	3812	3772	3808	3732	19.37	0.703	0.343
ADFI, g/d/bird	255	258	252	250	252	2.136	0.857	0.442
ADG, g/d/bird	60.00	60.37	59.24	60.26	58.08	1.317	0.708	0.346
F:G, g/g	4.25	4.27	4.25	4.16	4.35	0.028	0.340	0.698

[a,b]Mean values within a row with the same superscript letters were not significantly different ($P < 0.05$)

[1]Control group was fed a corn-soybean meal basal diet; CSM, cottonseed meal; CSM_{25}, CSM_{50}, CSM_{75}, and CSM_{100} indicate that 25, 50, 75, and 100% of the protein content provided by soybean meal in the control diet was replaced with CSM respectively

BW, body weight; ADFI, average daily feed intake; ADG, average daily gain; F:G, feed to gain ratio; SEM, standard errors of the mean

No effects on the free radical ($O_2{\bullet}^-$ and OH•) scavenging ability, SOD and CAT activities, the GSH concentration or GSH-related enzyme activities in the liver were observed (Table 8; $P > 0.05$).

Table 8
Effects of cottonseed meal on antioxidant capacity in the liver of goslings at 63 d of age

Item	Control[1]	CSM_{25}	CSM_{50}	CSM_{75}	CSM_{100}	SEM	*P*-value	
							ANOVA	Linear
$O_2 \cdot^-$ (U/mg protein)	1581	1543	1580	1547	1472	19.00	0.378	0.123
OH· (U/g protein)	192.3	199.3	184.1	219.0	167.9	7.012	0.214	0.547
SOD (U/mg protein)	151.8	149.8	154.8	152.1	140.1	2.501	0.414	0.245
CAT (U/mg protein)	35.53	36.95	38.01	41.08	33.44	0.995	0.155	0.995
GSH (mg/g protein)	56.58	60.76	63.13	66.84	62.41	1.788	0.504	0.177
GR (U/mg protein)	11.93	10.61	11.19	11.32	10.91	0.340	0.813	0.597
GSH-Px (U/mg protein)	175.0	161.9	171.8	164.6	155.0	2.756	0.146	0.053
GST (U/mg protein)	71.95	73.75	74.01	75.30	74.70	1.029	0.892	0.367

[a,b]Mean values within a row with the same superscript letters were not significantly different ($P < 0.05$)

[1]Control group was fed a corn-soybean meal basal diet; CSM, cottonseed meal; CSM_{25}, CSM_{50}, CSM_{75}, and CSM_{100} indicate that 25, 50, 75, and 100% of the protein content provided by soybean meal in the control diet was replaced with CSM respectively

$O_2 \cdot^-$, superoxide radical; OH·, hydroxyl radical; SOD, superoxide dismutase; CAT, catalase; GSH, reduced glutathione; GR, glutathione reductase; GSH-Px, glutathione peroxidase; GST, glutathione S-transferase; SEM, standard errors of the mean

The dietary CSM concentration had no effect on the concentrations of ROM or PC in the liver ($P > 0.05$; Table 9). However, the MDA content in the liver in the CSM_{100} group was lower than those in the other four groups ($P < 0.05$).

Table 9
Effects of cottonseed meal on the ROM, MDA, and PC concentrations in the liver of goslings at 63 d of age

Item	Control[1]	CSM_{25}	CSM_{50}	CSM_{75}	CSM_{100}	SEM	*P*-value	
							ANOVA	Linear
ROM(mmol H_2O_2/g protein)	18.79	19.34	18.09	18.12	19.89	0.355	0.446	0.700
MDA(nmol/mg protein)	1.43[a]	1.39[a]	1.60[a]	1.34[a]	0.70[b]	0.087	0.005	0.006
PC(nmol/mg protein)	2.63	2.38	2.36	2.55	2.36	0.058	0.448	0.351

[a,b]Mean values within a row with the same superscript letters were not significantly different ($P < 0.05$)

[1]Control group was fed a corn-soybean meal basal diet; CSM, cottonseed meal; CSM_{25}, CSM_{50}, CSM_{75}, and CSM_{100} indicate that 25, 50, 75, and 100% of the protein content provided by soybean meal in the control diet was replaced with CSM respectively

ROM, reactive oxygen metabolites; MDA, malondialdehyde; PC, protein carbonyl; SEM, standard errors of the mean

As shown in Table 10, increasing dietary CSM did not affect serum concentrations of ALT, AST, TP, ALB, GLOB, or UA ($P > 0.05$).

Table 10
Effect of cottonseed meal on serum biochemical parameters of goslings at 63 d of age

Item	Control	CSM_{25}	CSM_{50}	CSM_{75}	CSM_{100}	SEM	*P*-value	
							ANOVA	Linear
ALT (U/L)	25.00	27.17	25.33	23.17	34.33	2.741	0.180	0.175
AST (U/L)	52.73	64.24	79.56	64.79	73.92	4.018	0.272	0.134
TP (g/L)	42.33	43.50	40.33	43.83	40.67	0.767	0.508	0.589
ALB (g/L)	27.17	28.67	25.83	28.67	26.50	0.620	0.521	0.766
GLOB (g/L)	15.17	14.83	14.50	15.17	14.17	0.270	0.749	0.408
UA (mmol/L)	336.0	292.0	328.2	371.5	311.7	18.02	0.733	0.817

[a,b]Mean values within a row with the same superscript letters were not significantly different ($P < 0.05$)

[1]Control group was fed a corn-soybean meal basal diet; CSM, cottonseed meal; CSM_{25}, CSM_{50}, CSM_{75}, and CSM_{100} indicate that 25, 50, 75, and 100% of the protein content provided by soybean meal in the control diet was replaced with CSM respectively

ALT, Alanine aminotransferase; AST, Aspartate aminotransferase; TP, total protein; ALB, albumin; GLOB, globulin; UA, uric acid; SEM, standard errors of the mean

Discussion

To reduce production costs, some inexpensive local crop byproducts have been used in goose diets in China because of the strong tolerance and adaptability to roughage in geese [2–4]. CSM can be used as an inexpensive partial replacement for SBM in poultry diets. Swiatkiewicz et al. [5] reviewed the recent literature and concluded that CSM is an acceptable ingredient in poultry diets and can be safely consumed at a 10 to 15% dietary level, partially replacing SBM. Our previous study showed that low-gossypol CSM can completely replace SBM in the goose diet [4]. In the present study, goslings in the CSM_{50}, CSM_{75} and CSM_{100} groups had a lower ADFI and ADG, but a higher F:G from 1 to 28 d than those of the control group. Similar results in ducks were also reported by Zeng et al. [9], who found that ducks fed a diet containing 33.11% CSM (152.9 mg/kg FG in diet) had reduced ADFI and ADG from d 1 to 14. The reduction in BW gain and feed intake are common signs of gossypol toxicosis [7].

In addition to these general signs, severe feather pecking was also observed in the CSM_{100} group (experiment 1). One of the possible reasons is the imbalance in the amino acid composition in the diet. We also observed a 30% reduction in the ADFI in goslings fed the CSM_{100} diet, which may lead to protein, amino acid, and mineral deficiencies, increasing the risk of feather pecking [20]. These results indicated that CSM as a protein source in early goose diets (d 1–28) should be limited to 7.08%.

The liver may be one of the first tissues negatively affected by dietary gossypol because the liver had the highest concentration of gossypol residues among all the tissues [10, 21]. The mechanism of gossypol's effect on the liver is partly due to its involvement in the formation of free radicals [11], which alter the liver's redox state. In this experiment, we determined the $O2•^-$ and OH• scavenging abilities; SOD and CAT activities; GSH content; and GSH-related enzyme activities (including GR, GSH-Px, and GST activities) to evaluate the antioxidant capacity of the liver. $O2•^-$ and OH• are two representative ROS. When ROS levels exceed the scavenging capability of the antioxidant system, oxidative stress occurs [22]. In the present study, the OH• scavenging ability of the liver linearly decreased in response to the increase in dietary CSM. Hydrogen peroxide may be converted into water by the enzymes CAT and GSH-Px [11]. In this study, the decreases in CAT and GSH-Px activities in the liver demonstrated that gossypol reduced the antioxidant capacity in goslings. The data demonstrated that a high level of CSM in the feed suppressed antioxidant activity in the goose liver. Gossypol greatly accelerates the production of OH• from hydrogen peroxide by up to 8-fold in the presence of Fe^{3+}- EDTA [23].

ROM compounds are generated by the reaction of ROS with biomacromolecules [18]. Disruption of the intracellular redox balance leads to a state of oxidative stress, during which proteins, nucleic acids, lipids, and other macromolecules can suffer severe damage [24]. The MDA and PC concentrations represent the evaluation indicators of lipid peroxidation and protein oxidation by free radicals. Intriguingly, although the OH• scavenging ability and CAT and GSH-Px activities were decreased, dietary CSM concentrations had no effects on the ROM, MDA, and PC concentrations in geese livers on d 28. To maintain stability in ROM, MDA, and PC concentrations, goslings may have alleviated the negative effect of FG on free radical balance via a voluntary reduction in feed intake. Similarly, no differences were observed in serum ALT and AST, indicators of liver health status, in geese at 28 d of age.

The serum concentrations of TP, ALB, and GLOB decreased linearly, implying that the synthesis function of the liver had decreased. This observation is similar to that of Zeng et al. [10], who reported that the serum TP, ALB, and GLOB contents linearly decreased with increasing dietary CSM at 35 d of age in meat ducks. It has been reported that gossypol is transported in the blood by binding to the high-affinity binding site of serum ALB [25]. Furthermore, a linear increase in serum UA content was associated with a linear decrease in BW, serum TP, ALB, and GLOB, indicating that the utilization of protein decreased with increasing CSM or FG concentrations. These results are consistent with the findings of Zhu et al. [26], who also found that the serum UN concentration in ducklings fed diets with 80, 160, and 240 mg FG/kg was higher than that in birds fed the control diet at d 21.

In the current study, the growth performance (BW, ADFI, ADG, and F:G) from d 29 to 63, redox status indicators (except for MDA) in the liver, and serum biochemical parameters at d 63 were not affected by the different dietary CSM concentrations. However, many of these variables were significantly affected by dietary CSM levels in goslings from 1 to 28 d of age. Our results indicated that the tolerance of goslings to dietary FG concentration increased with age. Similar results were also reported in meat ducks by Zeng et al. [9]. However, the MDA content in the liver in the CSM_{100} group was lower than those in the other groups. The results show that gossypol has dual properties of pro-oxidant and antioxidant activity.

Gossypols stimulated the generation of free radicals in some studies [27] and inhibited it in others [28]. The role of gossypol as an antioxidant may depend on the dose or tissue type [29]. Gossypol was also found to have antioxidant properties in rat liver microsomes [30] and liver tissues [31].

Conclusions

The application of CSM in the gosling diet should not exceed 7.08% in the early growth stage (d 1 to 28) but can be increased to 22.65% from d 29 to 63. A high concentration of CSM may reduce the growth performance of goslings from d 1 to 28 due to decreases in feed intake, liver metabolism and antioxidant capacity.

Abbreviations

ADFI, average daily feed intake; ADG, average daily gain; ALB, albumin; ALT, Alanine aminotransferase; AST, Aspartate aminotransferase; BW, body weight; CAT, catalase; CSM, cottonseed meal; F:G, feed to gain ratio; GLOB, globulin; GR, glutathione reductase; GSH, reduced glutathione; GSH-Px, glutathione peroxidase; GST, glutathione S-transferase; MDA, malondialdehyde; $O_2^{\bullet -}$, superoxide radical; $OH^{\bullet}$, hydroxyl radical; PC, protein carbonyl; ROM, reactive oxygen metabolites; SEM, standard errors of the mean; SOD, superoxide dismutase; TP, total protein; UA, uric acid

Declarations

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Authors' contributions

JY, ZYW and HMY conceived and designed the experiments; JY, HMY and ZFY conducted the experiment 1; JY, QYS, XAX and HMY conducted the experiment 2; JY conducted laboratory analyses; JY and ZYW wrote and revised the manuscript. ZYW had primary responsibility for final content. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets supporting the conclusions of this article are included within the article.

Ethics approval and consent to participate

The experimental proposals and procedures for the care and treatment of the geese were approved by the animal care and use committee of Yangzhou University (Yangzhou, China).

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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