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Effects of fermented cottonseed meal on the growth performance, apparent digestibility, carcass traits, and meat composition in yellow-feathered broilers

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Abstract: The effects of supplementing broilers' diets with fermented cottonseed meal (FCSM) on the broilers' performance, apparent digestibility, carcass traits, and meat composition were studied. A total of 180 yellow-feathered chickens of 21 days old were randomly divided into three groups (six replicates of 10 broilers each): 1) a control group (Con), 2) a treatment group of FCSM by *Candida tropicalis* (Ct), and 3) a treatment group of FCSM by C. *tropicalis* plus *Saccharomyces cerevisiae* (Ct-Sc). Results showed that FCSM supplementation improved the average daily gain and gain-to-feed ratio from the 43rd to 64th and the 21st to 64th day, respectively ($P < 0.05$). The dietary nutrient digestibility of dry matter, crude protein, and crude ash significantly increased ($P < 0.05$) with FCSM supplementation. The abdominal fat content was lower ($P < 0.05$) in Ct and Ct-Sc than in Con (21st to 42nd day). The crude protein content was higher in the thigh muscles of treatment groups and breast muscles of Ct, and the crude fat (ether extract) in the breast muscles of Ct-Sc was higher than in Con throughout the entire duration ($P < 0.05$). In conclusion, FCSM is beneficial for broilers as it positively affects their growth and digestibility in addition to altering their meat compositions.

Key words: Fermented cottonseed meal, growth performance, digestibility, carcass traits, meat composition, broilers

1. Introduction

Deficient resources as well as the soaring prices of protein feed have induced a limiting bottleneck effect on the sustainable development of animal husbandry in China. A potential source of relief from this bottleneck is the application of cottonseed meal (CSM); however, despite being an inexpensive potential source of protein with high protein content, CSM application is limited due to the presence of free gossypol (FG), a toxic polyphenolic pigment (1). Numerous methods have been developed that could remove FG from CSM, including chemical treatment, solvent extraction, and microbial fermentation (2,3). These methods have played an important role in the detoxification of CSM. Microbial fermentation, in particular, is an especially promising detoxification method because fermentation products are usually some kind of exoenzyme (secreted by microbes) such as amylase, cellulolytic enzyme, protease, and lipolytic enzyme; some variety of vitamins; or some unknown active substances (4), which play a key role in the growth, development, and health of animals.

There have been many studies of fermented CSM (FCSM) that have reported the process of optimization of detoxified gossypol (2,3,5), the selection of detoxification strains (6), and nutrition characteristics (7,8). In addition, recent studies showed that replacing soybean meal with CSM fermented by *Bacillus subtilis* improves growth performance, immunity, beneficial bacteria in the intestinal tract, intestinal morphology, and digestive enzyme activity in broilers (9–11). However, there has been little information reported on the effect of CSM fermented with yeast compared to unfermented CSM on the yellow-feathered broiler, which is a valuable commercial chicken species that yields high-quality meat in China and is responsible for annual production approaching 3 billion (9).

This study's objectives were to study the effects of both FCSM on growth, digestibility of dietary components, carcass traits, and meat composition in yellow-feathered broilers in order to more fully understand FCSM function for poultry production. CSM was fermented by *Candida tropicalis* and *C. tropicalis* plus *Saccharomyces cerevisiae*. These strains could detoxify FG and improve the nutritional value in CSM in our previous study, and the strain *C. tropicalis* was more effective at detoxifying FG in CSM than S. *cerevisiae* (12,13). Considering the reduction of FG level, CSM was not treated with only S. *cerevisiae*.

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2. Materials and methods

2.1. Substrate treatment and preparation of FCSM

The *C. tropicalis* and *S. cerevisiae* strains that were used in this study were provided by the Feed Science Institute of Zhejiang University. CSM was obtained from Shihezi District (Xinjiang, China). The substrate material's treatment was performed according to the method of Zhang et al. (3), and then 1000 g of treated substrate was inoculated with 80 mL of liquid yeast inocula (10^8 cells/mL) that consisted of either *C. tropicalis* alone (Ct) or of *C. tropicalis* and *S. cerevisiae* together (Ct-Sc) at a ratio of 3:7. The inoculated substrate was evenly blended, encased in a plastic container (50 × 30 × 12 cm), and incubated in an incubator at 30 °C for 48 h. After fermentation, the fermented CSM samples were dried at 40 °C for 48 h in a draught drying cabinet. For a control, the same treatment process was performed for unfermented CSM with 80 mL of sterile culture medium. Chemical composition of substrates analyzed (14) were as follows: dry matter (DM), 953, 946, and 947 g/kg; crude protein (CP), 342, 376, and 375 g/kg; crude fat (ether extract, EE), 7.1, 7.2, and 7.4 g/kg; and crude ash (Ash) 53.2, 57.6, and 58.5 g/kg in unfermented CSM, Ct CSM, and Ct-Sc CSM, respectively. FG contents as determined by the method of the AOCS (15) were 126.7, 44.9, and 33.0 mg/kg, respectively.

2.2. Broiler breeding and management

The animal care and use protocol was approved by the Animal Welfare Committee of Shihezi University. A total of 300 Chinese yellow-feathered chickens of 1 day old were raised in a brooder house for 14 days and fed a commercial diet. The brooders were then fed the control diet for 7 days to acclimatize them to powder experimental feeds, and then 180 birds of 21 days old were selected and randomly divided into three groups consisting of six replicates (cages) each with 10 individuals. The three groups were fed three different diets: unfermented CSM (control group, Con), CSM fermented by *C. tropicalis* (treatment group, Ct), CSM fermented by *C. tropicalis* and *S. cerevisiae* (treatment group, Ct-Sc), and the yeast levels were at 2 × 10^6 cfu/g in the FCSM diets. The compositions and nutrition levels are presented in Table 1. This experiment consisted of a starter phase (21–42 days) and a finisher phase (43–64 days). The

Table 1. Ingredient compositions and nutrient contents of the experimental diets (air-dry basis).

Ingredient (%)	Starter (21–42 days)			Finisher (43–64 days)		
	Con	Ct	Ct-Sc	Con	Ct	Ct-Sc
Yellow corn	64.35	64.70	64.65	67.95	68.30	68.25
Soybean meal	17.15	16.80	16.85	13.25	12.90	12.95
Rapeseed meal	2.00	2.00	2.00	2.00	2.00	2.00
Cottonseed meal (CSM)	6.00	6.00	6.00	6.00	6.00	6.00
Unfermented CSM	6.00	-	-	6.00	-	-
Fermented CSM	-	6.00	6.00	-	6.00	6.00
Cottonseed oil	1.00	1.00	1.00	1.50	1.50	1.50
Dicalcium phosphate	1.20	1.20	1.20	1.10	1.10	1.10
Limestone	1.30	1.30	1.30	1.20	1.20	1.20
Premix[1]	1.00	1.00	1.00	1.00	1.00	1.00
Nutrient content[2] (%)						
ME (MJ/kg)	11.94	11.96	11.95	12.23	12.25	12.24
Crude protein	18.70	18.73	18.75	17.11	17.15	17.16
Calcium	0.87	0.85	0.84	0.81	0.79	0.82
Available phosphorus	0.36	0.34	0.35	0.33	0.35	0.34
Methionine + cystine	0.68	0.68	0.68	0.63	0.63	0.63
Lysine	1.04	1.03	1.03	0.89	0.89	0.89

[1]Premix (g or mg/kg diet): L-lysine•HCl, 2 g (1.5 g in finisher); DL-methionine, 1.1 g (1 g in finisher); NaCl, 3 g; choline chloride (50%), 1 g; Cu, 6 mg; Fe, 100 mg; Mn, 150 mg; Zn, 100 mg; Se, 0.3 mg; I, 0.4 mg; V_A, 25 mg; V_{D3}, 8 mg; V_E, 36 mg; V_K, 3 mg; V_{B1}, 2 mg; V_{B2}, 7.5 mg; V_{B6}, 4.5 mg; V_{B12}, 0.02 mg; V_{B5}, 12 mg; niacin, 50 mg; folic acid, 1.2 mg; biotin, 0.15 mg.

[2]Crude protein and calcium are analyzed values; other nutrition contents are calculated values.

ME = Metabolizable energy, Con = 6.0% unfermented cottonseed meal, Ct = 6.0% cottonseed meal fermented by *C. tropicalis*, Ct-Sc = 6.0% cottonseed meal fermented by *C. tropicalis* plus *S. cerevisiae*.

birds were housed in common rearing conditions with continuous light and ad libitum access to feed and water throughout the rearing period. Weekly, body weights and feed intakes of broilers were monitored on a replicate basis; from these data, the average daily feed intake (ADFI), average daily gain (ADG), and gain-to-feed ratio (G:F) were calculated.

2.3. Sample collection and related index assay

The final 3 days of each phase (40–42 days and 62–64 days), 10% of whole mixed excreta from each replicate was collected, and a certain proportion of 10% concentrated sulfuric acid (5 mL/100 g) was added to each sample for nitrogen fixation; the samples were then dried at 60 °C for 48 h. The dried samples were ground for chemical analyses. On days 42 and 64, 10 birds from each group that were similar in weight to the average weight were randomly selected and killed by cervical dislocation. Tissue samples were obtained from the abdominal fat, breast muscle, and thigh muscle for determining the carcass traits. Breast muscle and thigh muscle were freeze-dried at –80 °C for 24 h with a bench-top freeze-dryer (Millrock Technology Inc., Kingston, NY, USA) for analyzing the meat composition. The carcass traits were calculated as followed: carcass (%) = 100 × (carcass weight/live weight); eviscerated (%) = 100 × (total eviscerated yield weight/carcass weight); and tissue (%) = 100 × (tissue weight/total eviscerated yield weight).

The DM, CP, and Ash contents in the diet, feces, and meat were analyzed by the AOAC method (14). The EE of all samples was measured by extraction with diethyl ether in a Soxhlet apparatus. Acid (4 M HCl) insoluble ash (AIA) was determined as described by de Coca-Sinova et al. (16): digestibility (%) = 100 – [(excreta nutrient%/diet nutrient%) × (diet AIA %/excreta AIA%)] × 100.

2.4. Statistical analysis

One-way analysis of variance was performed using a least significant difference test from SPSS 16.0. A significance level of 0.05 was used.

3. Results

3.1. Growth performance

The effect of FCSM on growth performance of broilers is presented in Table 2. Throughout the experiment, all birds remained healthy in appearance. There was no significant difference in performance among the three groups during the starter phase. Compared to the birds that were fed the control diet, the ADFI in the broilers that were fed Ct CSM decreased by 4.94% in the starter phase and 4.33% in the finisher phase; however, those that were fed Ct-Sc CSM did not differ in their ADFI compared to the control diet ($P < 0.05$). During the finisher phase, the ADG was significantly higher in the treatment groups than in Con ($P < 0.05$). In general, for the entire experimental duration, the ADG was significantly higher in the broiler chickens that were fed Ct-Sc CSM ($P < 0.05$). Additionally, FCSM supplementation significantly increased the G:F during the whole experimental period ($P < 0.05$).

3.2. Apparent digestibility

The effect of FCSM on apparent digestibility of broilers is presented in Table 3. The results showed that apparent digestibility of the components in the FCSM diets was

Table 2. Effect of fermented cottonseed meal on the growth performance of broiler chickens during the starter (21–42 days), finisher (43–64 days), and whole experimental (21–64 days) periods.

Items	Con	Ct	Ct-Sc	Pooled SEM	P-value
Starter					
ADFI (g/day)	96.32	92.92	92.11	0.49	0.16
ADG (g/day)	35.23	35.33	35.26	0.40	0.98
G:F (g/g)	0.37	0.38	0.39	0.01	0.21
Finisher					
ADFI (g/day)	122.96^{a}	116.85^{b}	122.93^{a}	3.22	0.13
ADG (g/day)	36.86^{c}	38.34^{b}	40.30^{a}	0.92	0.01
G:F (g/g)	0.30^{a}	0.33^{b}	0.33^{b}	0.01	<0.01
Whole period					
ADFI (g/day)	109.64^{a}	104.89^{b}	107.52^{ab}	1.83	<0.01
ADG (g/day)	36.04^{b}	36.83^{b}	37.78^{a}	0.41	0.06
G:F (g/g)	0.33^{a}	0.35^{b}	0.35^{b}	0.01	<0.01

[a,b]: Means within a row that have different superscript letters are significantly different ($P < 0.05$). Data are means of six determinations. ADFI = Average daily feed intake, ADG = average daily gain, G:F = gain-to-feed ratio, Con = 6.0% unfermented cottonseed meal, Ct = 6.0% cottonseed meal fermented by *C. tropicalis*, Ct-Sc = 6.0% cottonseed meal fermented by *C. tropicalis* plus *S. cerevisiae*.

Table 3. Effect of fermented cottonseed meal on apparent digestibility for broiler chickens.

Items (%)	Con	Ct	Ct-Sc	Pooled SEM	P-value
40–42 days					
Dry matter	78.23[c]	80.87[a]	79.39[b]	0.11	<0.01
Crude protein	64.36[c]	68.78[a]	65.63[b]	0.45	<0.01
Crude fat	72.30	71.50	73.94	1.78	0.40
Crude ash	31.49[c]	41.57[a]	35.31[b]	1.34	<0.01
62–64 days					
Dry matter	78.32[c]	81.62[a]	79.61[b]	0.11	<0.01
Crude protein	67.35[c]	71.65[a]	69.20[b]	0.30	<0.01
Crude fat	82.22	84.28	83.04	1.07	0.21
Crude ash	23.74[c]	34.40[a]	29.30[b]	0.94	<0.01

[a,b]: Means within a row that have different superscript letters are significantly different (P < 0.05). Data are means of six determinations. Con = 6.0% unfermented cottonseed meal, Ct = 6.0% cottonseed meal fermented by *C. tropicalis*, Ct-Sc = 6.0% cottonseed meal fermented by *C. tropicalis* plus *S. cerevisiae*.

significantly higher than that of the control diet, except for EE digestibility (P < 0.05). Compared to Con, the digestibility of DM, CP, and Ash increased by 3.37%, 6.87%, and 32% in Ct and 1.48%, 1.97%, and 12.13% in Ct-Sc from day 40 to 42, respectively. In addition, the DM, CP, and Ash digestibility was augmented by 4.21%, 6.38%, and 44.9% in Ct and 1.65%, 2.75%, and 23.42% in Ct-Sc from day 62 to 64, respectively.

3.3. Carcass trait

The effect of FCSM on carcass trait of broilers is presented in Table 4. The abdominal fat content in the starter phase was significantly lower in the treatment groups than in Con (P < 0.05). The thigh muscle content was significantly increased after adding Ct-Sc CSM to the diet during the finisher phase (P < 0.05). Over the entire period, the carcass, eviscerated, and breast muscle yields in the treatment groups were not significantly different from those in Con (P > 0.05).

3.4. Meat composition

The effect of FCSM on meat composition of broilers is presented in Table 5. For the breast muscle, the intramuscular EE contents in Ct-Sc and the CP in Ct were significantly higher than in Con throughout the whole experimental period (P < 0.05). For the thigh muscle, the intramuscular EE contents increased in Ct-Sc and the CP decreased in both FCSM groups compared to Con during the starter phase. Additionally, the EE and CP contents were significantly higher and lower, respectively, in the FCSM groups than in Con during the finisher phase (P < 0.05). Finally, the Ash content in meat from the treatment groups was not different from Con (P > 0.05).

4. Discussion

4.1. Growth performance and digestibility

In the present study, there were no significant differences in growth performance when broilers ingested FCSM in the starter phase, consistent with previous studies (9,10). Furthermore, our results showed that FCSM significantly improved both the ADG and G:F of yellow-feathered broilers during the finisher and the whole periods. However, to our knowledge, there has not been any related study that has reported broiler performance in the later growth stage (43–64 days). The present results indicated that FCSM increased the nutrient digestibility of DM, CP, and Ash in broilers. Accordingly, a similar digestibility-promoting effect was found by Feng et al. (17) and Chang et al. (18). More specifically, Feng et al. (17) showed that soybean meal that had been fermented with *Aspergillus oryzae* could improve the apparent digestibility of dietary components in weaned piglets, and the results of Chang et al. (18) showed that protein feedstuffs that had been fermented with *A. oryzae* more efficiently increased nutrient digestibility than did the unfermented protein feedstuffs. Finally, Chiang et al. (19) obtained similar results by using fermented rapeseed meal.

Improved growth performance and apparent digestibility may be associated with the promotion of the nutritional value of fermented feed in addition to the elimination of antinutritional factors after fermentation. For instance, CSM fermented with *C. tropicalis* and *Aspergillus niger* could increase the crude protein content and in vitro digestibility as well as markedly decrease the FG content (12,13). Additionally, fermentation of

Table 4. Effect of fermented cottonseed meal on the carcass traits of broiler chickens.

Item (%)	Con	Ct	Ct-Sc	Pooled SEM	P-value
On day 42 of age					
Abdominal fat	3.19^{a}	2.45^{b}	2.41^{b}	0.07	<0.01
Carcass	87.94	88.03	88.22	0.50	0.85
Eviscerated	77.92	78.03	79.15	0.73	0.21
Breast muscle	15.14	15.41	15.24	0.29	0.65
Thigh muscle	18.08	18.16	18.09	0.30	0.96
On day 64 of age					
Abdominal fat	5.24	5.02	4.83	0.36	0.55
Carcass	90.46	90.61	91.44	0.72	0.37
Eviscerated	80.77	79.37	79.59	0.89	0.27
Breast muscle	14.10	14.11	14.18	0.59	0.99
Thigh muscle	17.16^{b}	17.36^{ab}	18.36^{a}	0.47	0.05

^{a,b}: Means within a row that have different superscript letters are significantly different (P < 0.05). Data are means of 10 determinations. Con = 6.0% unfermented cottonseed meal, Ct = 6.0% cottonseed meal fermented by *C. tropicalis*, Ct-Sc = 6.0% cottonseed meal fermented by *C. tropicalis* plus *S. cerevisiae*.

Table 5. Effect of fermented cottonseed meal on the meat composition of broiler chickens.

Item (%)	Con	Ct	Ct-Sc	Pooled SEM	P-value
On day 42 of age					
Crude fat of breast muscle	4.27^{b}	4.03^{b}	4.82^{a}	0.15	0.02
Crude protein of breast muscle	86.91^{b}	89.31^{a}	87.56^{b}	0.43	<0.01
Crude ash of breast muscle	4.24	4.31	3.92	0.52	0.74
Crude fat of thigh muscle	5.33^{b}	6.57^{ab}	8.28^{a}	0.21	0.02
Crude protein of thigh muscle	84.32^{a}	81.58^{b}	82.12^{b}	0.33	<0.01
Crude ash of thigh muscle	3.95	4.41	4.27	0.28	0.28
On day 64 of age					
Crude fat of breast muscle	6.04^{b}	5.94^{b}	6.82^{a}	0.27	0.03
Crude protein of breast muscle	87.41^{b}	88.89^{a}	87.77^{b}	0.32	0.01
Crude ash of breast muscle	4.44	4.65	4.53	0.11	0.19
Crude fat of thigh muscle	7.51^{b}	8.51^{a}	8.81^{a}	0.32	0.01
Crude protein of thigh muscle	81.07^{a}	79.44^{b}	77.17^{c}	0.33	<0.01
Crude ash of thigh muscle	4.52	4.32	4.03	0.22	0.13

^{a,b}: Means within a row that have different superscript letters are significantly different (P < 0.05). Data are means of six determinations. Con = 6.0% unfermented cottonseed meal, Ct = 6.0% cottonseed meal fermented by *C. tropicalis*, Ct-Sc = 6.0% cottonseed meal fermented by *C. tropicalis* plus *S. cerevisiae*.

soybean meal with *A. oryzae* could improve the nutritional value, reduce the trypsin inhibitor content, and result in degradation of large-size proteins (17,20). Furthermore, oil cakes as substrate in solid-state fermentation (SSF) could produce various enzymes (protease, amylase, lipase, phytase, etc.). These oil cakes are the ideal nutrient support in SSF for rendering both carbon and nitrogen sources, and they are reported to function as a good substrate for enzyme production using fungal species (21). Therefore, improving the growth performance and digestibility in animals might primarily be due to the exoenzymes present in fermented substrate. In addition, fermented CSM could enhance performance and digestibility through improving the cecal microflora, intestinal morphology, and digestive enzyme activity in broilers (11).

4.2. Carcass trait and meat composition

Little information is available about the effects of FCSM on the carcass traits and meat composition of chickens. The carcass traits remained unaltered, except for the abdominal fat content on day 42 and the thigh muscle on day 64, when the broilers digested FCSM-containing diet. On day 42, the abdominal fat content decreased in the treatment group compared to Con, which could have been caused by probiotics and metabolites included in the fermented substrate (4,22). In particular, Aluwong et al. (22) demonstrated that supplementing yeast probiotic in feed decreased the abdominal fat of broilers, and, in addition, some studies reported that fermented products decreased the fat content (23,24). Moreover, the percentage of thigh muscle during the finisher phase was higher in Ct-SC than in the other groups, and the EE was increased in Ct-SC and the CP was increased in Ct as compared to Con for the nutritional composition of the breast muscle. These results reflect the differences in the effect of different yeast species in the fermentation process. In addition, the feeding time is also an influencing factor in the altered thigh muscle percentage in the later stage growing rather than the early. The increased EE and the decreased CP in the thigh muscle were determined when broilers ingested FCSM-containing diets, which might be attributed to the enhanced anabolism of intramuscular fat or the weakened anabolism of intramuscular protein. Accordingly, a similar result was reported by Lee et al. (25), who found that supplementation by a diet with fermented apple decreased the CP content and tended to increase the EE content of the *longissimus dorsi* muscle in pigs in the finishing phase of fattening.

From this investigation, it can be concluded that supplementing FCSM to broilers' diet could result in improved growth performance and apparent digestibility. Both CSM fermented with *C. tropicalis* and *S. cerevisiae* and CSM fermented with *C. tropicalis* alone beneficially impacted the meat composition of meat-type chickens. This present study provides a practical approach for the application of FCSM as a functional feed and utilization of microbial fermented feedstuffs for poultry production.

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