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Experimental Study on Chemical Removal of Gossypol from Cottonseed Meal (Postprint)

Authors: Weiwei Wang, Li Aike, Han Fei, Zhou Tianbing, Song Dynasty military

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Abstract

This study aimed to investigate the optimal method for chemical detoxification of cottonseed meal. In Experiment 1, the effects of different moisture levels, temperatures, and hydrothermal treatment durations on gossypol detoxification rate were examined to determine the optimal hydrothermal conditions for cottonseed meal detoxification. The results demonstrated that within the ranges of 60~100 ℃, 8%~16% moisture, and 20 min~8 h, the detoxification rate of cottonseed meal increased with rising temperature, moisture addition, and treatment time. In Experiment 2, comparing the detoxification efficacy of 10 common single chemical detoxifying agents revealed that ferrous sulfate ($FeSO_4$), copper sulfate ($CuSO_4$), and hydrogen peroxide (H_2O_2) exhibited superior effects, all achieving detoxification rates exceeding 40%; however, cottonseed meal treated with $FeSO_4$ turned black and developed a rusty odor, making $CuSO_4$ and H_2O_2 the preferred single gossypol detoxifying agents. In Experiment 3, $CuSO_4$ and H_2O_2 were formulated into a mixed detoxifying agent to investigate its optimal reaction conditions and verify its efficacy. The findings indicated that 80 ℃, 16% detoxifying agent addition, and oven-drying for 1 h represented the optimal detoxification conditions, with detoxification rates for both free and bound gossypol exceeding 84% using this mixed agent; although the crude protein content of detoxified cottonseed meal decreased slightly ($P>0.05$), the crude protein digestibility was significantly enhanced ($P<0.05$). Therefore, moisture, temperature, and time are critical factors influencing chemical detoxification of cottonseed meal. Under controlled conditions of these three parameters (80 ℃, 16% detoxifying agent addition, and sealed oven-drying for 1 h), a mixed detoxifying agent prepared from H_2O_2 and $CuSO_4$ can effectively remove gossypol from cottonseed meal while improving its crude protein and energy digestibility, thereby providing robust theoretical and technical support for cottonseed meal detoxification processing.

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Full Text

Study on Detoxification of Gossypol in Cottonseed Meal by Chemical Methods

WANG Weiwei[1], ZHOU Tianbing[1,2]*, LI Aike[1], **HAN Fei[1], SONG Daijun[2]

(1. Academy of State Administration of Grain, Beijing 100037, China; 2. College of Animal Science and Technology, Southwest University, Chongqing 400715, China)

Abstract

This study investigated optimal chemical methods for detoxifying gossypol in cottonseed meal. In Experiment 1, we examined the effects of moisture content, temperature, and hydrothermal treatment duration on gossypol detoxification efficiency. Results demonstrated that within the ranges of 60–100 °C, 8–16% moisture, and 20 minutes to 8 hours, detoxification rates increased progressively with higher temperature, greater moisture addition, and longer treatment time. In Experiment 2, comparative evaluation of ten common single chemical detoxifying agents revealed that ferrous sulfate ($FeSO_4$), copper sulfate ($CuSO_4$), and hydrogen peroxide (H_2O_2) achieved superior detoxification performance, all exceeding 40% removal rates. However, $FeSO_4$ treatment caused black discoloration and imparted a metallic off-flavor to the meal, rendering $CuSO_4$ and H_2O_2 as the preferred single-agent options. In Experiment 3, we formulated a mixed detoxifying agent from $CuSO_4$ and H_2O_2 to determine optimal reaction conditions and validate its efficacy. The optimal conditions were identified as 80 °C, 16% detoxifying agent supplementation, and 1 hour of heating, which achieved detoxification rates exceeding 84% for both free and bound gossypol. Although crude protein content decreased slightly following treatment ($P>0.05$), crude protein digestibility improved significantly ($P<0.05$). These findings indicate that moisture, temperature, and time are critical factors influencing chemical detoxification of cottonseed meal. Under controlled conditions (80 °C, 16% mixed agent addition, and sealed heating for 1 hour), the $CuSO_4$-H_2O_2 mixed detoxifying agent effectively removes gossypol while enhancing crude protein and energy digestibility, providing robust theoretical and technical support for cottonseed meal detoxification processing.

Keywords: chemical detoxification; cottonseed meal; crude protein; digestibility; gossypol

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Author Introduction: WANG Weiwei (1984-), female, from Hohhot, Inner Mongolia, assistant researcher, Ph.D., engaged in research on development and utilization of new high-quality protein feed resources and application of encapsulated probiotics technology. E-mail: www@chinagrain.org

*Co-first author

**Corresponding author: LI Aike, professor, doctoral supervisor, E-mail: lak@chinagrain.org

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China ranks as the world' s largest cotton producer, with annual cotton output exceeding 6 million tons in recent years and cottonseed production surpassing 10 million tons. According to the National Grain and Oil Information Center, in 2013 China' s cottonseed production available for oil extraction reached 9.7276 million tons, yielding 1.1853 million tons of cottonseed oil and 4.3720 million tons of cottonseed meal. Cottonseed meal, a byproduct obtained after cottonseed delinting, decortication, kernel-shell separation, and pre-pressing extraction or direct solvent extraction, contains 39–45% protein, with protein content from extracted meal exceeding 50%—a nutritional value comparable to soybean meal and 4-5 times that of wheat protein. Given domestic soybean meal shortages and persistently high prices, cottonseed meal could partially substitute for soybean meal. However, its application is limited by the presence of toxic gossypol, a yellow-brown polyphenolic pigment concentrated in cottonseed pigment glands that is insoluble in water but soluble in organic solvents.

Gossypol exists in two forms: free gossypol (FG) and bound gossypol (BG). Toxic FG contains active aldehyde and hydroxyl groups, accounting for 0.8–0.9% of kernel weight. During oil processing, most FG combines with amino acids to form BG through heat treatment, rendering it non-toxic due to poor absorption in the animal digestive tract. The remaining FG persists in meal and oil products, exerting toxic effects. Consequently, untreated cottonseed meal must be used restrictively in animal diets, as excessive intake impairs growth and reproductive performance and may cause mortality. The *Feed Hygiene Standard* (GB 13078-2001) specifies maximum FG levels: $ $1,200 mg/kg in cottonseed cakes/meals, $ $100 mg/kg in broiler and growing chicken feeds, $ $20 mg/kg in laying hen feeds, and $ $60 mg/kg in growing-finishing pig feeds. Effective detoxification is therefore essential for expanded cottonseed meal utilization.

Current detoxification methods include chemical treatment, mixed solvent extraction, liquid-liquid-solid extraction, and microbial solid-state fermentation. Chemical detoxification offers operational simplicity, involving addition of chemical agents to cottonseed meal under specific conditions to destroy FG or convert it to non-toxic BG. For example, adjusting moisture, temperature, and pressure during processing can transform FG to BG, while additives like ferrous sulfate, calcium hydroxide, or urea-ferrous sulfate mixtures can reduce FG content by 20–93%. Dai et al. reported that hot alkaline treatment at pH 8–9, 60 °C for 3 hours reduced gossypol in cottonseed protein to 0.012%. However, these methods have drawbacks: $FeSO_4$ causes black discoloration, calcium hydroxide shows low efficiency and reduces vitamin bioavailability, and some methods cause substantial water-soluble protein loss. Additionally, certain BG may revert to FG during digestion. Therefore, ideal detoxification should reduce both FG and BG while preserving protein quality and digestibility.

Building on previous research regarding temperature, moisture, and reaction time effects, this study compared various single chemical agents ($FeSO_4$, $CuSO_4$, urea, NaOH, H_2O_2, etc.) and developed an optimized mixed detoxifying agent from the most effective candidates, evaluating its performance through crude protein content analysis and in vitro digestibility assessment.

1.1 Materials

Cottonseed meal with FG content of 500–2,000 mg/kg was prepared by hexane extraction of cottonseed kernels to remove oil, followed by hydrothermal treatment to achieve target FG concentrations (500–2,000 mg/kg).

1.2 Detection Methods

Free gossypol (FG) and total gossypol (TG) contents were determined using American Oil Chemists' Society standard methods AOCS Ba7-58 and AOCS Ba8-55, respectively.

1.2.1 Effects of Temperature, Moisture, and Hydrothermal Treatment Time on Detoxification Rate To examine temperature effects, three 50 g samples of cottonseed meal (10.93% moisture, 515 mg/kg FG) were each sprayed with 3 mL water, sealed in Erlenmeyer flasks, and heated at 60, 80, or 100 °C for 8 hours (sufficiently long to exclude time effects). Each treatment had three replicates, with FG content measured to assess temperature impacts.

For moisture effects, five 50 g samples of cottonseed meal (754 mg/kg FG, 7.8% moisture) received 4, 5, 6, 7, or 8 mL distilled water to achieve 8%, 10%, 12%, 14%, or 16% moisture addition, respectively. Samples were sealed in Erlenmeyer flasks and dried at 80 °C for 1 hour (three replicates per treatment) to evaluate moisture effects on FG detoxification.

To assess treatment duration, five 50 g samples of cottonseed meal (754 mg/kg FG, 7.8% moisture) were sprayed with 4.5 mL distilled water, sealed in flasks, and heated at 80 °C for 1, 2, 3, 4, or 5 hours (three replicates each), with FG content measured to determine time-dependent effects.

1.2.2 Optimization of Single Chemical Detoxifying Agents 1.2.2.1 Screening of Single Chemical Agents

Ten 50 g samples of cottonseed meal (1,383 mg/kg FG, 11.34% moisture) were treated with 2 mL of various solutions to achieve 15% final moisture: saturated $FeSO_4$, 1% $CuSO_4$, 2% lysine, 2% urea, 1% NaOH, 1% lysine + 0.1% NaOH, 1% $MgSO_4$, 1% $MnSO_4$, 1% $ZnSO_4$, or 30% H_2O_2 (analytical grade). After spraying, samples were sealed in Erlenmeyer flasks and heated at 80 °C for 1 hour (three replicates per treatment). FG content was measured to calculate detoxification rates and identify optimal single agents.

1.2.2.2 Condition Optimization for Single Agents

For $CuSO_4$ treatment, 50 g cottonseed meal samples (754 mg/kg FG, 7.8% moisture) were sprayed with 4.5 mL solution containing 0.25 g $CuSO_4$ (0.5% of meal weight), divided into five equal portions, sealed in flasks, and heated at 80 °C for 1, 2, 3, 4, or 5 hours (three replicates each) to determine time-dependent effects on FG detoxification.

For H_2O_2 treatment, 50 g cottonseed meal samples (754 mg/kg FG, 7.8% moisture) were sprayed with 4, 5, 6, 7, or 8 mL of 30% H_2O_2 to achieve 8%, 10%, 12%, 14%, or 16% addition levels, respectively. Samples were sealed in flasks and heated at 90 °C for 1 hour (three replicates per treatment) to evaluate H_2O_2 concentration effects on FG detoxification.

1.2.3 Optimization of Mixed Detoxifying Agent Conditions Based on single-agent results, a mixed detoxifying agent was formulated using $CuSO_4$ and H_2O_2. Fifty-gram cottonseed meal samples (754 mg/kg FG, 7.8% moisture) were treated with 4.5 mL solution containing 0.5% $CuSO_4$ and 30% H_2O_2 (adjusted to ~15% final moisture). A three-factor, three-level orthogonal experiment $L_9(3^4)$ was conducted using temperature (70, 80, 90 °C), reaction time (20, 40, 60 minutes), and detoxifying agent addition (8%, 12%, 16%) as variables .

1.2.4 Validation of Mixed Detoxifying Agent Efficacy Validation experiments were performed using cottonseed meals with FG contents of 1,792 mg/kg and 688 mg/kg to measure FG, BG, and TG contents. Crude protein content was determined using GB/T 6432-1994. Detoxified cottonseed meal samples were prepared and submitted to the Animal Feeding and Standardization Laboratory at the Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, for in vitro digestibility analysis. Dry matter digestibility, crude protein digestibility, and enzymatic hydrolyzate energy (poultry metabolic energy) were measured using a monogastric animal bionic digestive system (SDS-1) with duck simulated digestive fluid kits.

2.1 Effects of Temperature, Moisture, and Hydrothermal Treatment Time on Detoxification Rate

As shown in [Figure 1: see original paper]A, FG detoxification rates increased with temperature from 60 to 100 °C. [Figure 1: see original paper]B demonstrates that higher moisture addition yielded greater FG detoxification rates. [Figure 1: see original paper]C illustrates that longer treatment times reduced residual FG content and increased detoxification rates.

2.2 Optimization of Single Chemical Detoxifying Agents

Table 2 presents the detoxification effects of various chemical agents on cottonseed meal FG, revealing highly significant differences among the ten treatments ($P<0.01$). Saturated $FeSO_4$ showed the highest efficacy, followed by $CuSO_4$ and H_2O_2, all achieving >40% detoxification rates. However, $FeSO_4$ treatment caused black discoloration and pronounced metallic off-flavors, compromising product sensory quality. Therefore, $CuSO_4$ and H_2O_2 were selected for further condition optimization. Table 3 shows that treatment time with 0.5% $CuSO_4$ did not significantly affect detoxification rates ($P>0.05$). Table 4 demonstrates that H_2O_2 addition level had highly significant effects ($P<0.01$), with detoxification rates increasing progressively with H_2O_2 concentration ($P<0.01$).

2.3 Optimization of Mixed Detoxifying Agent Conditions

Table 5 shows that factors A, B, and C all significantly influenced detoxification rates (P =0.015, P =0.026, P =0.008), indicating that temperature, time, and agent addition level all substantially affected efficacy. The effect magnitude ranked as C>A>B, with agent addition showing the greatest impact, followed by temperature and reaction time. The optimal conditions were $A_3B_3C_3$ (90 °C, 16% agent addition, 1 hour reaction). Considering energy consumption and nutrient loss at higher temperatures, 80 °C, 1 hour, and 16% addition were selected as practical conditions.

2.4 Validation of Optimal Conditions

Based on orthogonal experiment results, practical detoxification conditions of 80 °C (sufficiently effective while minimizing energy costs), 1 hour, and 16% agent addition were selected for validation.

2.4.1 Validation of FG Content Reduction Using cottonseed meals with initial FG contents of 1,792 mg/kg and 688 mg/kg, the mixed detoxifying agent achieved >84% FG removal and ~87% reduction in both BG and TG (Table 6).

2.4.2 In Vitro Nutrient Digestibility of Detoxified Cottonseed Meal Table 7 shows that although crude protein content decreased slightly after detoxification, dry matter digestibility, crude protein digestibility, and enzymatic hydrolyzate energy all improved significantly ($P<0.05$). Specifically, dry matter digestibility increased by ~6.3%, crude protein digestibility by ~7.3%, and enzymatic hydrolyzate energy by ~14.5%.

3.1 Effects of Hydrothermal Treatment on Gossypol Removal

The active groups of FG (aldehyde and hydroxyl groups), particularly the ortho-aldehyde hydroxyl groups, readily interact with proteins, amino acids, and phospholipids under aqueous and thermal conditions to form complexes. FG can also be oxidized by oxidizing agents or chelated with Fe^{2+} and Cu^{2+} to reduce toxicity. While hydrothermal treatment during oil processing generates substantial BG, these complexes are unstable and may "hydrolyze back"in the digestive tract. This study therefore applied chemical agents under hydrothermal conditions to form hydrolysis-resistant chelates or degradation products. The temperature range of 60–100 °C was selected because higher temperatures damage protein nutritional value; moisture was limited to 8-16% to avoid excessive drying costs; and treatment time was constrained to 20 minutes-8 hours to minimize energy consumption. Results showed that increasing temperature, moisture, and time significantly improved detoxification rates, with temperature exerting the strongest effect. While previous reports indicated thermal treatment alone could achieve >70% detoxification, our constrained conditions yielded <60% removal, highlighting the need for chemical agents.

3.2 Selection of Single and Mixed Chemical Detoxifying Agents

As established, detoxification efficacy depends heavily on hydrothermal conditions. This study evaluated numerous reported chemical agents under controlled conditions, identifying $FeSO_4$, $CuSO_4$, and H_2O_2 as most effective. Toxic FG exists primarily as the stable dialdehyde tautomer, which reacts with Fe^{2+} and Cu^{2+} to form non-absorbable gossypol-iron/copper complexes that are excreted without adverse effects. While $FeSO_4$ and $CuSO_4$ can thus detoxify gossypol and reduce hepatic accumulation, $FeSO_4$ causes unacceptable black discoloration and metallic flavors. Oxidative detoxification uses strong oxidants to degrade FG but does not affect BG content.

Single-agent detoxification rates for $CuSO_4$ and H_2O_2 ranged only 43–52%, prompting investigation of a mixed agent under varying temperature, moisture, and time conditions through orthogonal experimentation. The optimal condition of 16% mixed agent at 90 °C for 1 hour was validated using two cottonseed meals with different initial FG levels. The mixed agent produced fragrant, normally colored meal with FG reduced to 219 and 110 mg/kg, achieving 88.89% and 84.01% removal rates—well below the United Nations Consultative Committee standard of $ $0.06% FG in edible cottonseed protein. Both TG and BG removal reached ~87%, likely because the 90 °C treatment simultaneously reduced BG content while preventing its reversion to FG, demonstrating superior detoxification efficacy. The slight crude protein reduction may result from oxidative effects of the detoxifying agents.

3.3 In Vitro Digestibility Assessment of Detoxified Cottonseed Meal

In vitro digestion tests demonstrated that despite modest crude protein content reduction, the mixed detoxifying agent improved crude protein digestibility by 9%, suggesting that treatment-induced protein denaturation enhanced digestibility. Furthermore, dry matter digestibility increased by 12% and energy digestibility by 14%, substantially improving cottonseed meal utilization efficiency.

This study systematically investigated hydrothermal effects on gossypol removal and developed an optimized $CuSO_4$-H_2O_2 mixed detoxifying agent that achieves >84% FG and ~87% BG removal at 80 °C, 1 hour, and 16% addition. While crude protein content decreased slightly, dry matter, crude protein, and energy digestibility all improved significantly. However, further research is needed to evaluate the application of chemically detoxified cottonseed meal in animal feeding and its effects on animal production performance.

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