# Detoxification Effect of Steam Explosion Technology on Free Gossypol in Cottonseed Meal (Postprint)

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## Abstract

This experiment aimed to investigate the detoxification efficacy of steam explosion technology on free gossypol in cottonseed meal. Commercially available cottonseed meal was subjected to seven different steam explosion treatments with steam pressure fixed at 2.0 MPa. The control treatment was steam explosion with a water-to-material ratio of 0 and holding time of 30 s. Treatments with water-to-material ratios of 30% and 50% were performed with holding times of 10, 20, and 30 s, respectively. Each treatment was replicated three times. Using free gossypol content and protein solubility as indicators, the optimal treatment was selected for determination of amino acid content and nutrient simulated porcine digestibility data. Results showed that at fixed steam pressure, the detoxification rate of free gossypol and protein solubility increased with prolonged steam holding time, and first increased then decreased with increasing water-to-material ratio. Based on the detoxification effect on free gossypol and protein solubility, the optimal steam explosion treatment was selected as steam pressure 2.0 MPa, water-to-material ratio 30%, and holding time 30 s. Under these conditions, free gossypol content in cottonseed meal was 85.0 mg/kg, the detoxification rate reached 87.0%, and protein solubility was 42.3%. Compared with the control group, steam explosion extremely significantly reduced lysine and arginine contents in cottonseed meal ($P < 0.01$), had no significant effect on in vitro dry matter digestibility and gross energy digestibility ($P > 0.05$), and extremely significantly reduced in vitro crude protein digestibility and lysine digestibility ($P < 0.01$). Therefore, steam explosion treatment can reduce free gossypol content, protein solubility, lysine content, arginine content, in vitro crude protein digestibility, and lysine digestibility of cottonseed meal. The optimal steam explosion treatment was steam pressure 2.0 MPa, water-to-material ratio 30%, and holding time 30 s.

## Full Text

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### Abstract

This experiment investigated the detoxification effect of steam explosion technology on free gossypol content in cottonseed meal. Commercially available cottonseed meal was subjected to seven different steam explosion treatments at a fixed steam pressure of 2.0 MPa. The control treatment used a water-to-material ratio of 0 with a 30-second retention time, while water-to-material ratios of 30% and 50% were tested at retention times of 10, 20, and 30 seconds. Each treatment was replicated three times. Free gossypol content and protein solubility were measured to identify optimal processing conditions, after which amino acid content and nutrient digestibility were evaluated using a porcine simulative digestion system. Results demonstrated that at constant steam pressure, both detoxification efficiency and protein solubility increased with longer retention times, but exhibited a pattern of initial increase followed by decrease as the water-to-material ratio increased. Based on free gossypol detoxification effectiveness and protein solubility, the optimal steam explosion parameters were identified as 2.0 MPa steam pressure, 30% water-to-material ratio, and 30-second retention time. Under these conditions, free gossypol content was reduced to 85.0 mg/kg, achieving an 87.0% detoxification rate, while protein solubility reached 42.3%. Compared with the control group, steam explosion treatment significantly reduced lysine and arginine contents ($P < 0.01$) and markedly decreased in vitro crude protein digestibility and lysine digestibility ($P < 0.01$), while showing no significant effect on in vitro dry matter digestibility or gross energy digestibility ($P > 0.05$). These findings indicate that steam explosion treatment effectively reduces free gossypol content, protein solubility, lysine and arginine contents, and the in vitro digestibility of crude protein and lysine in cottonseed meal. The optimal steam explosion conditions were determined to be 2.0 MPa steam pressure, 30% water-to-material ratio, and 30-second retention time.

**Keywords:** steam explosion; cottonseed meal; free gossypol; protein solubility; simulative digestion system

## Introduction

The shortage of protein feed resources represents a major challenge confronting the development of China' s animal husbandry and feed industries, with a substantial supply-demand gap necessitating large-scale imports. Although China produces over 6 million tons of cottonseed cake and meal annually, less than 30% of these resources have been effectively utilized, a situation that falls far short of meeting the practical demands of the feed industry, livestock sector, and agricultural development. The primary factor limiting the widespread application of cottonseed meal is the presence of free gossypol, a toxic component that can directly poison monogastric animals, damage myocardial tissue in severe cases, and reduce animal production performance, thereby severely restricting its use in animal feed.

Detoxification methods for free gossypol can be categorized into physical, chemical, and biological approaches. Physical methods primarily include screw pressing, heat treatment, and extrusion processing; chemical methods mainly involve ferrous sulfate and alkaline treatment; while biological methods encompass microbial fermentation, enzymatic hydrolysis, and genetic approaches. However, conventional extrusion detoxification exhibits low efficiency. Chemical passivation methods such as alkaline treatment require highly corrosion-resistant equipment and can reduce feed palatability. Solvent extraction methods may leave solvent residues, and microbial fermentation approaches suffer from long processing times and difficulties in cultivating and preserving superior strains. Consequently, there is an urgent need in this field for a rapid method to remove free gossypol from cottonseed meal that overcomes these limitations.

Steam explosion technology is a physicochemical reaction widely applied in the efficient utilization of biomass feedstocks, favored by researchers for its low cost, minimal energy consumption, and pollution-free characteristics. This technology involves brief high-temperature, high-pressure steam treatment (200–260 °C, 2.0–5.0 MPa) followed by instantaneous discharge of all material from the vessel within milliseconds. Due to its short duration, high energy density, and concentrated effects, steam explosion can alter the physicochemical structure of materials, partially degrading hemicellulose and lignin. The technology is extensively used in pretreatment processes for low-quality raw materials in papermaking and biomass energy utilization, with applications in the feed industry primarily focused on developing coarse feed resources from wheat, rye, oat straws, and corn stover. However, no studies have investigated its application in conventional feed ingredient development. Song et al. reported that extrusion processing could reduce free gossypol content in cottonseed meal to 120–140 mg/kg. Since both steam explosion and extrusion technologies operate through high temperature and pressure, we hypothesized that steam explosion could also reduce free gossypol content in cottonseed meal and achieve detoxification effects. This study aimed to investigate the detoxification efficacy of steam explosion technology on free gossypol in cottonseed meal, with the objective of developing a novel detoxification technique to provide a reference for the efficient utilization of cottonseed meal.

### 1.1 Experimental Materials

The commercially available cottonseed meal used in this experiment contained 642 mg/kg free gossypol, 33.2% crude protein, and had a protein solubility of 60.2%.

### 1.2 Experimental Equipment

Steam explosion experiments were conducted using a QBS-80B steam explosion test bench (Hebi, China).

### 1.3 Experimental Treatments

Commercial cottonseed meal was sieved through a 10-mesh screen. Distilled water was added to achieve specific water-to-material ratios, mixed thoroughly, and sealed in self-sealing bags for 8–10 hours to ensure complete moistening. The steam explosion apparatus was preheated and adjusted to the designated steam pressure. Cottonseed meal was loaded into the material chamber, maintained at pressure for a specified retention time, and then subjected to steam explosion treatment as outlined in Table 1 . Each treatment was exploded three times. Samples were collected, dried to constant weight in a 65 °C oven, and ground. Samples passing through a 40-mesh screen were used for determining free gossypol content, crude protein content, protein solubility, and porcine nutrient simulative digestibility, while samples passing through a 60-mesh screen were used for amino acid hydrolysis analysis.

### 1.4 Measurement Methods

Free gossypol content was determined according to the national standard GB 13086-91 "Method for Determination of Free Gossypol in Feeds." Crude protein and protein solubility (using 0.2% potassium hydroxide solution method) were measured with a FOSS KJELTEC 8000 Kjeldahl nitrogen system (Denmark). Amino acid hydrolysis (using 6 mol/L hydrochloric acid at 110 °C for 24 hours) was performed using a Hitachi L-8800 automatic amino acid analyzer (Japan). All measurements followed the methods described in Zhang' s "Feed Analysis and Feed Quality Detection Technology." In vitro digestibility in pigs was determined using the "SDS-II Monogastric Animal Simulative Digestion System" and methodology developed by the Institute of Animal Science, Chinese Academy of Agricultural Sciences. Energy content of samples before and after digestion was analyzed using a PARR 1281 automatic oxygen bomb calorimeter (USA) according to ISO 9831:1998, while nitrogen content was determined by the DUMAS method (AOAC 990.03) using a Rapid N III combustion nitrogen analyzer (Germany).

### 1.5 Statistical Methods

Data were analyzed using SAS 9.2 statistical software. Differences were considered extremely significant at $P < 0.01$ and significant at $P < 0.05$.

## Results

### 2.1 Effects of Steam Explosion on Free Gossypol Content and Protein Solubility in Cottonseed Meal

As shown in Figure 1 [Figure 1: see original paper], steam explosion treatment significantly reduced free gossypol content in cottonseed meal compared with the untreated control (642 mg/kg), with free gossypol levels ranging from 85.0 to 293.6 mg/kg, representing reductions of 54% to 87%. Within the range of retention times tested, both detoxification effectiveness and protein solubility followed the order: 30 s > 20 s > 10 s, with longer retention times yielding better detoxification results. Within the range of water-to-material ratios tested, detoxification effectiveness and protein solubility ranked as: 30% water-to-material ratio > 50% water-to-material ratio > 0% water-to-material ratio. Compared with steam explosion at 0% water-to-material ratio, the addition of water significantly mitigated the decline in protein solubility.

Steam explosion treatment markedly reduced protein solubility in cottonseed meal while decreasing free gossypol content. The protein solubility of untreated cottonseed meal was 60.18%, whereas steam-exploded samples exhibited solubility values of 23.8% to 42.3%. All treatments showed that protein solubility increased with longer retention times and higher water-to-material ratios, though solubility decreased when the water-to-material ratio reached 50%.

Comprehensive evaluation of both free gossypol content and protein solubility indicators revealed that at a fixed steam pressure of 2.0 MPa, the optimal water-to-material ratio was 30% with a 30-second retention time. Under these conditions, free gossypol content reached 85.0 mg/kg, achieving an 87.0% detoxification rate, while protein solubility was 42.3%.

### 2.2 Effects of Steam Explosion Treatment on Amino Acid Content in Cottonseed Meal

As presented in Table 2 , steam explosion treatment (30% water-to-material ratio, 2.0 MPa pressure, 30-second retention time) significantly reduced lysine content in cottonseed meal ($P < 0.01$) by 38.77%, and also significantly decreased arginine content ($P < 0.01$) by 28.81%. No significant effects were observed on other amino acid contents ($P > 0.05$).

### 2.3 Effects of Steam Explosion Treatment on In Vitro Nutrient Digestibility in Cottonseed Meal

Porcine simulative digestion trials (Table 3 ) demonstrated that compared with the control group, steam explosion treatment (30% water-to-material ratio, 2.0 MPa pressure, 30-second retention time) had no significant effect on in vitro dry matter digestibility or gross energy digestibility ($P > 0.05$). However, the treatment significantly reduced in vitro crude protein digestibility and lysine digestibility ($P < 0.01$) by 20.99% and 11.24%, respectively, while showing no significant impact on the digestibility of other amino acids ($P > 0.05$).

## Discussion

Cottonseed meal represents an unconventional protein feed resource for animal production, with gossypol content being the bottleneck limiting its widespread application. The optimal steam explosion treatment in this study reduced free gossypol content to 85.0 mg/kg with an 87.0% detoxification rate, comparable to the biological detoxification efficacy reported by Zhu et al. (85.9 mg/kg) and substantially superior to results from other fermentation studies: Qiu et al. (197.68 mg/kg), Ma et al. (640 mg/kg), Tang et al. (250 mg/kg), and Nie et al. (43.0% degradation rate).

As a physical processing technology, steam explosion achieved superior detoxification compared to extrusion processing reported by Song et al. (120–140 mg/kg free gossypol) and chemical methods such as ferrous sulfate with high-speed grinding (324.5 mg/kg) or ferrous sulfate alone (82.08% detoxification rate) reported by Yao et al. and Saimaiti et al., respectively.

Free gossypol content in cottonseed meal varies with production processes, generally being lowest in screw-pressed meal, intermediate in pre-pressed solvent-extracted meal, and highest in direct solvent-extracted meal. This study demonstrates that integrating steam explosion technology into conventional processing can achieve highly efficient detoxification (87% efficiency within 30 seconds). Compared with existing detoxification technologies, steam explosion treatment requires only water adjustment of the water-to-material ratio, hydration at room temperature, involves no pollution or corrosion, does not affect feed palatability, requires no fermentation temperature control, and demands only a one-time equipment investment with energy consumption limited to water, electricity, and liquefied gas. The 30-second processing time enables rapid, efficient removal of 87% of free gossypol, yielding a final content of 85.0 mg/kg that far exceeds requirements set by the World Health Organization (gossypol content $< 0.04\%$) and China' s national feed hygiene standards (free gossypol $< 1,200$ mg/kg in cottonseed cake and meal).

Steam explosion efficacy is influenced by three factors: material moisture (water-to-material ratio), steam pressure, and retention time. High-temperature, high-pressure steam softens the material and penetrates its structure, weakening intermolecular bonds. The sudden pressure release within milliseconds causes rapid material expansion and explosive effects that alter physicochemical structure, leading to free gossypol dissociation or formation of bound gossypol.

Under the conditions tested, both detoxification rate and protein solubility increased with longer retention times at constant steam pressure. This occurs because appropriate extension of retention time allows more complete steam penetration into the material' s internal structure, enabling optimal adjustment of chemical structure during steam explosion. This enhances detoxification efficiency while promoting protein solubility, indicating that the tested retention times remained within the ascending phase of the detoxification response curve and had not yet reached a plateau.

Similarly, detoxification rate and protein solubility increased with higher water-to-material ratios but declined when the ratio reached 50%. Appropriate moisture increase facilitates material swelling, ensures uniform heating, and improves steam explosion efficacy while mitigating adverse effects of high temperature on protein solubility. However, excessive moisture hinders steam penetration, reducing treatment effectiveness—a finding consistent with results reported by Wang.

Compared with untreated controls, steam explosion reduced protein solubility, lysine content, arginine content, and in vitro digestibility of crude protein and lysine. This occurs because high-temperature, high-pressure conditions promote Maillard reactions in protein feedstuffs. Excessive heat treatment causes binding between reducing sugars and amino acid N-termini, particularly the -side chain of lysine, destroying lysine structure and biological activity. This significantly reduces protein solubility, lysine content, and animal protein digestibility, with excessive steam explosion converting considerable digestible amino acids into "artificial fiber." Therefore, establishing appropriate steam explosion parameters is critically important.

Protein solubility in 0.2% potassium hydroxide solution serves as an in vitro indicator for evaluating excessive heating of protein feedstuffs, showing strong correlation with animal in vitro digestibility and serving as a precise indicator for assessing protein utilization efficiency, particularly for evaluating excessive heating of soybean and rapeseed meals. Although protein solubility is not a sensitive indicator for cottonseed meal evaluation, it can indirectly reflect animal utilization efficiency of protein feedstuffs and help comprehensively assess the detoxification effects of steam explosion as a physical method on cottonseed meal.

Based on protein solubility and detoxification rate, the optimal steam explosion conditions were determined to be 30% water-to-material ratio, 2.0 MPa pressure, and 30-second retention time. These conditions produced minimal changes in dry matter and gross energy digestibility but substantial reductions in crude protein digestibility (20.99% decrease) and lysine digestibility (11.24% decrease). The reduction in lysine digestibility was associated with both decreased lysine content (38.77% reduction) and reduced digestibility of residual lysine (11.24%

decrease).

In vitro simulative trials revealed that steam explosion effects on amino acid digestibility did not consistently align with changes in amino acid content, indicating that while protein solubility and amino acid content correlate with nutrient digestibility and can serve as evaluation criteria for feed processing, they cannot replace animal digestibility trials. Further animal studies are needed to assess nutrient digestibility comprehensively.

## Conclusion

Comprehensive evaluation indicates that at a fixed steam explosion pressure of 2.0 MPa, the optimal water-to-material ratio is 30% with a 30-second retention time. Under these conditions, cottonseed meal exhibits free gossypol content of 85.0 mg/kg, an 87.0% detoxification rate, protein solubility of 42.3%, lysine content of 1.21%, in vitro dry matter digestibility of 51.20%, gross energy digestibility of 61.84%, crude protein digestibility of 65.31%, and lysine digestibility of 55.23%.

Key findings: (1) Steam explosion treatment reduces free gossypol content, protein solubility, lysine and arginine contents, and in vitro digestibility of crude protein and lysine in cottonseed meal. (2) The optimal steam explosion parameters are 2.0 MPa steam pressure, 30% water-to-material ratio, and 30-second retention time.

## References

[1] LI Aike, HAO Shuhong, WU Songling. Research progress on new technologies for development and utilization of plant protein feed resources[J]. Feed and Animal Husbandry, 2006(10): 5-9.

[2] TOUSSAINT B, EXCOFFIER G, VIGNON M R. Effect of steam explosion treatment on the physico-chemical characteristics and enzymic hydrolysis of poplar cell wall components[J]. Animal Feed Science and Technology, 1991, 32(1/2/3): 235-242.

[3] YU Z D, ZHANG B L, YU F Q, et al. A real explosion: the requirement of steam explosion pretreatment[J]. Bioresource Technology, 2012, 121: 335-341.

[4] ZHU Junjun, YONG Qiang, CHEN Shangxing, et al. Analysis of degradation products from corn stover steam explosion[J]. Chemistry and Industry of Forest Products, 2009, 29(2): 22-26.

[5] VIOLA E, ZIMBARDI F, CARDINALE M, et al. Processing cereal straws by steam explosion in a pilot plant to enhance digestibility in ruminants[J]. Bioresource Technology, 2008, 99(4): 681-689.

[6] YANG Xuexia, CHEN Hongzhang, LI Zuohu. Study on production of feed from steam-exploded corn stover by solid-state fermentation[J]. Cereal and Feed

Industry, 2001(2): 27-29.

[7] LIU Dongbo, WANG Xiuran, CHEN Shan, et al. Study on feeding safety of corn straw feed treated by steam explosion[J]. Journal of Jilin Agricultural University, 2003, 25(6): 657-660.

[8] CHANG Juan, YIN Qingqiang, REN Tianbao, et al. Effect of steam explosion pretreatment and microbial fermentation on degradation rate of corn straw[J]. Transactions of the Chinese Society of Agricultural Engineering, 2011, 27(4): 277-280.

[9] CHANG Juan, YIN Qingqiang, JIANG Yibao, et al. Study on effect of biological straw on apparent metabolic energy in broilers and optimal substitution ratio for corn[J]. Chinese Journal of Animal Nutrition, 2012, 24(8): 1557-1563.

[10] SONG Xiaomin, WU Desheng. Experimental process and mechanism analysis of cottonseed meal extrusion processing[J]. Cereals and Oils Processing, 2006(8): 47-49.

[11] ZHANG Liying. Feed Analysis and Feed Quality Detection Technology[M]. Beijing: China Agricultural University Press, 2007.

[12] ZHUANG Xiaofeng, ZHANG Hongfu, CHEN Liang. Effects of different dialysis bags on in vitro digestibility of dry matter and phosphorus in pig feed ingredients evaluated by simulative digestion method[J]. Chinese Journal of Animal Nutrition, 2012, 24(8): 1527-1533.

[13] ZHU Dewei, LIU Zhipeng, CAI Guolin, et al. Screening of highly efficient gossypol-degrading strains and study on detoxification process of cottonseed meal by fermentation[J]. China Oils and Fats, 2010, 35(2): 24-28.

[14] QIU Liangwei, LI Aike, CHENG Maoji, et al. Study on nutritional value of cottonseed meal produced by two different fermentation processes[J]. Feed Industry, 2012, 33(13): 32-36.

[15] MA Li, ZHANG Rijun, CHEN Fushui, et al. Experiment on effects of fermented cottonseed meal replacing part of soybean meal on growth performance of growing pigs[J]. Zhejiang Journal of Animal Science and Veterinary Medicine, 2012(4): 28-30.

[16] TANG Jiangwu, WU Yifei, SUN Hong, et al. Effects of fermented cottonseed meal on growth performance, serum biochemical indices and immune function of broilers[J]. Chinese Journal of Animal Science, 2011, 47(5): 29-34.

[17] NIE Pengbo, TANG Jiangwu, LIANG Yunxiang. Study on screening of detoxification strains for cottonseed meal and fermentation conditions[J]. Zhejiang Agricultural Sciences, 2009(1): 120-122.

[18] YAO Zhong, TAN Hequn. Mechanochemical effect of free gossypol in cottonseed meal during high-speed grinding[J]. Feed Research, 2007(4): 39-41.

[19] Saimaiti · Aimaiti, OUYANG Hongfei, ZHAO Li, et al. Comparative study on detoxification effects of five different methods on cotton by-products[J]. Feed Industry, 2008, 29(1): 27-30.

[20] WANG Anping, LÜ Yunfeng, ZHANG Junmin, et al. Investigation on nutrient composition and gossypol content of cottonseed meal and cottonseed protein in China[J]. Acta Agriculturae Boreali-Sinica, 2010, 25(Suppl.): 301-304.

[21] WANG Xin. Research progress on steam explosion pretreatment technology and its application in cellulosic ethanol bioconversion[J]. Chemistry and Industry of Forest Products, 2010, 30(4): 119-121.

[22] ANDERSON-HAFERMANN J C, ZHANG Y, PARSONS C M. Effects of processing on the nutritional quality of canola meal[J]. Poultry Science, 1993, 72(2): 326-333.

[23] ZHOU Yanmin, WU Di, LIU Feng, et al. Study on evaluation of several major oilseed meals by protein solubility method[J]. Cereal and Feed Industry, 1996(6): 36-39.

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