Effects of Steam Explosion and Post-Steam-Explosion Fermentation on the Nutritional Value of Cotton Stalk: Postprint

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Abstract

This experiment aimed to investigate the effects of steam explosion and post-explosion fermentation on the nutritional value of cotton straw. Cotton straw was subjected to four treatments: untreated (control group), grinding (S group), steam explosion (SE group), and steam explosion followed by fermentation (SEF group). Samples were collected using multi-point sampling and quartering method to determine the contents of Gross Energy (GE), Crude Protein (CP), Ether Extract (EE), Neutral Detergent Fiber (NDF), Acid Detergent Fiber (ADF), Acid Detergent Lignin (ADL), Ash, Calcium (Ca), Phosphorus (P), and Free Gossypol (FG) in cotton straw. The Grading Index (GI) was calculated to comprehensively evaluate the effects of different treatments on the nutritional value of cotton straw. The results showed that: 1) The CP content in cotton straw of the SE and SEF groups increased by 10.79% and 14.60% compared with the S group ($P<0.01$), respectively; the CP content in cotton straw of the SEF group increased by 5.40% compared with the control group ($P<0.05$). The EE content in cotton straw of the S, SE, and SEF groups increased by 29.29%, 61.09%, and 59.83% compared with the control group ($P<0.01$), respectively, and the EE content in cotton straw of the SE and SEF groups increased by 24.60% and 23.62% compared with the S group ($P<0.01$), respectively. The GE of cotton straw in the SEF group was the highest, increasing by 7.89%, 24.56%, and 10.27% compared with the SE, S, and control groups ($P<0.01$), respectively. 2) Compared with the S group, the NDF content in cotton straw of the SE and SEF groups increased by 10.83% and 9.85%, respectively, and the ADF content increased by 23.94% and 14.52% ($P<0.01$), respectively; the ADL content in cotton straw was highest in the S group, followed by the SE group, and lowest in the SEF group, with extremely significant differences among all groups ($P<0.01$). 3) After different treatments, the FG content in cotton straw varied extremely significantly ($P<0.01$), with the SEF group being the lowest.

The GI values of the SEF and SE groups increased by 11.60% (P<0.01) and 11.58% (P<0.05) compared with the control group, respectively, while the GI value of the S group increased by 2.11% (P>0.05). In conclusion, steam explosion and post-explosion fermentation treatments can improve the nutritional value of cotton straw and reduce the ADL and FG contents, with post-explosion fermentation showing the best effect.

Full Text

Effects of Steam Explosion and Fermentation after Steam Explosion on the Nutritional Value of Cotton Stalk

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Abstract

This experiment investigated the effects of steam explosion and subsequent fermentation on the nutritional value of cotton stalk. Cotton stalk samples were subjected to four treatments: untreated (control, CK), smashed (S), steam-exploded (SE), and fermented after steam explosion (SEF). Samples were collected using multipoint sampling and quartering methods, then analyzed for gross energy (GE), crude protein (CP), ether extract (EE), neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL), ash, calcium (Ca), phosphorus (P), and free gossypol (FG) content. The graded index (GI) was calculated to comprehensively evaluate the effects of different treatments on cotton stalk nutritional value. The results showed: (1) Compared with the S group, CP content in SE and SEF groups increased by 10.79% and 14.60%, respectively (P<0.01). The SEF group also showed a 5.40% increase in CP content compared with the control group (P<0.05). EE content in S, SE, and SEF groups increased by 29.29%, 61.09%, and 59.83% compared with the control group (P<0.01), while SE and SEF groups showed 24.60% and 23.62% increases compared with the S group (P<0.01). The GE content was highest in the SEF group, exceeding SE, S, and control groups by 7.89%, 24.56%, and 10.27%, respectively (P<0.01). (2) Compared with the S group, NDF and ADF contents in SE and SEF groups increased by 10.83%, 9.85% and 23.94%, 14.52%, respectively (P<0.01). ADL content was highest in the S group, followed by SE group, and lowest in SEF group, with highly significant differences among all groups (P<0.01). (3) FG content varied significantly across treatments (P<0.01), with the SEF group showing the lowest level. GI values increased in processed groups, with SEF and SE groups showing 11.60% (P<0.01) and 11.58% (P<0.05) increases over the control group, respectively, while the S group showed a non-significant 2.11% increase (P>0.05). In conclusion, steam explosion and fermentation after steam explosion can improve the nutritional value of cotton stalk by reducing ADL and FG contents, with the combined treatment showing the best results.

Keywords: cotton stalk; steam explosion; fermentation; nutritional value; acid detergent lignin; free gossypol

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As a pillar industry of agriculture in Xinjiang, cotton cultivation generated nearly 8 million tons of cotton stalk in 2013. According to Wei Min [1], cotton stalk from the Kuitun area of Xinjiang contains 6.5% crude protein (CP), 15.2% lignin (ADL), 44.1% cellulose, 10.7% hemicellulose, 0.65% calcium (Ca), 0.09% phosphorus (P), and 0.03% free gossypol (FG), demonstrating good potential as a roughage source for livestock. Although the CP content of cotton stalk is higher than that of wheat and rice straw, its high lignification, presence of toxic FG, and poor palatability limit its utilization as animal feed. Reducing ADL and FG contents through appropriate treatments could substantially improve the feeding value of cotton stalk and help alleviate forage shortages in Xinjiang. Various processing methods such as soaking, ammoniation, alkalization, and biological fermentation have been applied to crop residues including corn stover, wheat straw, and rice straw, with varying effectiveness [2-5]. Recent research has advanced steam explosion technology for crop residue processing. Steam explosion can reduce cellulose, hemicellulose, and ADL contents [6-8], and steam-exploded rice straw, eucalyptus, and cereal straws have shown improved digestibility in ruminants through in vitro rumen fermentation [9-11]. This study combined steam explosion with solid-state fermentation to treat cotton stalk, utilizing the high temperature and pressure to disrupt cell walls, break covalent bonds between ADL and hemicellulose, and release cellulose and hemicellulose, thereby exposing nutrients for subsequent microbial degradation through solid-state fermentation. By analyzing routine nutritional components, this experiment evaluated the effectiveness of this combined physical-biological treatment to provide a theoretical basis for producing high-nutritional-value cotton stalk feed and its utilization in animal production.

1.1 Experimental Materials

Cotton stalk used in this experiment was harvested from Bayingolin Mongolian Autonomous Prefecture, Xinjiang, using mechanical harvesters producing stalk pieces approximately 10 cm in length. The air-dried cotton stalk had a moisture content of 10.4%. The active bacterial inoculant used for fermentation was Haixing Zihuan straw fermentation agent, containing *Bacillus subtilis*, *Lactobacillus plantarum*, *Saccharomyces cerevisiae*, and other microorganisms, with viable bacterial counts reaching 20 billion CFU/g.

1.2 Steam Explosion Treatment of Cotton Stalk

Cotton stalk processing was conducted at Xinjiang Hongrui Fiber Co., Ltd. Following the production process for steam-exploded cotton stalk, the smashed stalk (2-3 cm length) was first moistened with water to reach 40% moisture content. The material was then fed into an expansion and detoxification machine (ZL2014208627577) via a feeding device. Steam was injected to achieve a pressure of 2.5 MPa at 220°C, maintained for 2-3 minutes, then rapidly released to cause instantaneous expansion under natural pressure differential. The exploded material was discharged into a collection tank, then sequentially passed through a discharge screw conveyor and elevator before entering a counter-flow cooler. For fermentation treatment, steam-exploded cotton stalk was sprayed with fermentation solution (inoculant:water = 1 g:350 kg) at approximately 500 kg per ton to adjust moisture content to 60-65% for optimal fermentation conditions.

1.3 Sampling Methods

Four treatment groups were established: (1) Untreated cotton stalk (control, CK): Samples were collected from the raw material pile using the five-point method, with the center point at the diagonal intersection and four additional points at equal distances along the diagonals. One kilogram was collected from each point, the 5 kg composite was mixed thoroughly, and 1 kg was obtained by quartering for analysis. Samples were sealed and stored at -20°C. Three batches were collected. (2) Smashed cotton stalk (S group): Cotton stalk from the control group was smashed to 2-3 cm length. Samples (0.5 kg each) were collected before entering the steam explosion chamber, sealed and stored at -20°C. Sampling followed the same method as the control group, with three corresponding batches. (3) Steam-exploded cotton stalk (SE group): Smashed stalk from the S group was steam-exploded. After cooling, samples were collected from the explosion chamber, sealed and stored at -20°C. Sampling followed the same method as the control group, with three corresponding batches. (4) Fermented after steam explosion (SEF group): Steam-exploded stalk from the SE group was cooled, inoculated with 1% active bacteria, packed in anaerobic bags, sealed after air removal, and fermented for 45 days at temperatures not lower than 15°C. Samples were then collected, sealed and stored at -20°C. Sampling followed the same method as the control group, with three corresponding batches.

1.4 Analytical Methods

1.4.1 Routine Nutrient Components Samples were analyzed in the laboratory for dry matter (DM), gross energy (GE), CP, ether extract (EE), acid detergent fiber (ADF), neutral detergent fiber (NDF), ADL, ash, Ca, and P contents. CP content was determined by the Kjeldahl method (GB/T 6432-94) using a Haineng K1100 analyzer. EE content was measured by petroleum ether extraction (GB/T 6433-2006) using a Soxhlet extractor. Ca content was determined by atomic absorption spectroscopy (GB/T 6436-2002) using an East-West Electronics AA-7000 spectrometer. P content was measured by spectrophotometry (GB/T 6437-2002) using a GC2010 spectrophotometer. NDF, ADF, and ADL contents were determined by digestion methods (GB/T 20806-2006, NY/T 1459-2007, GB/T 20805-2006). Moisture content was measured by oven drying (GB/T 6435-1986). Ash content was determined by incineration (GB/T 6438-2007). GE was measured by bomb calorimetry using an OR2014 calorimeter.

1.4.2 Graded Index of Roughage The graded index (GI) is a scientific indicator for evaluating roughage quality proposed by Chinese animal nutritionist Lu Dexun in 2001. The formula is:

$$GI = ME \times DMI \times CP / NDF$$

where ME is the metabolic energy of roughage (MJ/kg), using NEL for dairy cows; DMI is the dry matter intake (kg) of roughage; CP is the crude protein content (% DM); and NDF is the neutral detergent fiber content (% DM). DMI was corrected for a 40 kg standard body weight sheep (1.4 kg/d). ME was calculated using Liu Jie' s formula [12]: ME (MJ/kg DM) = 3.866 + 0.285 × CP (% DM). Other data were measured values, and calculations were converted from air-dry basis to DM basis.

1.4.3 Free Gossypol Content Feed samples were ground using a FOSS MILL with water circulation cooling and sent to a third-party testing agency for FG content determination using the method GB/T 13086-1991.

1.5 Statistical Analysis

Data were initially processed using Excel 2007, then subjected to one-way ANOVA using the SAS 9.0 GLM model. Multiple comparisons were performed using the Tdiff method. Significance was declared at $P<0.01$ (highly significant) and $P<0.05$ (significant).

2.1 Effects of Steam Explosion and Fermentation on Routine Nutrient Components of Cotton Stalk

As shown in Table 1 , CP content in SE and SEF groups increased by 10.79% and 14.60% compared with the S group ($P<0.01$). The SEF group also showed a 5.40% increase in CP content compared with the control group ($P<0.05$). EE content in S, SE, and SEF groups increased by 29.29%, 61.09%, and 59.83% compared with the control group ($P<0.01$), while SE and SEF groups showed 24.60% and 23.62% increases compared with the S group ($P<0.01$). Variations in Ca and P contents among groups were minor, with the SEF group showing relatively higher values and the SE group lower values. The SEF group also had the highest ash content, while the S group had the lowest. GE content was highest in the SEF group, exceeding SE, S, and control groups by 7.89%, 24.56%, and 10.27%, respectively (P<0.01).

Table 1 Effects of steam explosion and fermentation after steam explosion on routine nutrient ingredients of cotton stalk (DM basis)

Item	Groups				P-value
	Control	S	SE	SEF	
Crude protein CP	6.85Ab	6.30Bc	6.98Aa	7.22Aa	<0.0001
Ether extract EE	2.39Cc	3.09Bb	3.85Aa	3.82Aa	<0.0001
Ash	7.77ABab	6.84Bb	7.95ABab	8.17Aa	0.0003
Ca	0.59Cc	0.66Bb	0.48Dd	0.68Aa	<0.0001
P	0.19Bb	0.18Bb	0.12Cc	0.23Aa	<0.0001
GE (MJ/kg)	16.74Cc	14.82Dd	17.11Bb	18.46Aa	<0.0001

In the same row, values with no letter or the same letter superscripts mean no significant difference (P>0.05), while different lowercase letters indicate significant difference (P<0.05) and different capital letters indicate highly significant difference (P<0.01). The same as below.

2.2 Effects of Steam Explosion and Fermentation on Fiber Content of Cotton Stalk

Compared with the S group, NDF and ADF contents in SE and SEF groups increased by 10.83%, 9.85% and 23.94%, 14.52%, respectively (P<0.01). ADL content was highest in the S group, followed by the SE group, and lowest in the SEF group, with highly significant differences among all groups (P<0.01).

Table 2 Effects of steam explosion and fermentation after steam explosion on fiber content of cotton stalk (DM basis) %

Item	Groups				P-value
	Control	S	SE	SEF	
Neutral detergent fiber NDF	58.86Aa	51.69Cc	57.29Bb	56.78Bb	<0.0001
Acid detergent fiber ADF	40.60Cc	36.84Dd	45.66Aa	42.19Bb	<0.0001
Acid detergent lignin ADL	26.62Cc	30.59Aa	28.82Bb	22.51Dd	<0.0001

2.3 Effects of Steam Explosion and Fermentation on FG Content and GI Value of Cotton Stalk

As shown in Table 3 , FG content varied significantly across treatments ($P<0.01$), with the SEF group reaching levels below the safety limit for post-weaning ruminants (200 mg/kg) [13]. GI values also improved with processing, showing increases of 11.60% ($P<0.01$) and 11.58% ($P<0.05$) in SEF and SE groups compared with the control group, respectively, while the S group showed a non-significant 2.11% increase ($P>0.05$).

Table 3 Effects of steam explosion and fermentation after steam explosion on FG content and GI value of cotton stalk (DM basis)

Item	Groups				P-value
	Control	S	SE	SEF	
Free gossypol FG (mg/kg)	483.6Aa	309.45Bb	205.16Cc	95.22Dd	<0.0001
GI	0.95c	0.97c	1.00b	1.06a	<0.0001

3 Discussion

3.1 Effects of Steam Explosion and Fermentation on Nutritional Value of Cotton Stalk

Steam explosion treatment primarily causes chemical modifications and physical structural changes through high temperature and pressure, while fermentation promotes cellulose degradation and synthesis of other nutrients through microbial action. He Liwen et al. [14] reported that steam explosion increased CP content by 0.45% in corn stover but decreased it by 0.8% and 0.1% in wheat and rice straw, respectively. Steam explosion can cause DM loss, which relatively increases the proportions of crude ash, CP, and EE in DM, but also increases neutral detergent insoluble nitrogen (NDIN) and acid detergent insoluble nitrogen (ADIN) contents [15-17]. In this experiment, steam explosion alone increased CP content by 10.79% compared with smashed cotton stalk, while the combined treatment increased CP content by 14.60%, indicating that steam explosion followed by fermentation can effectively increase CP content in cotton stalk. Similar to He Liwen et al. [14], both steam explosion and the combined treatment increased crude ash, Ca, and P contents. The steam explosion process can rupture fat cells, allowing released fat to combine with starch matrix and form fat-starch complexes that reduce fat deterioration and facilitate storage [18]. This experiment demonstrated that steam explosion and fermentation significantly increased EE content and GE in cotton stalk compared with the smashed material. Following steam explosion, the deconstruction of lignified structures makes cellulose and hemicellulose more accessible for microbial attachment and enzymatic digestion. Meanwhile, high temperature and pressure partially degrade nutrients into soluble sugars and organic acids that promote microbial proliferation and improve digestibility for animals [19-20].

3.2 Effects of Steam Explosion and Fermentation on Fiber Content of Cotton Stalk

The plant cell wall is primarily composed of ADL, which firmly binds with small amounts of starch, pectin, and protein, making it difficult for animals to digest and negatively affecting the degradation of other nutrients. Breaking down this lignified structure to release nutrients and reduce ADL content could significantly improve feed utilization efficiency. Steam explosion alone may not be particularly effective for ADL degradation in cotton stalk, as this experiment showed only a 5.79% reduction in ADL content compared with the smashed treatment. However, fermentation after steam explosion reduced ADL content by 26.41%. Steam explosion had minimal effects on NDF and ADF contents, with only minor changes. Different crop residues respond differently to steam explosion treatment. For instance, steam-exploded reed showed 19.2% increased cellulose content, 5.5% decreased ADL content, and 4.51% increased reducing sugar content [21]. Zheng Lili et al. [22] reported that steam explosion reduced ADL content in banana leaves from 14.62% to 6.52% (55.40% degradation rate). Under 2.5 MPa pressure for 200 seconds, corn stover showed reductions of 26.44%, 82.99%, and 35.12% in cellulose, hemicellulose, and ADL contents, respectively [23]. Steam explosion can break the bonds between ADL and cellulose, increasing the internal surface area of fibers and providing more contact points for microbial degradation. Additionally, steam explosion produces reducing sugars that serve as carbon sources for microbial growth, promoting metabolic activity and enhancing lignin degradation by key enzymes.

3.3 Effects of Steam Explosion and Fermentation on FG Content of Cotton Stalk

The results demonstrate that fermentation after steam explosion not only increased the GI value of cotton stalk but also degraded most of the FG content, removing a major obstacle to its utilization in animal feed. Zhong Yingchang et al. [4] isolated five microbial strains that achieved 60-74% detoxification rates for free gossypol in unpressed cottonseed meal. Wang Qinghua et al. [24] reported that under optimal conditions of 2.0 MPa steam pressure, 30% water-to-material ratio, and 30 seconds retention time, FG content in cottonseed meal decreased to 85.0 mg/kg, achieving an 87.0% detoxification rate. In this experiment, FG content varied significantly across treatments, with the SEF group reaching levels below the safety limit for post-weaning ruminants (200 mg/kg) [13], indicating that the combined steam explosion and fermentation treatment can effectively eliminate FG toxicity in cotton stalk.

4 Conclusion

1. Fermentation after steam explosion resulted in the lowest ADL content in cotton stalk, demonstrating the best degradation effect.

2. The combined treatment achieved the best FG degradation effect (95 mg/kg), which is below the 200 mg/kg safety standard for adult ruminant feed.

3. The SEF group showed the highest GI value among all treatments.

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