The Cotton Stalk and Its Utilization as Ruminant Feed Resource in Xinjiang

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Abstract Two separate experiments were carried out: the first one involved a study of the chemical and some intrinsic feed characteristics of raw cotton stalk (CS) and the efficiency of chemical, biological treatments on the improvement of the feed value of CS. CS was treated with chemical treatment by mixing 3% $Ca(OH)_2$ (CS + Ca), 3% urea (CS + U), 3% urea + 3% $Ca(OH)_2$ (CS + Ca + U). Moreover, CS was treated with biological treatment by inoculation of either *Lentinus edodes* (CS + Po) or *Pleurotus ostreatus* (CS + Pl) or crushing CS by adding minor ingredient processed into pellet feed, samples from all treatment were analyzed for the nutrients, *In vitro* dry matter digestibility (INDMD) and free gossypol detoxification rate. The second investigated was the use of crushed or granulation CS as a test feed followed by voluntary feed intake and preference test with sheep. CS + Ca + U treatment can reach up the CP and IVDMD to 9% and 36%, respectively. CS + Po decreased the fiber component and improved CP content and IVDMD. The free gossypol content declined and the free gossypol detoxification rate reached more than 50%. By feeding sheep under the same level of concentrate and corn silage, the voluntary feed intake and daily weight gain in granulation group is higher than in crushing group ($P < 0.05$). Crushing CS by adding minor ingredient processed into a pellet feed was best in terms of improved nutritional quality, feed intake and preference, and it was safe and practical method that can be widely used in sheep production systems, to promote the CS feed utilization in similar areas.

Key words Cotton stalk, Pre-treatment, Granulation, Feed intake, Sheep

1 Introduction

Cotton is one of the major crops in Xinjiang, China, and it's cultivated about almost 1.46 million ha as about one third of the total crops on arable land in Xinjiang. A large area for planting cotton for livestock industry can provide a lot of cotton by-product as feed resources such as cotton seed meal, cotton seed hulls and cotton stalk (CS). The CS is a kind of low cost feed resource in Xinjiang, but except a few of them used to make paper and board or feeding sheep in winter, most of the CS were burned directly to field. CS leaves and boll shells are rich in nutrients, and they can be used as roughage feed (Wei *et al.*, 2003). Zhang and Ji (2005) reported that CS treated with the mixture solution contained 2.5% lime + 3.5% urea + 3% salt, and it became so soft and fragrant and it can improve palatability and the feed conversion efficiency. Saimaiti, (2008) reported that using 4%, 6% and 8% of urea treatment to CS can not only remove gossypol, but also improve palatability, digestibility and feed conversion efficiency. Processing CS fermented feed can improve the CP content and palatability, it can also reduce fiber and free gossypol content (Yang, 2003; Xu *et al.*, 1998; Zhang *et al.*, 2014). Currently, all researches of CS as animal feed focus on component analysis and pretreatment methods. Therefore, this paper aims to investigate the chemical composition and the feed characteristics of CS, followed by pre-treatment of CS by chemical and biological methods, explore the possibility of improving feed values and detoxification and to assess the effect of granulation by adding sub-material on voluntary intake and performance of sheep.

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2 Materials and methods

CS samples were obtained from several regions in Xinjiang and the constituent part and nutrients were were analyzed. The crushed CS through chemical and biological treatments was mixed into pellet feed to determine the improvement efficiency on the feed quality of CS. Voluntary feed intake from crushing or granulation of CS affecting the performance of sheep was studied.

2.1 Experiment 1

2.1.1 Chemical treatment. Crushed CS was treated by addition of 3% $Ca(OH)_2$, 3% urea or 3% $Ca(OH)_2$ and 3% urea mixed with 1% salt, and water was added to adjust the moisture content according to water ratio of 1:1. The samples were stored for 30 days sampling for chemical analysis. The CP, NDF, ADF, hemicellulose, ADL, free gossypol contents and IVDMD were measured before and after treatments.

2.1.2 Biological treatment. *Lentinus edodes* and Pleurotus ostreatus were used for inoculation. After the crushed CS mixed with other components (Table 1) and steam was sterilized, the mixed medium was inoculated with each of edible basidiomycetes and cultured at Hyphal compartment (about 20 – 22℃, humidity 70%) for 30 days. After 30 days, CP, NDF, ADF, hemicellulose, ADL, IVDMD and free gossypol content were determined. The feed slices were compared using electron microscope (Transmission Electron Microscopy, TEM) and optical microscope.

2.1.3 Granulation processing. Dry CS was crushed by grinding machine (XI DA 9F-45B) and 85% of crushed CS was combined with 6% of corn, 5% of cotton seed meal, 1% of urea, 3% of premix and 8% of water and mixed by mixer (9HWP – 1000) used to make pellet (300 type). The raw material and CS pellet samples were used for determination of the nutrients, free gossypol content and IVDMD.

Table 1 The composition of the culture medium for each basidiomycete species (%)

Composition	*Lentinus edodes*	*Pleurotus ostreatus*
Raw material from(% of dry matter)		
Cotton stalk	80.0	80.0
Wheat bran	17.0	14.0
Urea	0.5	0.5
Plaster	1.0	1.0
Lime	–	3.0
Sucrose	1.0	1.0
Calcium superphosphate	0.5	0.5

2.2 Experiment 2 The study was conducted in June to July 2014, in the new rural cooperative sheep farm of Aksu Shaya County. 20 sheep were randomly divided into 2 groups according to their age, gender and body weight for the feeding experiment. Feed or granulation CS was crushed ad libitum under the same level of formula concentrate and silages. Water was available at all times during feeding period. The period lasted for 20 days and consisted of 14 days of diet adaptation followed by 6 days of sample collection. To determine fecal DM output, 20 g chromium pellet containing approximately 10% Cr was used for feeding before the diet for each sheep at 0800 and 2000 h from day 5 to 18 of the period. After 20 days of adaption period, feed intake (ad libitum access), refusal and total fecal output were recorded for each sheep. Besides, body weight was recorded with an electronic scale at the beginning and the end of experimental period.

2.3 Analysis and calculation method The DM concentration of sample was analyzed by drying at 60℃ for 24 h. The dried samples were ground through a 1 mm screen of chemical composition (Zhu, 2009). Content of nitrogen (N) was measured by the Kheldal method (AOAC, 1990). The CP was calculated as N × 6.25. Content of neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) was determined by the method of Van Soest *et al*. (1991). Free gossypol content was also determined by phloroglucinol colorimetric method (Tang *et al*., 2005). Free gossypol detoxification effects were calculated by free gossypol content in sample before and after treatment. The IVDMD was evaluated by using the method of Tilley and Terry (1963). The sheep intake through the formal pilot phase (after the start of two weeks) was determined in accordance with the feeding and the remaining amount of feed. When the trial began and every 15 days, in the morning on an empty stomach before feeding weighing, the weight gained was calculated. Using the initial weight, each test weight and the number of breeding days were calculated by the weight and the average daily gained weight. Sheep defecation quantity was calculated by chromium oxide, and according to the feed intake and defecation of sheep, the DM digestibility was calculated.

2.4 Statistical method According to test data, SPSS 21.0 statistical software was used for statistical analysis.

3 Results

3.1 Feed characteristics of cotton stalks In Xinjiang, using cotton planting density method, it was found that there were $1.1 \times 10^4 - 1.2 \times 10^4$ plants per hectare in cotton fields. Plant height of CS is approximately 60 – 80 cm and 3.0 – 3.5 t of CS per hectare was in cotton fields. The CS consisted of 41% of main stalks, 16% of slender stalks, 22% of leaves and 21% of bolls on a dry matter basis. The cotton plant included 5.88% of CP, 73.4% of NDF, 7.4% of hemicelluloses and 17.2% of ADL, and the content of NDF and ADL was highest in main stalks (Table 2). The fiber content of the whole plant of cotton was higher than that of cereal straws such as corn stalk and wheat straw.

Table 2 Chemical composition of each part of the cotton plant (% of dry matter)

Item	Whole cotton plant	Parts of cotton plant			
		Main stalks	Slender stalks	Leaves	Bolls
Proportion (%)	–	41.00	16.00	22.00	21.00
Chemical composition					
Crude protein	5.88 ±0.19	5.80 ±1.23	6.90 ±0.21	11.20 ±0.12	5.60 ±1.00
NDF	73.40 ±0.98	78.60 ±0.25	68.90 ±1.02	30.50 ±0.32	62.70 ±2.01
ADF	66.00 ±0.43	67.80 ±1.05	62.10 ±0.62	18.90 ±0.74	53.50 ±0.02
Hemicellulose	7.40 ±2.91	10.80 ±0.32	6.90 ±0.11	11.60 ±0.14	9.30 ±0.71
ADL	17.20 ±0.45	19.80 ±0.01	16.10 ±0.15	11.60 ±0.22	13.00 ±1.02
Crude ash	6.80 ±0.12	8.30 ±0.52	10.20 ±0.12	13.60 ±0.03	12.10 ±0.63
Calcium	0.65 ±0.02	0.23 ±0.01	0.22 ±0.02	3.83 ±0.01	0.14 ±0.04
Phosphate	0.11 ±0.01	0.08 ±0.04	0.09 ±0.01	0.24 ±0.00	0.16 ±0.02
Free gossypol	0.020 – 0.030	0.020 – 0.025	0.030 – 0.035	0.030 – 0.035	0.035 – 0.040

3.2 The effects of pre-treatment experiment feed nutritional value and detoxification

3.2.1 Chemical treatment. CS + Ca, CS + Ca + U treatments significantly decreased the NDF content of treated CS ($P < 0.05$), but had not significant effect on ADF and hemicellulose content. CS + U or CS + Ca + U treatments significantly improved the CP content and IVDMD compared with untreated CS (Table 3).

3.2.2 Inoculation of edible basidiomycete. Mycelium of Pleurotus ostreatus extended faster than *Lentinus edodes*, however, the hyphae of two strains can cover the whole medium 30 days after inoculation. The basidiomycete decreased the fiber components, increased CP content and improved IVDMD (Table 4), but the Pleurotus ostreatus basidiomycetes effect was better than *Lentinus edodes* ($P<0.01$). The cotton stalk as the main medium inoculated with edible basidiomycete can improve the cellulose activity and dissolve the cell wall of CS (Fig. 1).

Table 3 Effects of chemical treatments on chemical composition and IVDMD of CS (% of dry matter)

Item	Non-treated CS	Chemical treatment		
		CS + Ca	CS + U	CS + Ca + U
NDF	78.6 ± 0.9^{b}	76.3 ± 0.4^{b}	78.6 ± 0.4^{a}	73.4 ± 0.4^{c}
ADF	70.3 ± 1.9	67.8 ± 0.9	70.4 ± 0.2	67.8 ± 0.7
Hemicellulose	7.3 ± 2.9	8.5 ± 1.3	8.3 ± 0.6	5.7 ± 0.4
ADL	18.0 ± 0.4	17.5 ± 0.3	17.6 ± 0.6	17.8 ± 0.7
CP	5.9 ± 0.2^{c}	6.7 ± 0.3^{b}	8.7 ± 0.6^{a}	9.0 ± 0.2^{a}
IVDMD	22.8 ± 3.0^{b}	37.2 ± 1.2^{a}	35.5 ± 0.3^{a}	36.1 ± 0.8^{a}

Note: a, b, c means different superscripts in the same row differ significantly ($P<0.05$); NDF (neutral detergent fiber); ADF (acid detergent fiber); ADL (acid detergent lignin); CP (crude protein); IVDMD (in vitro dry matter digestibility).

Table 4 Effect of edible basidiomycete inoculated (30 d) on each treatment group culture medium nutrients (%)

Item	CS + Le	CS + Po	*P* value
Moisture(%)	60.59 ±0.42	60.75 ±0.82	>0.05
Nutrition(% of dry matter)			
NDF	64.59 ±1.66	60.24 ±0.86	>0.05
ADF	48.65 ±0.30	46.94 ±1.18	<0.05
HC	15.90 ±2.07	13.31 ±0.32	<0.01
ADL	17.56 ±0.05	17.36 ±0.96	>0.05
CP	11.46 ±0.53	11.65 ±0.59	>0.05
IVDMD	54.89 ±1.57	59.54 ±0.37	<0.01

Note: NDF (neutral detergent fiber); ADF (acid detergent fiber); ADL (acid detergent lignin); CP (crude protein); IVDMD (in vitro dry matter digestibility).

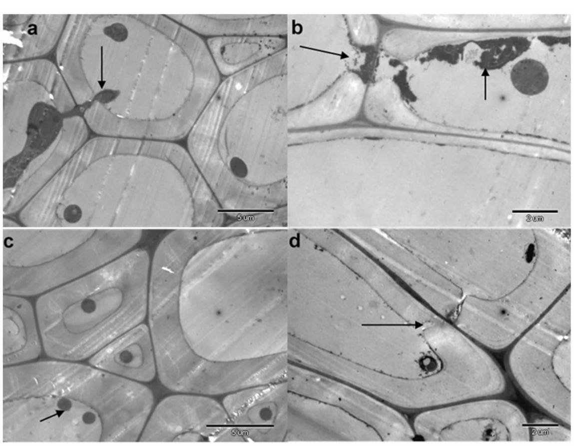

Fig. 1 Effect of edible basidiomycete inoculated on cell wall structure of cotton stalk (a, b Pleurotus ostreatus, c, d *Lentinus edodes*)

3.2.3 Comparative analysis of the effects of various treatments. Comparative analysis on the improved nutrition and detoxification effects of different treatments are presented in Table 5. In chemical treatment, the crushed CS with CS + Ca + U can increase the INDMD to 59.09%, the free gossypol content by 121.0 mg/kg, 97.0 mg/kg and 76.0 mg/kg, to reduce 30.46, 44.25 and 56.32%. In the biological treatments, 80% of CS-based ingredients inoculated with Pleurotus ostreatus cultured one month can significantly increase the CP and IVDMD content to 59.59 and 18.87%, free gossypol content by 83.4mg/kg and 72.8 mg/kg, to reduce 44.03 and 51.14%. In granulation treatment, the efficiency of IVDMD was higher than in other treatments and decreased free gossypol content of diet, and this may be treated with 10% corn supplement.

3.3 Feeding effect of crushing and granulation CS Under an equal amount of concentrate and corn silage feeding conditions (Table 6), the voluntary feed intake of fattening sheep from granulation CS was significantly higher than in crushed CS group ($P<0.01$). Dry matter digestibility was higher in sheep feeding with granulation CS ($P<0.01$), and the average weight gained during 60 days increased 18 kg gross weight. The average daily gain reached 0.303kg in granulation CS group.

4 Discussions

4.1 Feed characteristics of cotton stalk Cotton stalks, the lignocelluloses by-products from cotton production, have considerable economic and ecological importance. In China, it is estimated that more than 20 million t (dry weight) of cotton stalk are generated annually (Deng *et al.*, 2011). And Xinjiang is one of the major cotton-producing areas in China; large planting area of cotton has provided the cotton products as feed resources such as cotton stalk, cotton seed hulls, and cottonseed cake for the animal husbandry sector in Xinjiang. Xu and Wei (2005) determined CS different part nutrient and free gossypol content, and the results showed that CS leaves and boll shell had rich nutrient, especially the content of leaves crude protein reached 17.8%, and whole cotton plant CP also reached 6%, which can be used as fiber feed. CP content was slightly higher and the ADL content was 2 – 3 times in CS than grain straws (Wei, *et al*., 2003). Our result shows that CP content of CS is 5.88%, NDF and ADF content 73.40 and 66.00%, IVDMD content only 22.77%, and it is low nutrition and digestibility roughage.

Table 5 Improvement and detoxification effects of different treatments on chemical composition, IVDMD and free gossypol content of feed mixed with CS (%)

Processing	Chemical			Biological		Granulation
	CS + Ca	CS + U	CS + Ca + U	CS + Le	CS + Po	
NDF	-1.68	1.28	-5.36	-9.57	-10.10	-13.92
ADF	-3.50	0.19	-3.54	-2.60	-6.03	-17.62
CP	-14.14	48.21	29.87	56.99	59.59	-11.58
IVDMD	63.37	56.32	59.09	9.58	18.87	120.17
Detoxification rate	-30.46	-44.25	-56.32	-44.03	-51.14	-55.80

Note: NDF (neutral detergent fiber); ADF (acid detergent fiber); ADL (acid detergent lignin); CP (crude protein); IVDMD (in vitro dry matter digestibility).

Table 6 The feed intake and performance of fattening sheep

Items	Crushing CS	Granulation CS	P-value
Dry matter intake(kg/d)	2.03 ± 0.02	2.30 ± 0.05	$P < 0.05$
Concentrate feed	1.04 ± 0.05	1.04 ± 0.04	NS
Corn silage	0.35 ± 0.06	0.36 ± 0.04	NS
Cotton stalk	0.63 ± 0.05	0.90 ± 0.21	$P < 0.01$
Fecal output (kg/d)	0.78 ± 0.34	0.84 ± 0.21	$P < 0.05$
Dry matter digestibility (%)	61.79 ± 0.03	63.44 ± 0.02	$P < 0.01$
Initial weight (kg)	49.8 ± 2.67	48.0 ± 2.30	$P > 0.05$
Final weight (kg)	60.2 ± 2.79	66.0 ± 3.95	$P < 0.05$
Daily gain (kg/d)	0.173 ± 0.06	0.303 ± 1.44	$P < 0.05$

4.2 Pre-treatment effects In resent years, CS utilization research is mainly concentrated in cellulose extraction (Reddy N., 2009), ethanol fermentation (Chen, 2007) and edible fungus cultivation substrate materials (Abdurrahman *et al*., 2009; Orly *et al*., 1998). Alkali treatment can reduce fiber content, improve the feed value and IVDMD (Zhang, 2005), and after processing of CS, the texture and aromatic content were enhanced. Pre-treatment of cotton stalk with ionic liquids including 2-hydroxy ethyl ammonium formate enhances biomass digestibility (Nazife *et al*., 2013). In this study, different treatment methods were used on CS for comparative analysis after the treatment of CS nutrient, IVDMD and free gossypol detoxification rate. In the chemical treatment, the crushed CS with CS + Ca + U can be increased and the INDMD can be up to 59.09%. This was the same result as that of Chen (2007) who uses 2.5% lime + 3.5% urea + 3% salt for processing straw. After processing the CS, CP content and IVDMD result was the same as that of Mao *et al*. (1991). Inoculation with *Lentinus edodes* and Pleurotus ostreatus after 30 d can increase medium CP content by 34.6 – 67.1%. Due to the high protein content of edible Basidiomycete, about 40 – 50% (Liu, 2003), during the cultivation, a large amount of hyphae helps increase CP content. Rice straw is as the main raw material of the medium, and inoculation with Pleurotus ostreatus for 30 days increases CP content by 45.85% compared with control group (Chen, 2001). Use of white-rot fungus dealing with rice straw can degrade or destroy most of the lignins (Yutaka *et al*., 2000). Straw nutrition after white-rot fungi treatment can be improved, and fermentation of straw, fragrance, texture and palatability can be also obviously improved (Guo *et al*., 2003). In our biological treatment, CS + Le is faster than CS + Po, however, the hyphae of two strains can cover the whole medium 30 days after inoculation. The basidiomycete decreased the fiber components, increased CP content and improved the IVDMD, but the CS + Po effect was better than CS + Le. We recommend this result, because of hyphal elongation and penetration in the cell associated with the decomposition of cellulose enzyme. Free gossypol with alkali, heat and light is unstable, easily oxidized or decomposed (Zhang, 1989). For biological treatment, in the fermentation period, part of the free gossypol is combined with microbes to secrete amino acid or free amino in the active protein, forming non-toxic bound gossypol (Xiao, 2008). When CS is inoculated with *Lentinus edodes* and Pleurotus ostreatus, free gossypol detoxification affects CS + Po training, and the detoxification rate is more than 50%. In granulation treatment, detoxification rate also reaches over 50%. The main reason is that when pellet feed is processed, granulator machine has the inside temperature of over 90℃. All kinds of processing methods can improve the nutritional value of CS, but for the safer use, it now lacks CS feed processing methods about applicability to improve the feed value and free gossypol detoxification. Use of chemical, biological and granulation treatment on CS, compared with raw material, can improve nutrition and decrease free gossypol content, but granulation treatment is widely used for CS processing in

Xinjiang, because the use of physical treatment is simple and safe.

4.3 Feeding effects CS is widely used in Xinjiang, and currently part of the by-products of cottonseed are processed in Xinjiang. Mostly, it's used as animal feed resource. Especially the CS used for grazing sheep or after being crushed is mostly used for ruminant feeding. In this experiment, it shows the effects of feeding crushed and granulation CS on the feed intake and performance of the sheep. The results show that feeding sheep under 1.0 kg/d of formula concentrate and 1.5 kg/d (fresh) of corn silage, the voluntary feed intake of sheep in granulation CS group is significantly higher than in crushing CS group. Wei (2003) reports that crushed CS free feed intake of sheep under the condition of the limit to 350 g concentrate can reach 0.693 kg/d. Yue (2010) adds different proportion of crush CS to the sheep diet, and the proportion of 40% is advisable. The results show that after feeding crushed or granulation CS, sheep CS intake was 0.63 ± 0.05 and 0.90 ± 0.21 kg/d, average daily gain was 0.173 ± 0.06 and 0.303 ± 1.44 kg/d, indicating that the voluntary feed intake of sheep in granulation CS group was significantly higher than in crushed CS group. However, the granulation pellet feeding method is simple and safe, and there is effective detoxification of free gossypol in CS. Stalk granulation technology widely used in modern animal husbandry system can increase the feed intake and the feed characteristics of CS, providing a viable way to reduce free gossypol content. Among them, the granulation treatment has obvious effect, and the feed intake is high than in crushed CS treatment. In practice, granulation is simple, detoxification effect is obvious, and it can improve sheep body weight, so it is the safest and most effective treatment method for CS.

5 Conclusions

CS is a kind of high cellulose and lignin roughage resources. Crushed CS treated with CS + Ca + U can improve the CP content and the dry matter digestibility. However, it will also reduce the free gossypol content of cotton stalk by half. The chemical and the biological treatment methods are modified to? improve the nutritional value of the CS, but from the security and scale, granulation processing is better than the crushed CS. All in all, it is evident that pelleting CS can be prepared to improve feed intake, digestibility and feeding effect.

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