Nutritional Value of Cotton Stalk and Its Effects on Nutrient Digestion and Metabolism, Growth, and Mutton Safety in Sheep (Postprint)

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Abstract

This study aimed to conduct a comprehensive evaluation of the feed characteristics and nutritional value of cotton stalk, the nutritional value of cotton stalk diets, and the safety of meat from sheep fed cotton stalk diets, by determining the proportions of cotton stalk parts and their proximate nutrients, the digestibility of cotton stalk in sheep, voluntary feed intake of sheep fed cotton stalk diets, digestion and metabolism, and the effects of feeding cotton stalk diets on sheep growth, slaughter performance, and meat safety. In this study, Experiment 1 determined the proportions of different cotton stalk parts and proximate nutrients; Experiment 2 measured the digestibility of cotton stalk in sheep under restricted feeding conditions using the difference method; Experiment 3 employed a self-control design with corn stover diet as control to measure the digestion and metabolism of cotton stalk diet in sheep; Experiment 4 investigated the effects of corn stover diet and cotton stalk diet on sheep growth and slaughter performance through a 120-day feeding trial, and determined the contents of free gossypol and major pesticide residues in tissue samples of sheep in the cotton stalk group. The results showed that the proportions of coarse stems, fine branches, leaves, and cotton boll shells in cotton stalk were 31.48%, 10.99%, 17.12%, and 40.41%, respectively, and the contents of organic matter, crude protein, cellulose, hemicellulose, and lignin in cotton stalk were 90.00%, 6.40%, 34.80%, 15.60%, and 10.30%, respectively. The (apparent) digestibility of dry matter, organic matter, crude protein, cellulose, hemicellulose, energy, calcium, and phosphorus of cotton stalk in sheep was 38.20%, 38.00%, 1.58%, 60.50%, 58.10%, 44.10%, 57.80%, and 45.00%, respectively, with a digestible energy of 10.28 MJ/kg DM. The apparent digestibility of organic matter, cellulose, and energy in sheep fed cotton stalk diet was 5.30%, 10.00%, and 13.60% lower than that in sheep fed corn stover diet, respectively, while the apparent digestibility of calcium and phosphorus was 52.40% and 36.70% higher, respectively; there was no significant difference in voluntary feed intake and apparent digestibility of crude protein between sheep fed the two diets (P>0.05). There were no significant differences in average daily gain, carcass weight, and carcass lean weight between sheep fed cotton stalk diet and corn stover diet (P>0.05). The detection results of free gossypol and major pesticide residues in tissue samples of sheep fed cotton stalk diet were not detected. These results indicate that the sum of the proportions of cotton boll shells, fine branches, and leaves in cotton stalk is approximately 70%, with a relatively large amount of consumable and utilizable parts; cotton stalk is a roughage with lower energy nutrition but higher calcium and phosphorus nutrition than corn stover, with relatively high lignin content and extremely low apparent digestibility of crude protein (which can be considered as zero in practice); there was no significant difference in the effects of cotton stalk diet group and corn stover diet group on sheep weight gain and slaughter performance, with equivalent feeding effects; free gossypol and major pesticide residues were not detected in tissue samples of sheep fed cotton stalk diet, and its food safety was not affected.

Full Text

Nutritional Value of Cotton Stalk and Its Effects on Digestion and Metabolism of Nutrients, Growth and Mutton Safety in Sheep

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Abstract

This study comprehensively evaluated the feed characteristics, nutritional value of cotton stalk and cotton stalk diet, and the safety of mutton from sheep fed cotton stalk diet. The investigation included determination of cotton stalk part ratios and approximate nutrients, overall digestibility of cotton stalk in sheep, free intake, digestion and metabolism, and effects on growth, slaughter performance, and mutton safety.

Experiment 1 determined the proportions of different cotton stalk parts and their approximate nutrient contents. Experiment 2 measured the digestibility of cotton stalk in sheep under restricted feeding conditions using the difference method. Experiment 3 employed a self-control design with corn stalk diet as control to measure digestion and metabolism of cotton stalk diet (70%) in sheep under free intake conditions. Experiment 4 conducted a 120-day feeding trial to compare the effects of corn stalk diet and cotton stalk diet on growth and slaughter performance, and to determine free gossypol and major pesticide residues in tissue samples from the cotton stalk group.

Results showed that cotton stalk consisted of 31.48% main stem, 10.99% branches, 17.12% leaves, and 40.41% cotton boll shell. The contents of organic matter, crude protein, cellulose, hemicellulose, and lignin were 90.00%, 6.40%, 34.80%, 15.60%, and 10.30%, respectively. The apparent digestibility of dry matter, organic matter, crude protein, cellulose, hemicellulose, energy, calcium, and phosphorus in cotton stalk for sheep was 38.20%, 38.00%, 1.58%, 60.50%, 58.10%, 44.10%, 57.80%, and 45.00%, respectively, with a digestible energy value of 10.28 MJ/kg DM.

Compared with corn stalk diet, cotton stalk diet resulted in 5.30%, 10.00%, and 13.60% lower apparent digestibility of organic matter, cellulose, and energy, respectively, but 52.40% and 36.70% higher apparent digestibility of calcium and phosphorus. No significant differences were observed between the two diets in free intake or crude protein apparent digestibility ($P>0.05$). Average daily gain, carcass weight, and carcass lean weight did not differ significantly between groups ($P>0.05$). No free gossypol or major pesticide residues were detected in tissue samples from sheep fed cotton stalk diet.

These findings indicate that the combined proportion of cotton boll shell, branches, and leaves accounts for approximately 70% of cotton stalk, representing substantial available resources. Cotton stalk is a roughage with higher lignin content and extremely low crude protein apparent digestibility (effectively zero in practice), lower energy but higher calcium and phosphorus content than corn stalk. The effects on weight gain and slaughter performance were comparable between cotton stalk and corn stalk diets. The absence of detectable free gossypol and pesticide residues confirms that feeding cotton stalk diet does not compromise food safety.

Keywords: cotton stalk; sheep; digestion and metabolism; growth; mutton safety

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Introduction

China has extensive cotton cultivation, yet research on cotton stalk as a roughage remains limited. Xinjiang is the primary cotton-producing region in China, with approximately 1.93 million hectares planted in 2017, accounting for over 60% of the national total. Following cotton harvest, using cotton fields as winter grazing areas is common practice, representing enormous resource potential. With the continuous expansion of cattle and sheep production, roughage shortage has become increasingly prominent, particularly in southern Xinjiang. Consequently, feed utilization of cotton stalk has attracted widespread attention.

While no international studies on cotton stalk as roughage have been reported, domestic research remains scarce. Xu et al. [1] documented the approximate nutrient content of different cotton stalk parts, suggesting that cotton stalk is relatively rich in key nutrients and can be used as fiber feed. Wei et al. [2] conducted digestion and metabolism trials of cotton stalk diet in sheep, demonstrating that ground cotton stalk can be consumed and utilized. Fang et al. [3] investigated the effects of pelleting cotton stalk diet on digestion and metabolism in sheep, finding that ground cotton stalk as sheep feed has nutritional value second only to corn stalk and superior to wheat straw and rice straw. Several reports [4-10] indicate that cotton stalk resources can be utilized as cattle and sheep feed. However, existing studies lack systematic investigation, and data on cotton stalk as sheep roughage remain insufficient.

This paper systematically investigated the proportions and nutritional value of cotton stalk parts (leaves, branches, stems, etc.), determined cotton stalk digestibility in live sheep, and evaluated the effects of cotton stalk diet on free intake, digestion and metabolism, weight gain, slaughter performance, and mutton safety using corn stalk diet as control, aiming to provide scientific and objective evaluation of cotton stalk feed utility.

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1. Materials and Methods

1.1 Experimental Design and Animals

This study comprised four experiments: Experiment 1 for cotton stalk analysis, Experiments 2 and 3 for digestion and metabolism trials, and Experiment 4 for feeding trials.

Experiment 1 analyzed cotton stalk divided into whole plant, main stem, branches, leaves, and cotton boll shell to determine each part' s proportion and approximate nutrient content for preliminary nutritional evaluation.

Experiment 2 used six non-pregnant Small-tailed Han ewes (approximately 2 years old, 48.50±1.50 kg) in a self-control design with two-period digestion trials under restricted feeding. Sheep received identical concentrate mixtures plus either 400 g cotton stalk (Diet 1) or 800 g cotton stalk (Diet 2) to determine apparent digestibility of dry matter, organic matter, and crude protein. Cotton stalk apparent digestibility was estimated by the difference method, calculated as the difference in digested amount between the two diet groups divided by the difference in intake, multiplied by 100 [11]:

$$\text{Cotton stalk apparent digestibility (\%)} = \frac{\text{Digested amount}_{\text{Diet 2}} - \text{Digested amount}_{\text{Diet 1}}}{\text{Intake}_{\text{Diet 2}} - \text{Intake}_{\text{Diet 1}}} \times 100$$

Experiment 3 employed six non-pregnant Small-tailed Han ewes (approximately 2 years old, 52.60±2.20 kg) in a self-control design under free intake conditions, using corn stalk diet (70%) as control (Diet 3) to evaluate digestion and metabolism of cotton stalk diet (70%) (Diet 4).

Experiment 4 utilized twelve 6-month-old Small-tailed Han ram lambs (29.20±0.80 kg), divided into two groups (n=6 each) by body weight. Under free intake conditions, corn stalk diet (60%) served as control (Diet 5) and cotton stalk diet (60%) as treatment (Diet 6) for 120 days to compare slaughter performance and determine free gossypol and major pesticide residues in tissue samples.

Diet composition, nutrient levels, and feeding methods for all six diets are presented in Table 1. Cotton stalk was supplemented with heptahydrate sulfate at 2.00 g/kg.

All sheep were housed individually and fed twice daily at 10:00 and 18:00. Concentrate was fed before roughage, with each feeding providing half the daily amount. Water was freely available.

1.2.1 Digestion and Metabolism Trials

During digestion-metabolism periods, sheep were placed in metabolic cages for a 14-day preliminary period followed by an 8-day collection period (total 22 days). Feces and urine were collected continuously for 8 days, with total daily collections at 09:00 each day. Fecal samples (10% of daily fecal weight) were air-dried in a ventilated area. At each period's end, all fecal samples from each sheep were mixed, air-dried, weighed, and stored at room temperature. Approximately 100 g was ground to pass a 40-mesh sieve before analysis. Urine samples were weighed, filtered through gauze, and 10% was collected into numbered 600 mL plastic bottles containing 2 mL of 1:1 HCl (1 volume concentrated HCl + 1 volume deionized water, approximately 6 mol/L) added during first collection, then stored at 4°C. Refusals were collected daily at 09:00, weighed, air-dried, and stored.

Starting one day before each collection period, 100 g of mixed concentrate and 250 g of ground cotton or corn stalk were sampled daily, weighed, air-dried, and stored using quartering method. Diets and refusals were ground to pass a 40-mesh sieve. Daily feed intake was recorded for each sheep.

1.2.2 Slaughter Trials

Sheep were fasted for 24 h and deprived of water for 12 h before slaughter by exsanguination. Carcasses were weighed after 30 min of resting, then divided into shoulder-back, rump, neck, chest, abdomen, foreleg, and hind shank at room temperature. Meat was separated from bone in each part, with care taken not to damage periosteum. Weights of bone, lean meat, and fat were recorded separately.

During slaughter, muscle, heart, liver, kidney, and testis samples were collected from both groups. Fresh samples were homogenized for determination of free gossypol and major pesticide residues. Detection limits for major pesticide residues are shown in Table 2.

1.4 Sample Analysis

Dry matter, organic matter, crude protein, cellulose, hemicellulose, lignin, energy, and phosphorus in feed, urine, feces, and refusals were determined by conventional feed analysis methods [13]. Specifically:

- Cellulose (%) = Acid detergent fiber (ADF) value - residue after 72% sulfuric acid treatment
- Hemicellulose (%) = Neutral detergent fiber (NDF) value - ADF value
- Acid detergent lignin (ADL) (%) = Residue after 72% sulfuric acid treatment - ashed residue

Calcium content was determined by o-cresolphthalein colorimetry [14]. Free gossypol in cotton stalk samples was measured according to SN 0535-1996 [15], while tissue samples were analyzed by GB 5009.148-2014 [16]. Pesticide residues (thiamethoxam, imidacloprid, avermectin, endosulfan, chlorpyrifos, profenofos, chlormequat) were determined by GB/T 20772-2008 LC-MS/MS [17], SN/T 1873-2007 GC-MS/MS (endosulfan) [18], SN/T 1973-2007 LC-MS/MS (avermectin) [19], and LC-MS/MS (chlormequat) [12].

1.5 Statistical Analysis

Data are expressed as mean ± standard deviation. Independent samples t-test was performed using SPSS 17.0. Multiple comparisons were conducted for nutrient content among cotton stalk parts in Experiment 1. $P>0.05$ indicated no significant difference, $P<0.05$ significant difference, and $P<0.01$ highly significant difference.

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2. Results

2.1 Composition of Cotton Stalk

As shown in Table 3, cotton stalk consisted of 31.48% main stem, 10.99% branches, 17.12% leaves, and 40.41% cotton boll shell.

Table 4 presents the approximate nutrient content of whole cotton stalk and corn stalk, and cotton stalk parts. Whole cotton stalk contained 90.00% organic matter, 6.41% crude protein, 34.80% cellulose, 15.60% hemicellulose, and 10.30% lignin.

2.2 Digestibility of Cotton Stalk in Sheep

Results showed that dry matter and organic matter intake of Diet 2 increased by 56.20% (P<0.01) compared with Diet 1. Digested amounts of dry matter and organic matter increased by 37.60% (P<0.01) and 36.10% (P<0.01), respectively.

Using the difference method, cotton stalk digestibility is presented in Table 5. Sheep demonstrated good digestibility of cellulose, hemicellulose, and calcium (all >57%), and moderate digestibility of dry matter, organic matter, energy, and phosphorus (all >38%). Digestible energy of cotton stalk was 10.28 MJ/kg DM (44.05% of gross energy) (Table 4).

2.3 Digestion and Metabolism of Cotton Stalk Diet

As shown in Table 6, no significant difference (P>0.05) was observed in free intake between cotton stalk and corn stalk diet groups.

Table 7 indicates that apparent digestibility of dry matter, organic matter, crude protein, cellulose, hemicellulose, and energy in cotton stalk diet was 5.00% (P>0.05), 5.30% (P<0.05), 3.50% (P>0.05), 10.00% (P<0.05), 9.60% (P>0.05), and 13.60% (P<0.01) lower than corn stalk diet, respectively. Digestibility of fiber components and energy was notably lower in cotton stalk diet.

Digestible amounts of hemicellulose, energy, and phosphorus were 22.80% (P<0.01), 14.40% (P<0.01), and 23.00% (P<0.01) lower in cotton stalk diet, while calcium digestibility increased significantly (P<0.01). Dry matter and organic matter digestibility tended to decrease, whereas cellulose and crude protein digestibility showed increasing trends (P>0.05).

Table 8 demonstrates that nitrogen intake increased by 14.20% (P<0.01) with cotton stalk diet, while fecal nitrogen increased by 9.70% (P<0.05) and urinary nitrogen decreased by 17.20% (P<0.01). Nitrogen retention amount and rate improved by 47.90% (P<0.01) and 29.50% (P<0.01), respectively. Calcium retention amount and rate increased by 82.90% (P<0.01) and 70.20% (P<0.01), while phosphorus retention amount and rate increased by 61.20% (P<0.01) and 31.50% (P<0.01), respectively.

2.4 Effects of Cotton Stalk Diet on Growth and Slaughter Performance

Results (Table 9) showed that free intake of cotton stalk diet was 6.9% lower than corn stalk diet, but 120-day average daily gain did not differ significantly between groups (P>0.05).

Slaughter data (Table 10) revealed no significant differences (P>0.05) in live weight, carcass weight, dressing rate, or carcass lean yield between diets. However, fat weight was significantly lower (P<0.01) and carcass bone weight was

13.23% higher (P<0.01) in cotton stalk diet group. Meat-bone ratio was lower in cotton stalk diet group.

2.5 Free Gossypol and Pesticide Residues in Tissue Samples

No free gossypol or major pesticide residues were detected in muscle, heart, liver, kidney, or testis samples from sheep fed cotton stalk diet.

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3. Discussion

3.1 Digestive Characteristics of Cotton Stalk

Cotton stalk has traditionally been considered a roughage with high lignin content, low approximate nutrients, and poor digestibility, but objective scientific data have been lacking. This study demonstrates that compared with corn stalk, cotton stalk has higher lignin content, lower hemicellulose, but comparable crude protein, cellulose, calcium, phosphorus, and gross energy contents. These findings contrast with conventional perceptions.

Research on cotton stalk digestibility in animal trials is scarce. Nylon bag and in vitro gas production techniques showed dry matter effective degradation of 33.30% [20], whereas this study found 38.20% apparent digestibility by the difference method—14.71% higher. This discrepancy may relate to abundant and effective fiber-degrading bacteria in sheep rumen, limited direct contact in nylon bag methods, and incomplete replication of rumen conditions in vitro. Additionally, the cotton stalk used in this trial retained leaves, cotton boll shells, and branches relatively intact, providing more available parts with lower lignin content, thereby enhancing effective digestibility. Wei et al. [20] estimated cotton stalk digestible energy as 6.38 MJ/kg DM using nylon bag method, while Fang et al. [21] reported 6.57-7.66 MJ/kg DM in sheep trials. This study determined 10.28 MJ/kg DM. Variations in cotton varieties, harvest timing between northern and southern Xinjiang, and resulting differences in fiberization degree and lignin content likely contribute to these differences, with timely harvest benefiting cotton stalk utilization as sheep roughage.

This study found cotton stalk crude protein apparent digestibility of only 1.58%, indicating extremely low utilizable crude protein. This relates to high lignin content and the adsorption of nitrogen (including endogenous nitrogen) by cellulose and lignin, hindering crude protein utilization. Therefore, in practice, nitrogen content of cotton stalk-based diets should be considered as zero.

Apparent digestibility of organic matter, cellulose, hemicellulose, calcium, and phosphorus in cotton stalk was 38.00%, 60.50%, 58.10%, 57.80%, and 45.00%, respectively, indicating good digestibility of fiber components, calcium, and phosphorus. Lower organic matter digestibility relates to high fiberization. Increased cotton stalk intake significantly reduced apparent digestibility of dry matter, organic matter, crude protein, calcium, and phosphorus, but did not significantly affect cellulose and hemicellulose digestibility. Lignin content directly affects digestibility. Wei et al. [20] and Fang et al. [21] reported lignin contents of 15.30% and 17.10%, respectively, whereas this study found 10.29%, possibly due to cotton variety, fermentation method, and whole-plant nutritional value.

In summary, cotton stalk possesses certain nutritional value and can be utilized as cattle and sheep roughage.

3.2 Nutritional Value of Cotton Stalk Diet

Halida et al. [22] studied effects of ground or pelleted cotton stalk on sheep weight gain, finding that cotton stalk diet improved fattening benefits, with pelleted form superior to ground form, indicating good nutritional value as fattening roughage. This study found no significant differences in free intake, average daily gain, or slaughter performance (except fat weight and carcass bone weight) between corn stalk and cotton stalk diets. Reduced fat and increased bone weight align with cotton stalk's lower digestible energy and higher calcium-phosphorus content. From a production perspective, cotton stalk diet provides slightly less energy but better calcium-phosphorus nutrition. Overall feeding effects were nearly identical, indicating comparable nutritional value between cotton stalk and corn stalk diets.

The second phase of this research investigated new methods to improve cotton stalk diet nutritional value and utilization. Optimal utilization of roughage in ruminants can be achieved through appropriate nutritional supplementation and rumen environment optimization. Physical and chemical processing methods provide limited nutritional quality improvement, whereas nutritional supplementation is fundamental for developing roughage potential [23]. Studies agree that appropriate nutritional supplementation can regulate microbial flora growth to improve roughage utilization efficiency [24-26]. Subsequent trials in this study used cotton stalk-specific nutritional additives with scientific processing, with preliminary results showing significantly improved cotton stalk utilization.

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4. Conclusions

Based on this study, the following conclusions can be drawn:

1. The combined proportion of cotton boll shell, branches, and leaves accounts for approximately 70% of cotton stalk, representing substantial available and utilizable resources.

2. Regarding lignification degree, the nutritional value ranking of cotton stalk parts is: leaves > cotton boll shell > branches > main stem.

3. Cotton stalk has relatively high lignin content and extremely low crude

protein apparent digestibility. Its energy content is lower but calcium-phosphorus nutrition is higher than corn stalk.

4. No significant differences exist in free intake or crude protein digestibility between sheep fed cotton stalk diet and corn stalk diet.
5. No significant differences in weight gain or slaughter performance were observed between diets, with comparable feeding effects.
6. No free gossypol or major pesticide residues were detected in tissue samples from sheep fed cotton stalk diet, indicating no compromise in food safety.

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