Effects of Different Anaerobic Alkaline Treatments on Nutrient Composition, Ultrastructure, and In Vitro Fermentation Parameters of Fresh Wheat Straw (Postprint)

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Abstract

This study aimed to investigate the effects of different anaerobic alkaline treatments on the nutritional composition, ultrastructure, and in vitro fermentation parameters of fresh wheat straw. Fresh wheat straw was treated anaerobically for 40 days with 2.5% sodium bicarbonate (sodium bicarbonate group), 4% urea (urea group), and 2.5% sodium bicarbonate + 4% urea (combination group) based on dry matter weight, and changes in its nutritional composition and ultrastructure under scanning electron microscopy were analyzed. Using rumen fluid from three healthy Laoshan dairy goats fitted with permanent rumen fistulas as donors, a 48-hour in vitro fermentation experiment was conducted via the in vitro gas production technique to determine the in vitro degradability, gas production, and fermentation parameters of the treated wheat straw. The results showed: 1) The crude protein (CP) content in the combination group was extremely significantly higher than that in the sodium bicarbonate and urea groups ($P<0.01$), and the urea group was extremely significantly higher than the sodium bicarbonate group ($P<0.01$). The neutral detergent fiber (NDF) content in the combination group was significantly lower than that in the sodium bicarbonate group ($P<0.05$), with no significant difference from the urea group ($P>0.05$). The hemicellulose (HCEL) content in the combination group was extremely significantly lower than that in the sodium bicarbonate group ($P<0.01$), with no significant difference from the urea group ($P>0.05$). There was no significant difference in acid detergent fiber (ADF) content among the three groups ($P>0.05$). 2) Under scanning electron microscopy, the degree of structural damage to wheat straw by different anaerobic alkaline treatments was observed as combination group > urea group > sodium bicarbonate group, and the erosion intensity of alkaline solution on wheat straw tissues was sclerenchyma cells >

parenchyma cells > vascular bundles. 3) The dry matter degradability (DMD) of the combination group was extremely significantly higher than that of the sodium bicarbonate group (P<0.01), and the neutral detergent fiber degradability (NDFD) and acid detergent fiber degradability (ADFD) of the combination group were both significantly higher than those of the sodium bicarbonate group (P<0.05). The DMD, NDFD, and ADFD of the combination group were all higher than those of the urea group, but the differences were not significant (P>0.05). The 48-hour in vitro gas production and the yields of acetate, propionate, butyrate, and total volatile fatty acids (TVFA) in the combination group were significantly or extremely significantly higher than those in the sodium bicarbonate group (P<0.01 or P<0.05). The acetate yield in the combination group was significantly higher than that in the urea group (P<0.05), while the ammonia nitrogen yield was significantly lower than that in the sodium bicarbonate and urea groups (P<0.05). There were no significant differences in pH or acetate/propionate ratio among the three groups (P>0.05). In conclusion, the combined anaerobic treatment with 2.5% sodium bicarbonate + 4% urea was superior to sodium bicarbonate or urea treatment alone, increasing the CP content of wheat straw, reducing the contents of NDF and HCEL, and improving the in vitro degradability, gas production, and VFA yield of the straw. This method can be promoted and utilized as an approach to improve the feeding value of low-quality roughage.

Full Text

Effects of Different Anaerobic Alkalization Treatments on Nutrients, Ultrastructure and in vitro Fermentation Parameters of Fresh Wheat Straw

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Abstract

This experiment investigated the effects of different anaerobic alkalization treatments on the nutritional composition, ultrastructure, and in vitro fermentation parameters of fresh wheat straw. Fresh wheat straw was treated anaerobically for 40 days with either 2.5% sodium bicarbonate (sodium bicarbonate group), 4% urea (urea group), or a combination of 2.5% sodium bicarbonate and 4% urea (compound group), all applied on a dry matter weight basis. Nutritional composition was analyzed, and ultrastructural changes were examined using scanning electron microscopy. Using rumen fluid from three healthy Laoshan dairy goats fitted with permanent rumen fistulas as donors, a 48-hour in vitro

fermentation trial was conducted via gas production techniques to determine the in vitro degradation rate, gas production, and fermentation parameters of the treated wheat straw.

The results showed: (1) The crude protein (CP) content in the compound group was significantly higher than in both the sodium bicarbonate and urea groups ($P<0.01$), while the urea group was significantly higher than the sodium bicarbonate group ($P<0.01$). The neutral detergent fiber (NDF) content in the compound group was significantly lower than in the sodium bicarbonate group ($P<0.05$), with no significant difference from the urea group ($P>0.05$). The hemicellulose (HCEL) content in the compound group was significantly lower than in the sodium bicarbonate group ($P<0.01$), with no significant difference from the urea group ($P>0.05$). No significant differences in acid detergent fiber (ADF) content were observed among the three groups ($P>0.05$). (2) Under scanning electron microscopy, the degree of structural damage to wheat straw followed the pattern: compound group > urea group > sodium bicarbonate group, with alkali erosion affecting tissues in the order: sclerenchyma cells > parenchyma cells > vascular bundles. (3) The dry matter degradation rate (DMD) in the compound group was significantly higher than in the sodium bicarbonate group ($P<0.01$), while the neutral detergent fiber degradation rate (NDFD) and acid detergent fiber degradation rate (ADFD) were significantly higher than in the sodium bicarbonate group ($P<0.05$). Although DMD, NDFD, and ADFD in the compound group were higher than in the urea group, the differences were not significant ($P>0.05$). The 48-hour in vitro gas production and the yields of acetic acid, propionic acid, butyric acid, and total volatile fatty acids (TVFA) in the compound group were significantly or highly significantly higher than in the sodium bicarbonate group ($P<0.01$ or $P<0.05$). Acetic acid yield in the compound group was significantly higher than in the urea group ($P<0.05$), while ammonia nitrogen yield was significantly lower than in both the sodium bicarbonate and urea groups ($P<0.05$). No significant differences in pH or acetic/propionic acid ratio were observed among the three groups ($P>0.05$).

In conclusion, the combined anaerobic treatment with 2.5% sodium bicarbonate and 4% urea was superior to single treatments, increasing CP content while reducing NDF and HCEL contents, and improving in vitro degradation rate, gas production, and VFA yield. This approach can be promoted as an effective method for enhancing the feeding value of low-quality roughage.

Keywords: anaerobic alkalization treatment; fresh wheat straw; nutrients; ultrastructure; in vitro gas production; degradation rate

Introduction

Straw represents an important roughage resource in China. However, direct feeding is limited by its high degree of fiber lignification, low crude protein (CP) content, low nutrient digestibility, and poor palatability. Appropriate treatment can increase straw degradation rate and improve energy utilization efficiency by

20%, which is significant for alleviating the shortage of high-quality roughage [1-2]. Alkali treatment of straw weakens hydrogen bonding within fibers, breaks ester or ether linkages, causes fiber molecules to swell, and dissolves hemicellulose (HCEL) and partial lignin, facilitating infiltration of rumen fluid and action of rumen microorganisms, thereby improving palatability and increasing intake and digestibility [3].

Adding urea to straw not only achieves alkalization but also generates ammonium salts through reactions between ammonia released from urea decomposition and straw organic matter, effectively increasing CP content [4]. However, urea is a weak base and less effective for fiber treatment than strong alkalis [5]. Combined alkalization and ammoniation treatments generally outperform single treatments. Sodium bicarbonate, a common feed buffer that forms a weak alkaline solution, can increase animal intake, improve the rumen environment, and enhance degradation, digestion, and absorption of carbohydrates including cellulose, HCEL, and sugars when added to ruminant diets [6]. Therefore, this study examined the effects of different alkaline substances under anaerobic fermentation on the nutritional composition and ultrastructure of fresh wheat straw, and utilized in vitro gas production methods to measure degradation rate, gas production, rumen culture pH, ammonia nitrogen, and volatile fatty acid (VFA) yields, aiming to identify suitable treatment methods for low-quality roughage and improve its utilization value.

1.1 Preparation of Anaerobically Alkalized Wheat Straw

Fresh wheat straw harvested immediately after grain collection was chopped to 3–5 cm lengths. Sodium bicarbonate (2.5% of straw dry matter), urea (4% of straw dry matter), or a combination of both (2.5% sodium bicarbonate + 4% urea) were added and thoroughly mixed. Water was sprayed to achieve approximately 30% moisture content before compaction and sealing in clean, sterile 1 L plastic buckets. Each treatment had three replicates and was stored at room temperature (approximately 25°C) for 40 days. After storage, partial straw samples were frozen at -20°C for ultrastructural observation, while the remainder was dried at 65°C to create air-dried samples for nutritional analysis and use as in vitro fermentation substrates.

The urea used was analytical grade (nitrogen content $ $46%) purchased from Yangmei Group Yantai Juli Chemical Fertilizer Co., Ltd., while sodium bicarbonate (food-grade baking soda, purity $ $99%) was purchased from Qingdao Soda Ash Industrial Development Co., Ltd. The nutritional composition of the fresh wheat straw before treatment is shown in Table 1 .

1.2 Experimental Animals

Three healthy 2-year-old male Laoshan dairy goats weighing 58.15$±$1.66 kg and fitted with permanent rumen fistulas were selected. The basal diet composition and nutritional levels are presented in Table 2 . Animals were fed at

06:00, 11:30, and 18:00 daily, with ad libitum access to feed and clean water.

1.3 Scanning Electron Microscopy Analysis

Representative stems were selected from each group and cut with a blade into 0.3 cm × 0.1 cm samples of transverse sections, outer surfaces, and inner surfaces. After a series of pretreatments [8], samples were fixed to copper platforms with conductive adhesive, sputter-coated with gold four times using a JFC-1600 instrument, and observed with a JSM-7500F scanning electron microscope (SEM).

1.4 Preparation of Rumen Fermentation Culture

Rumen fermentation culture was prepared according to the method of Menke et al. [9]. Rumen fluid was collected from the three experimental goats 2 hours after morning feeding, filtered through four layers of gauze, mixed thoroughly, and immediately transferred to a thermos flask filled with carbon dioxide (CO_2) to a 39°C incubator. Artificial buffer solution consisted of 400 mL distilled water, 200 mL mineral solution, 200 mL buffer solution, 0.1 mL trace element solution, 1 mL resazurin (0.1%), and 40 mL reducing solution (freshly prepared). The pH was adjusted to 6.8–7.0 with CO_2. Rumen fluid and artificial buffer were mixed at a 1:2 ratio for use.

1.5 Determination of in vitro Gas Production and Rumen Fermentation Parameters

Accurately weighed 300 mg samples were placed in 400-mesh nylon bags (4.0 cm × 1.5 cm), sealed, labeled, and inserted into 100 mL glass syringes. Medical Vaseline was evenly applied to the middle third of each syringe barrel before preheating to 39°C. Thirty milliliters of fresh artificial rumen culture were rapidly added to each syringe, initial scale values were recorded, and each group had six replicates with three blank controls without substrate. After addition, syringes were immediately transferred to a 39°C incubator. Gas production scale values were recorded at 2, 4, 8, 12, 24, 36, and 48 hours.

After 48 hours, syringes were removed and ice-bathed. Nylon bags were retrieved, washed until odorless, and dried at 65°C for determination of dry matter degradation rate (DMD), neutral detergent fiber degradation rate (NDFD), and acid detergent fiber degradation rate (ADFD). Culture fluid was poured into 50 mL centrifuge tubes for pH measurement, then centrifuged at 3,556×g for 20 minutes. The supernatant was stored at -20°C for ammonia nitrogen (NH_3-N) and VFA analysis.

pH was measured using an OHAUS pH meter, NH_3-N using a FOSS automatic Kjeldahl nitrogen analyzer, and VFAs using an Agilent 6890N gas chromatograph with an FFAP column (30 m × 0.32 mm × 0.25 m). Operating parameters included: injector temperature 250°C, detector temperature 250°C, column oven programmed at 60°C for 5 minutes, then 10°C/min to 180°C for 5 minutes,

nitrogen carrier gas flow rate 1.9 mL/min, split ratio 2:1, and injection volume 1 L.

1.6 Measurement Indicators and Calculation Methods

Moisture content was determined according to GB/T 6435-2006, and dry matter (DM) content was calculated accordingly. CP content was measured per GB/T 6432-1994, crude ash content per GB 6438-2007, and neutral detergent fiber (NDF) and acid detergent fiber (ADF) contents were analyzed using the Van Soest method [10] with an ANKOM A200i automatic fiber analyzer. HCEL content was calculated as: HCEL (%) = NDF (%) -ADF (%).

Gas production, nutrient degradation rates, and total volatile fatty acids (TVFA) were calculated using the following formulas:

- Gas production (mL/g) = [Cumulative gas production in the period (mL) -Average blank gas production in the corresponding period (mL)] / Sample mass (g)
- Nutrient degradation rate (%) = 100 × [Nutrient content before rumen fluid action (g) -Nutrient content after rumen fluid action (g)] / Nutrient content before rumen fluid action (g)
- TVFA (mmol/L) = Acetate (mmol/L) + Propionate (mmol/L) + Butyrate (mmol/L)

1.7 Statistical Analysis

Experimental data were initially processed using Excel 2010, followed by one-way ANOVA using SPSS 17.0 software. Duncan' s multiple comparison test was used to examine significant differences between groups. Data are expressed as mean ± standard error, with $P<0.05$ and $P<0.01$ as significance thresholds.

Results

2.1 Effects of Different Anaerobic Alkalization Treatments on Nutrient Content of Wheat Straw

As shown in Table 3 , CP content in the urea and compound groups was significantly higher than in the sodium bicarbonate group ($P<0.01$), increasing by 87.84% and 107.80%, respectively. The compound group was significantly higher than the urea group ($P<0.01$), showing a 10.62% increase.

For NDF content, the compound group was significantly lower than the sodium bicarbonate group ($P<0.01$), decreasing by 3.02%, while the urea group was significantly lower than the sodium bicarbonate group ($P<0.05$). No significant difference was observed between the compound and urea groups ($P>0.05$). HCEL content in the urea and compound groups was significantly lower than in the sodium bicarbonate group ($P<0.01$), decreasing by 5.50% and 8.14%, respectively, with no significant difference between the compound and urea

groups (P>0.05). No significant differences in ADF content were found among the three groups (P>0.05). For ash content, the compound and sodium bicarbonate groups were significantly higher than the urea group (P<0.01), with the compound group significantly higher than the sodium bicarbonate group (P<0.05).

2.2 Effects of Different Anaerobic Alkalization Treatments on Ultrastructure of Wheat Straw

Figure 1 [Figure 1: see original paper] illustrates the ultrastructure of wheat straw under scanning electron microscopy following different anaerobic alkalization treatments. The straw structure in cross-section consists of three parts: epidermis, ground tissue, and vascular bundles.

The epidermis comprises a single layer of tightly arranged cells covered externally by a cuticle (Figures 1-4, 1-5, 1-6). Epidermal cells include long cells and short cells, with the latter consisting of suberized cork cells and silica-containing silicified cells. Stomatal apparatus composed of two dumbbell-shaped guard cells and two semicircular subsidiary cells are distributed on the epidermis. The inner surface of the straw wall forms a membranous structure from the disintegration of young pith parenchyma cells during development (Figures 1-7, 1-8, 1-9). After alkalization, the epidermis and inner surface remained essentially intact without obvious cuticle damage. The sodium bicarbonate group showed a relatively smooth outer surface, while the urea and compound groups exhibited rough, undulating surfaces.

Inside the epidermal cells, 4–5 layers of sclerenchyma cells provide mechanical support, while parenchyma cells near the inner surface have thickened cell walls in later developmental stages. The straw stem contains two rings of vascular bundles: larger inner bundles within the parenchyma tissue and smaller outer bundles within the sclerenchyma tissue. Following alkalization, parenchyma cells showed distortion and deformation, while sclerenchyma cells were generally flattened outward (Figures 1-1, 1-2). The walls of larger sclerenchyma cells adjacent to small vascular bundles often appeared disintegrated (Figure 1-3). Vascular bundle size changed little. The erosion degree on straw cross-sections generally followed: sodium bicarbonate group < urea group < compound group. Examination of cell wall thickness changes revealed that while smaller sclerenchyma cells showed minimal change, both parenchyma cells and larger sclerenchyma cells in treated straw exhibited thinning trends.

2.3 Effects of Different Anaerobic Alkalization Treatments on in vitro Nutrient Degradation Rate of Wheat Straw

As shown in Table 4 , DMD in the compound group was significantly higher than in the sodium bicarbonate group (P<0.01), increasing by 20.79%, with no significant difference from the urea group (P>0.05). The urea group was significantly higher than the sodium bicarbonate group (P<0.05), increasing by

10.88%. For NDFD and ADFD, the compound group was significantly higher than the sodium bicarbonate group ($P<0.05$), with no significant difference from the urea group ($P>0.05$). No significant differences were observed between the urea and sodium bicarbonate groups ($P>0.05$).

2.4 Effects of Different Anaerobic Alkalization Treatments on in vitro Fermentation Parameters of Wheat Straw

As shown in Table 5 , the compound group showed the highest gas production at all time points except 8 hours, followed by the urea group and then the sodium bicarbonate group. No significant differences were observed among groups at 2 and 4 hours ($P>0.05$). At 6 and 8 hours, the compound group was significantly or highly significantly higher than the urea and sodium bicarbonate groups ($P<0.05$ or $P<0.01$). At 48 hours, the compound and urea groups were significantly higher than the sodium bicarbonate group ($P<0.05$), with no significant difference between the compound and urea groups ($P>0.05$).

No significant differences in pH were found among the three groups ($P>0.05$). For NH_3-N production, the compound group was significantly lower than the sodium bicarbonate and urea groups ($P<0.05$). For VFA production, acetic acid in the compound group was significantly higher than in the urea group ($P<0.05$) and highly significantly higher than in the sodium bicarbonate group ($P<0.01$), increasing by 11.74% and 22.68%, respectively. No significant difference was observed between the urea and sodium bicarbonate groups ($P>0.05$). Propionic acid production in the compound and urea groups was significantly higher than in the sodium bicarbonate group ($P<0.05$), with no significant difference between the compound and urea groups ($P>0.05$). Butyric acid in the compound group was highly significantly higher than in the sodium bicarbonate group ($P<0.01$), with no significant difference from the urea group ($P>0.05$). For TVFA production, the compound group was highly significantly higher than the sodium bicarbonate group ($P<0.01$), and the urea group was significantly higher than the sodium bicarbonate group ($P<0.05$), increasing by 26.69% and 15.71%, respectively, with no significant difference between the compound and urea groups ($P>0.05$). No significant differences in the acetic/propionic acid ratio were observed among the three groups ($P>0.05$).

Discussion

3.1 Effects of Different Anaerobic Alkalization Treatments on Nutrient Content of Wheat Straw

CP is a crucial indicator for evaluating protein nutrition in ruminant diets. In this study, urea treatment significantly increased wheat straw CP content, with the compound group showing a further 10.62% increase over the urea group. After urea treatment, ester bonds between lignin and cellulose/hemicellulose in straw are broken, destroying the embedded structure and allowing cell wall fibrous materials to combine with urea and other macromolecular organic sub-

stances through adsorption, thereby increasing CP content [11]. Sodium bicarbonate addition elevated pH, enhancing straw' s adsorption capacity for urea and improving nitrogen retention. Yan et al. [12] treated wheat straw with 2% urea combined with 2%, 4%, 6%, 8%, and 10% calcium hydroxide, finding that all combined treatments significantly increased CP content compared to untreated straw, with nitrogen retention rate increasing with calcium hydroxide dosage.

NDF represents cell wall components that remain intact after neutral detergent washing, comprising the sum of cellulose, hemicellulose, and lignin. In this study, NDF and HCEL contents were lowest in the compound group, followed by the urea group, then the sodium bicarbonate group. This may be because urea decomposition produces ammonia that forms a weak alkaline solution, and the addition of weakly alkaline sodium bicarbonate improved treatment efficacy compared to urea alone, dissolving hemicellulose. HCEL comprises various polysaccharides extractable by alkaline solutions [13], and the HCEL fraction in NDF is unstable, dissolving in alkaline solutions to generate pentoses and hexoses [14], thereby reducing NDF content. After acid detergent treatment, cell contents and hemicellulose are dissolved, leaving ADF comprising cellulose and lignin. No significant differences in ADF content were observed among the three anaerobic alkalization treatments. Similar findings were reported by Lü et al. [15] and Lu et al. [16], who found no ADF reduction in wheat straw treated with urea or urea + calcium hydroxide compared to controls.

3.2 Effects of Different Anaerobic Alkalization Treatments on Ultrastructure of Wheat Straw

Wheat straw structure consists of epidermal tissue, vascular bundles, and ground tissue. Epidermal cell walls are not only cutinized but also highly silicified. Liu [8] treated rice straw with 4% NaOH, 5% urea, and 10% ammonium bicarbonate, observing no obvious deformation or detachment of epidermal layer tissue structures under SEM. Shen et al. [17] reported that urea could dissolve small portions of the cuticular wax-silicon layer on straw stems and leaves. In this study, epidermal tissues and inner medullary cavity surfaces remained intact across all three groups, with the urea and compound groups showing rougher epidermal surfaces, likely because urea dissolved portions of the cuticular wax-silicon layer and silicates in short cells, depositing them on the stem surface in an undulating pattern.

All three groups showed twisted and deformed parenchyma tissue, flattened sclerenchyma cells, and thinning cell walls. This likely occurred because alkaline conditions broke ester or ether bonds between hemicellulose and lignin, causing hemicellulose hydrolysis. Alkali can hydrolyze ester bonds at room temperature (1 mol/L NaOH, 25°C), while higher concentrations and temperatures (4 mol/L NaOH, 170°C) are required for ether bond hydrolysis [18]. Liu et al. [19] found that after 10 days of treatment with 2.5% sodium bicarbonate, 4% urea, and their combination, pH values were 6.74, 8.47, and 8.66, respectively, with the

compound group being most alkaline and the sodium bicarbonate group least alkaline, confirming that the compound group caused the greatest structural damage observed under SEM, followed by the urea group, then the sodium bicarbonate group.

Cell wall thinning may also result from alkaline substances breaking linkages between lignin and structural polysaccharides and between lignin monomers, causing varying degrees of lignin component reduction in cell wall layers [20]. When the three-dimensional structure formed by fibrous polymers (cellulose, hemicellulose, lignin, partial proteins) with acetyl and phenolic acid compounds is destroyed, cellular mechanical support capacity decreases. Notably, although parenchyma cells are theoretically more susceptible to alkaline substances due to their thinner walls, they only showed distortion and deformation, while sclerenchyma cells were flattened and even partially disintegrated beside small vascular bundles. This may be because the hollow cylindrical straw structure allowed alkali solution to penetrate from the epidermis, dissolving some silicates through the highly cutinized and silicified epidermis before acting on outer sclerenchyma cells and vascular bundles. While vascular bundles are difficult to degrade, the highly lignified secondary walls of sclerenchyma cells allowed alkali to break hemicellulose-lignin connections, reducing lignin and causing cell wall disintegration. After passing through densely arranged sclerenchyma cells, the alkali solution quantity and concentration decreased, so its action on inner parenchyma cells could only cause deformation rather than complete disintegration.

3.3 Effects of Different Anaerobic Alkalization Treatments on in vitro Degradation Rate of Wheat Straw

The DMD of roughage reflects its digestibility in animals and is an important factor affecting dry matter intake [21]. NDFD and ADFD accurately reflect the availability of fiber in feed and are important indicators for evaluating the nutritional value of ruminant roughage. Studies show that in vitro DMD of roughage is highly significantly negatively correlated with NDF content and significantly positively correlated with CP content [22], while in vitro NDFD is significantly negatively correlated with NDF content [23]. Wheat straw has high fiber content, and combined alkalization and ammoniation can break ester bonds between lignin and hemicellulose, reduce hemicellulose and NDF contents, and significantly increase CP content. Ruminants can utilize cellulase secreted by rumen bacteria, fungi, and protozoa to degrade fibrous materials [24]. Urea-treated straw shows a fluffy fiber structure with increased voids and enlarged enzyme adsorption surface area, facilitating cellulase hydrolysis [25].

He [26] reported that combined treatment of corn straw with 4% urea + 4% calcium hydroxide significantly improved degradation rate, with 48-hour in situ DMD, NDFD, and ADFD increasing by 45.54%, 50.56%, and 36.81% compared to untreated straw. Wang et al. [27] found that total silicon content in chemically treated straw decreased linearly with increasing alkalization or ammoni-

ation doses, with a highly significant negative correlation between total silicon and rice straw DMD (r=-0.560). Sun et al. [28] studied different combinations of urea and calcium hydroxide (both at 2%, 3%, and 4%) on rumen degradation rates of wheat straw DM and fiber, finding that 72-hour DMD, NDFD, and ADFD generally increased with additive proportions, reaching maximum improvements of 90.98%, 92.06%, and 139.38% with the optimal 4% urea + 2% calcium hydroxide combination compared to untreated straw.

In this study, NDF content followed the pattern: compound group < urea group < sodium bicarbonate group, while CP content showed the opposite trend. Nutrient degradation rates followed: compound group > urea group > sodium bicarbonate group, with the compound group' s 48-hour DMD, NDFD, and ADFD increasing by 20.79%, 21.45%, and 15.47% compared to the sodium bicarbonate group, and by 8.94%, 5.93%, and 5.21% compared to the urea group. The combined urea and sodium bicarbonate treatment of fresh wheat straw was superior to single treatments, substantially improving straw nutritional value, consistent with reports by Xu et al. [29].

3.4 Effects of Different Anaerobic Alkalization Treatments on in vitro Gas Production and Fermentation Parameters of Wheat Straw

In vitro rumen fermentation trials, gas production, VFA, pH, and NH_3-N are important indicators reflecting dietary fermentation efficacy. Gas production primarily comprises methane and carbon dioxide generated by rumen microbial action and can reflect dietary energy level, with studies demonstrating positive correlations between in vitro gas production and nutrient digestibility [30-31]. VFAs, mainly acetic, propionic, and butyric acids (accounting for ~95% of total rumen VFA production), are the primary form of energy utilization in ruminants. Increased TVFA production indicates improved substrate digestibility.

pH is influenced by VFAs and other metabolites, reflecting dietary fermentation degree in the rumen and is affected by dietary quality, saliva secretion, rumen osmotic pressure, and VFA production [32]. NH_3-N, produced from deamination of amino acids derived from degraded exogenous proteins and endogenous nitrogenous substances under suitable pH conditions, is an important substrate for microbial protein synthesis. Rumen NH_3-N production reflects the balance between protein degradation and synthesis, being influenced by dietary nitrogenous nutrient degradation rate and microbial uptake/utilization rate.

In this study, the compound and urea groups showed higher 48-hour gas production than the sodium bicarbonate group, with the compound group producing the highest values. Acetic, propionic, butyric, and TVFA production also followed the pattern: compound group > urea group > sodium bicarbonate group, further confirming that the compound group had higher nutrient degradation rates. This may be related to the compound group' s lower NDF content. Total gas production and yields of CH_4, CO_2, and VFAs are significantly negatively correlated with NDF content and significantly positively correlated with

non-structural carbohydrate content. Combined alkalization and ammoniation reduces NDF content, loosens cell wall structure, increases carbon sources for microbial fermentation, and provides nitrogen from urea, increasing microbial (primarily bacterial and protozoal) numbers and activity, promoting straw DM and fiber degradation. Carbohydrate degradation produces VFAs and generates CH_4 and CO_2, increasing in vitro gas production.

Zhao et al. [33] treated rice straw with different urea levels (0, 2%, 4%, 6%, 8%) for in vitro rumen fermentation, finding that increasing urea levels enhanced alkalinity, reduced NDF content, and significantly improved gas production and TVFA and acetic acid yields, consistent with this study. Notably, combined urea and sodium bicarbonate treatment did not increase NH_3-N production but significantly decreased it, possibly because improved digestibility of fibrous materials increased carbon source availability, enhancing microbial protein synthesis. Similar findings were reported by Huo et al. [34] in studies on combined hydrogen peroxide and urea treatment of wheat straw.

The acetic/propionic ratio reflects microbial fermentation patterns, with no significant differences among groups indicating that none of the treatments altered rumen fermentation mode, which remained acetic acid-dominant. Additionally, pH was maintained between 6.85–6.87 across groups with no significant differences, and cellulase activity remained within the suitable pH range (6.5–7.0) [35], consistent with Li [36] who reported no significant pH differences after 72-hour in vitro rumen fermentation of peanut vines treated with different calcium bicarbonate-urea combinations.

Conclusion

The combined anaerobic alkalization treatment with 2.5% sodium bicarbonate and 4% urea significantly improved wheat straw quality compared to single treatments. Specifically, CP content increased by 107.80% and 10.62% compared to the sodium bicarbonate and urea groups, respectively, while NDF and HCEL contents decreased by 3.02% and 8.14% compared to the sodium bicarbonate group, and by 1.58% and 2.80% compared to the urea group. Ultrastructural damage followed the pattern: compound group > urea group > sodium bicarbonate group, with alkali erosion affecting tissues in the order: sclerenchyma cells > parenchyma cells > vascular bundles. The compound group' s 48-hour DMD, NDFD, and ADFD increased by 20.79%, 21.45%, and 15.47% compared to the sodium bicarbonate group, and by 8.94%, 5.93%, and 5.21% compared to the urea group. The combined treatment significantly increased in vitro gas production and VFA yields while reducing NH_3-N production, without altering rumen fermentation pH or pattern.

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