Postprint: In Vitro Fermentation Characteristics of Different Crop Straws in Tibet

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Abstract

This experiment aimed to evaluate the nutritional value of different crop straws in Tibet using the in vitro gas production method. Five common types of crop straws from Tibet (pea straw, corn straw, highland barley straw, wheat straw, and rapeseed straw) were selected. Through a single-factor experimental design, the in vitro gas production method was employed to assess various indicators of the five crop straws after 48 h of in vitro fermentation, including gas production, theoretical maximum gas production (Vf), methane (CH4) production, Logistic-Exponential (LE) model gas production parameters, fermentation broth pH and NH3-N concentration, main volatile fatty acid (VFA) production, in vitro dry matter digestibility (IVDMD), and in vitro neutral detergent fiber digestibility (IVNDFD). The results showed that the 48 h in vitro gas production of the five crop straws decreased in the order of corn straw, highland barley straw, pea straw, wheat straw, and rapeseed straw. After 48 h of in vitro fermentation, corn straw exhibited significantly higher Vf, IVDMD, IVNDFD, CH4 production (except for pea straw), propionate and total VFA production compared to the other four crop straws ($P<0.05$), and its in vitro fermentation broth pH was also significantly lower than that of the other straws ($P<0.05$). The results indicated that corn straw demonstrated the best in vitro fermentation effect and was more readily degraded and utilized by rumen microorganisms compared to the other experimental crop straws.

Full Text

Study on in Vitro Fermentation Characteristics of Different Crop Straws in Tibet Region

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Abstract

This experiment aimed to evaluate the nutritional value of different crop straws in Tibet region using in vitro gas production technology. Five common types of crop straws (common vetch straw, maize straw, hulless barley straw, wheat straw, and rape straw) from Tibet were selected to determine their 48 h gas production, theoretical maximum gas production (Vf), methane (CH_4) production, logistic-exponential (LE) model gas production parameters, fermentation fluid pH and NH_3-N concentration, main volatile fatty acid (VFA) production, in vitro dry matter degradability (IVDMD), and in vitro neutral detergent fiber degradability (IVNDFD) through a single-factor experimental design. The results showed that the 48 h gas production of the five crop straws decreased in the order of maize straw, hulless barley straw, common vetch straw, wheat straw, and rape straw. After 48 h of in vitro fermentation, the theoretical maximum gas production, IVDMD, IVNDFD, CH_4 production (except for common vetch straw), propionic acid, and total VFA yields of maize straw were significantly higher than those of the other four crop straws ($P < 0.05$), while its in vitro fermentation fluid pH was significantly lower than that of the other straws ($P < 0.05$). These results indicate that maize straw exhibits the best in vitro fermentation effect and is more easily degraded and utilized by rumen microorganisms compared with the other crop straws tested.

Keywords: Tibet; crop straws; in vitro fermentation

Introduction

Grassland animal husbandry has long been a crucial economic foundation in Tibet, accounting for approximately 60% of the total agricultural output value for decades. However, with increasing population and further development of animal husbandry in Tibet in recent years, the traditional production model relying solely on natural grasslands can no longer meet livestock forage requirements. Simultaneously, as livestock numbers continue to grow, the conflict between forage supply and livestock demand has become increasingly prominent, leading to continuous ecological environment deterioration that seriously affects the balance, stability, and development of animal production systems in Tibet.

Tibet produces approximately 700,000 tons of crop straw annually, with hulless barley straw being one of the main crop straw resources. According to statistics, the annual output of hulless barley straw alone reaches about 400,000 tons, of which approximately 60% is used as livestock feed. Therefore, the full utilization of abundant crop straw resources in Tibet' s agricultural areas is of great significance for meeting the forage demand of animal husbandry production, maintaining ecological balance and stability, and promoting economic development in Tibetan regions.

In recent years, numerous studies have investigated forage and crop straws in Tibet. Qu Guangpeng conducted introduction trials of forage and fodder crops in Tibetan agricultural areas, demonstrating that five forage species—oats, Italian ryegrass, forage maize, green wheat, and red amaranth—are suitable for cultivation in Tibetan agricultural areas and river valleys, though no nutritional evaluation was performed on these forages. Zhang Zhongyue evaluated the nutritional value and rumen degradation characteristics of 28 forage species in Tibet, providing valuable baseline data for further research on Tibetan forages. Zhang Ji et al. studied the effects of additives on the fermentation quality of mixed oat and common vetch silage in Tibet, finding that molasses addition significantly improved silage quality. Sun Xiaohui et al. and Li Junfeng et al. obtained high-quality silage by adding 4% molasses, 3.5% ethanol, or 0.4% acetic acid to mixed oat and alfalfa silage in Tibet. Zhao Qingjie et al. significantly improved the fermentation quality of mixed hulless barley straw and perennial ryegrass silage by adding molasses and lactic acid bacteria. Yuan Xianjun conducted research on mixed silage of crop straws and forages in Tibet, effectively improving fermentation quality. In summary, current research on forages and crop straws in Tibet has primarily focused on silage materials, with few reports on the rumen degradation characteristics of crop straw hay.

Therefore, this study employed in vitro gas production technology to investigate rumen in vitro fermentation parameters of common crop straws in Tibet. Through analysis of experimental data, we aimed to identify crop straws that are easily degraded and utilized by rumen microorganisms, providing a scientific basis for the development of agriculture and animal husbandry in Tibet.

Materials and Methods

Sample Collection and Processing

Experimental crop straws (common vetch, maize, hulless barley, wheat, and rape straw) were collected from Jinna Village pasture, Gangdui Town, Gongga County, Tibet Autonomous Region. The crop straws were dried at 65 °C for 24 h, ground, and passed through a 1 mm sieve before use.

Experimental Animals and Diet

Three healthy Holstein dairy cows with permanent rumen fistulas, similar body weight [(500 ± 50) kg], served as rumen fluid donors and were provided by Shenghe Dairy Farm, Bairuopu Town, Wangcheng District, Changsha City, Hunan Province. During the experimental period, the dairy cows were fed a diet formulated according to NRC (2001) standards, consisting of rice straw and concentrate supplement with a concentrate-to-forage ratio of 40:60. Diet composition and nutrient levels are presented in Table 1 , consistent with reference [12].

Nutritional Analysis of Crop Straws

Processed crop straws were analyzed for dry matter (DM), crude protein (CP), ether extract (EE), crude fiber (CF), ash, neutral detergent fiber (NDF), and acid detergent fiber (ADF) using feed analysis methods provided by Yang Sheng [13]. Results are shown in Table 2 .

Preparation of In Vitro Fermentation Fluid

Anaerobic buffer solution was prepared according to the method described by Menke et al. [14]. Rumen contents were collected from the three fistulated cows before morning feeding, filtered through eight layers of gauze, and the filtrates were mixed in equal volumes and transferred to a pre-warmed thermos flask (39.5 °C) filled with CO_2. The mixed rumen fluid was quickly transported to the laboratory and combined with anaerobic buffer solution preheated in a 39.5 °C water bath (V_{buffer} solution:V_{rumen} fluid = 9:1) while continuously flushing with CO_2.

In Vitro Incubation

Approximately (0.5000 ± 0.0003) g of ground crop straw was weighed into 145 mL fermentation bottles and preheated in a 39.5 °C incubator. Before adding fermentation fluid, CO_2 was flushed through each bottle for 1 min, followed by addition of 50 mL fermentation fluid. Bottles were immediately sealed with stoppers and caps while continuing CO_2 flushing. A needle was used to release gas and equalize internal and external pressure before bottles were quickly returned to the incubator for static cultivation at 39.5 °C for 48 h. Each fermentation substrate was sampled at three time points (12, 24, and 48 h) with three replicates per time point, meaning three fermentation bottles were removed for sampling at each time point.

Measurement of In Vitro Gas Production

In vitro gas production was measured using the method described by Wang et al. [12]. Cumulative gas production data were fitted using the logistic-exponential (LE) model proposed by Wang et al. [15-16]:

$$V = \frac{V_f}{1 + \exp(-b - kt)}$$

where: V represents gas production from substrate at time t (mL); Vf represents theoretical maximum gas production (mL); k represents fractional gas production rate (h^{-1}); b and d are curve shape parameters, with b > 0 indicating an S-shaped curve and b < 0 indicating a non-S-shaped curve; FRD_0 represents initial gas production rate (<12 h) (mL/h); $t_{0 \cdot 5}$ represents time required to reach half of maximum gas production (h).

Measurement of In Vitro Fermentation Parameters

Methane (CH_4) Measurement and Calculation At 12, 24, and 48 h of in vitro fermentation, fermentation bottles were removed and 5 mL of headspace gas was extracted with a syringe and injected into pre-evacuated gas collection bottles, followed by injection of 25 mL high-purity N_2. Parameters were measured according to the method provided by Li et al. [17]. CH_4 production was calculated as:

$$CH_4 = 6 \times V_t \times C$$

where: 6 represents the dilution factor; Vt represents total gas volume in the fermentation bottle at time t (mL); C represents CH_4 concentration measured at fermentation time t (%); CH_4 represents CH_4 volume produced at fermentation time t (mL).

pH Measurement At 12, 24, and 48 h of in vitro fermentation, fermentation bottles were removed and fermentation fluid was filtered through 400-mesh nylon cloth. Five mL of filtrate was immediately measured for pH using a pH meter (REX PHS-3C, Shanghai Instrument Equipment Factory).

Ammonia Nitrogen (NH_3-N) Concentration Measurement At 12, 24, and 48 h of in vitro fermentation, fermentation fluid was filtered through 400-mesh nylon cloth and 4 mL of filtrate was aliquoted into two 2 mL centrifuge tubes and stored at -20 °C for NH_3-N concentration determination. NH_3-N concentration was measured using the method provided by Feng Zongci et al. [18].

Major Volatile Fatty Acid (VFA) Production Measurement At 12, 24, and 48 h of in vitro fermentation, 2 mL of fermentation fluid was collected from each bottle and centrifuged at 15,000 r/min for 15 min. Then 1.5 mL of supernatant was transferred to a 2 mL centrifuge tube with 0.15 mL of 25% metaphosphoric acid added and stored overnight at -20 °C. Major VFA production was measured according to the method described by Wang et al. [19].

In Vitro Dry Matter Degradability (IVDMD) Measurement and Calculation At 12, 24, and 48 h of in vitro fermentation, fermentation fluid was filtered through 400-mesh nylon cloth. The filtered residue was completely transferred to a quartz crucible, rinsed repeatedly with hot distilled water, dried in an oven at 105 °C for 8 h, and the remaining dry matter mass was measured to calculate IVDMD:

$$IVDMD = \frac{M_1 - M_2}{M_1} \times 100\%$$

where: M_1 represents dry matter mass of fermentation substrate before fermentation (g); M_2 represents dry matter mass remaining after fermentation (g).

In Vitro Neutral Detergent Fiber Degradability (IVNDFD) Measurement and Calculation After determining residual dry matter from IVDMD measurement, NDF mass was determined according to the method provided by Hall et al. [20] and IVNDFD was calculated:

$$IVNDFD = \frac{m_1 - m_2}{m_1} \times 100\%$$

where: m_1 represents NDF mass in fermentation substrate before fermentation (g); m_2 represents NDF mass remaining after fermentation (g).

Statistical Analysis

Experimental data were statistically analyzed using the MIXED procedure of SAS 8.2, with statistical significance defined as $P < 0.05$.

Results

Effects of Different Crop Straws on In Vitro Fermentation Gas Production

The in vitro fermentation gas production of different crop straws is shown in Figure 1 [Figure 1: see original paper]. During the initial fermentation stage (1–4 h), no significant differences in gas production were observed among the five crop straws. After 4 h of in vitro fermentation, the gas production rate gradually increased. Between 6–24 h of in vitro fermentation, the slopes of the gas production curves for all five crop straws reached their maximum values, indicating that the gas production rates also peaked during this period. After 24 h of in vitro fermentation, the curve slopes decreased, suggesting that gas production rates gradually declined and gas production stabilized. Maize straw consistently showed higher gas production and rate than the other four crop straws after 4 h of fermentation. At 24 and 48 h of in vitro fermentation, maize straw gas production was 75.33 and 97.84 mL, respectively, both higher than the other four crop straws. Rape straw showed the lowest gas production at 48 h (58.53 mL). The 48 h gas production of the five crop straws decreased in the order of maize straw, hulless barley straw, common vetch straw, wheat straw, and rape straw.

Effects of Different Crop Straws on In Vitro Fermentation Gas Production Parameters and CH_4 Production

The effects of different crop straws on in vitro fermentation gas production parameters and CH_4 production are presented in Table 3 . The theoretical maximum gas production (Vf) was highest for maize straw (101.92 mL), significantly higher than other crop straws ($P < 0.05$), while rape straw had the lowest Vf (59.09 mL), significantly lower than the other four crop straws ($P < 0.05$). No significant differences in initial gas production rate (FRD_0) were observed among the five crop straws ($P > 0.05$). The time required to reach half of maximum gas production ($t_{0\cdot5}$) was highest for wheat straw (17.74 h), which showed no significant difference from hulless barley straw ($P > 0.05$) but was significantly higher than common vetch, maize, and rape straws ($P < 0.05$), with no significant differences among the latter three ($P > 0.05$). CH_4 production was relatively high for common vetch and maize straws, with no significant difference between them ($P > 0.05$), but both were significantly higher than CH_4 production from hulless barley, rape, and wheat straws ($P < 0.05$), with no significant differences among the latter three ($P > 0.05$).

Effects of Different Crop Straws on IVDMD and IVNDFD

The effects of different crop straws on IVDMD and IVNDFD are shown in Table 4 . Significant differences in both IVDMD and IVNDFD were observed among the five crop straws ($P < 0.05$). IVDMD was highest for maize straw (57.35%) and decreased significantly in the order of maize straw, common vetch straw, hulless barley straw, rape straw, and wheat straw ($P < 0.05$). For IVNDFD, maize straw also showed the highest value (53.73%), decreasing significantly in the order of maize straw, hulless barley straw, common vetch straw, rape straw, and wheat straw ($P < 0.05$).

Effects of Different Crop Straws on In Vitro Fermentation pH and NH_3-N Concentration

The effects of different crop straws on in vitro fermentation pH and NH_3-N concentration are presented in Table 5 . Maize straw fermentation fluid had the lowest pH (6.42), significantly lower than the other four crop straws ($P < 0.05$), while no significant differences were observed among the other four straws ($P > 0.05$). NH_3-N concentration in common vetch straw fermentation fluid was significantly higher than in the other four crop straws ($P < 0.05$), while maize, hulless barley, and wheat straws showed relatively low NH_3-N concentrations, significantly lower than common vetch and rape straws ($P < 0.05$).

Effects of Different Crop Straws on Main VFA Production

The effects of different crop straws on main VFA production are shown in Table 6 . After 48 h of in vitro fermentation, maize straw showed the highest production of acetic acid, propionic acid, butyric acid, and total VFA. Acetic acid production from maize straw was significantly higher than from common vetch, hulless barley, and wheat straws ($P < 0.05$). Propionic acid and total VFA production were significantly higher than from the other four crop straws ($P < 0.05$). Butyric acid production was significantly higher than from common vetch, rape, and wheat straws ($P < 0.05$). The acetate-to-propionate ratio was lower for maize and hulless barley straws, with no significant difference between them ($P > 0.05$), but both were significantly lower than the other three crop straws ($P < 0.05$).

Discussion

Effects of Different Crop Straws on In Vitro Fermentation Gas Production

Research indicates that differences in in vitro fermentation gas production exist among the five crop straws, with maize straw showing the highest values, likely due to variations in the amount and composition of carbohydrates among different crop straws. The primary source of gas production from crop straw fermentation is carbohydrates, though proteins also contribute some gas during fermentation. However, the contribution of proteins to total gas production is far lower than that of carbohydrates throughout the fermentation process. Cone et al. found that protein fermentation produced only 30% of the gas volume generated by carbohydrates after 72 h of in vitro fermentation. Additionally, among the five crop straws used in this experiment, common vetch is a legume, rape belongs to the Brassicaceae family, and the other three crop straws are grasses. Different plant families may be one reason for differences in gas production characteristics of roughages. Previous reports indicate that different types of roughage show substantial differences in in vitro fermentation gas production characteristics, with generally smaller intraspecific differences and larger interspecific differences. Hai Cunxiu evaluated the nutritional value of natural pastures on the Qinghai Plateau using castrated yaks and found that average gas production increased sharply during 12–48 h of in vitro fermentation and gradually plateaued after 48 h. In this experiment, gas production from all five crop straws increased sharply during 6–24 h and plateaued after 36 h, differing from Hai Cunxiu's results, possibly due to differences in fermentation substrates and fermentation fluid composition.

Effects of Different Crop Straws on In Vitro Fermentation Gas Production Parameters and CH_4 Production

During in vitro fermentation, the degree of substrate utilization by rumen microorganisms can be reflected by cumulative gas production. In this experiment, significant differences in Vf among crop straws may be caused by different ratios of soluble non-structural carbohydrates to CP. Tang Shaoxun et al. reported that Vf increases with larger ratios of soluble non-structural carbohydrates to CP and decreases with smaller ratios, meaning that in vitro fermentation intensifies with increasing non-structural carbohydrate content in forages. Gao Wei et al. demonstrated that neutral detergent soluble (NDS) fractions from corn straw silage contributed the majority of cumulative gas production.

FRD_0 represents the gas production rate before 12 h of in vitro fermentation, while $t_{0 \cdot 5}$ represents the time required to reach half of maximum gas production. Typically, larger FRD_0 values correspond to smaller $t_{0 \cdot 5}$ values. In this experiment, no significant differences in FRD_0 were observed among the five crop straws, though hulless barley straw showed a relatively higher FRD_0, possibly due to differences in NDF/CP ratios. NDF is a refractory component while CP is readily degradable; higher ratios result in lower degradability, whereas lower ratios lead to faster fermentation rates. Muck et al. reported that 65%–70% of total gas production occurs within the initial 9–10 h of in vitro fermentation. However, $t_{0 \cdot 5}$ values for the five crop straws in this experiment ranged from 10–19 h, notably higher than Muck et al.' s findings, likely due to differences in fermentation methods and substrates.

Methane in the rumen of ruminants is produced through anaerobic fermentation of carbohydrates by rumen microorganisms. CH_4 generation represents a major source of energy loss during rumen fermentation, with approximately 6%–15% of dietary energy reportedly lost as CH_4. CH_4 production correlates with dietary CP, ADF, NDF, NFE content, and IVDMD. In this study, differences in CH_4 production among crop straws were observed, with maize straw showing the highest values, consistent with previously reported results. These differences may be attributed to variations in fermentable carbohydrate types and components related to CH_4 production among different crop straws. Reports indicate that fiber content is an important factor affecting CH_4 production, possibly because fiber-rich feeds promote symbiosis between fiber-degrading bacteria and methanogenic bacteria, which can couple carbohydrate degradation products and utilize hydrogen to reduce carbon dioxide for CH_4 synthesis.

Effects of Different Crop Straws on IVDMD and IVNDFD

Dry matter degradability (DMD) and neutral detergent fiber degradability (NDFD) are important indicators reflecting roughage utilization during rumen fermentation. Roughage degradation in the rumen results from interactions between microorganisms and enzymes they secrete, with degradation efficiency related to nutrient structure, microbial attachment capacity to substrates, and catalytic ability of microbial enzymes. In this experiment, differences in IVDMD among crop straws were observed, with maize straw showing the highest values. These differences may be caused by variations in non-structural carbohydrate and digestible organic matter contents among different crop straws. Maize straw contains higher non-structural carbohydrate and digestible organic matter contents than other crop straws, suggesting that maize straw is readily degraded and utilized by rumen microorganisms.

Since NDF degradability affects animal performance and crop straw degradability varies considerably in the rumen, NDFD serves as an important indicator for evaluating forage quality. In this study, maize straw showed the highest IVNDFD among the five crop straws, with differences among other straws possibly related to microbial attachment capacity and substrate structure. Maize straw may more readily adsorb fiber-degrading bacteria compared with other crop straws. Fernando et al. reported that bacterial attachment capacity to substrates is an important factor affecting substrate digestibility. Xu Jun et al. reported that the degradation rate and extent of alfalfa stems by rumen microorganisms are influenced by tissue structure and composition, and that inconsistent microbial adsorption patterns to plant tissues may also contribute to differences in fiber degradability among substrates.

Effects of Different Crop Straws on In Vitro Fermentation pH and NH_3-N Concentration

Rumen fluid pH is an important indicator for evaluating the rumen internal environment, and maintaining normal rumen pH is essential for ensuring normal fermentation. The normal pH range for dairy cow rumen fluid is 5.5–7.5. In this experiment, the pH range of fermentation fluid from the five crop straws was 6.32-6.59, all within the normal range. Reports indicate that rumen microorganisms achieve maximum growth rate when pH exceeds 5.7, suggesting that in vitro fermentation of all five crop straws in this study was conducive to microbial growth. Rumen fluid pH is influenced by various factors including saliva secretion and organic acid production, absorption, and excretion, but its fluctuation fundamentally depends on dietary structure. Maize straw showed the lowest fermentation fluid pH (Table 5), likely due to higher VFA production during fermentation. The pH values of fermentation fluid from each crop straw generally corresponded to their respective total VFA production levels.

NH_3-N in the rumen serves as the primary raw material for rumen microorganisms to synthesize microbial protein and body protein, and is an important nitrogen source for microbial growth. Its concentration can reflect the balance between protein degradation and synthesis in the rumen to some extent. Wanapat et al. reported that the optimal NH_3-N concentration range in rumen fluid is 6.2-27.5 mg/dL. In this experiment, NH_3-N concentrations in fermentation fluid from the five substrates ranged from 6.26-19.74 mg/dL, all within the reported optimal range. Significant differences in NH_3-N concentration were observed among the five crop straws, likely due to different protein contents. Common vetch straw showed the highest NH_3-N concentration, significantly higher than other crop straws, possibly because common vetch is a legume while the other straws (except rape) are grasses. Reports indicate that legumes contain higher protein content than grasses, suggesting that legume common vetch straw has higher protein content than grass species (maize, hulless barley, and wheat straws), resulting in higher NH_3-N concentration in fermentation fluid.

Effects of Different Crop Straws on In Vitro Fermentation VFA Production

VFAs are the main end products of rumen microbial anaerobic fermentation of dietary nutrients, providing 70%-80% of the total energy requirement for ruminants and thus playing a crucial role in ruminant carbohydrate nutrition. The main VFAs include acetic, propionic, butyric, valeric, isobutyric, and isovaleric acids, with acetic, propionic, and butyric acids being most important for animal metabolism, accounting for approximately 95% of total rumen VFA production. Acetic acid is the most abundant VFA, comprising about 70%-75% of total VFA production when feeding roughages. In this experiment, differences in VFA production and composition were observed among crop straws. Maize straw showed significantly higher production of acetic, propionic, butyric, and total VFAs than other crop straws, but had a relatively lower acetate-to-propionate ratio. Wheat straw showed relatively lower total VFA production but a higher acetate-to-propionate ratio. These differences may be caused by variations in fermentable organic matter and NDF content among different crop straws. Guo Dongsheng reported that VFA production 主要取决于可发酵有机物, while Li Wang reported that dietary mineral elements also affect VFA production and that VFA composition is significantly influenced by dietary NDF and non-NDF content. Additionally, significant differences exist in VFA production from in vitro fermentation of plant cell walls from different sources. Zhang Yuanqing et al. reported significant differences in total VFA production and individual VFA components (except butyric acid) from fermentation of six different plant cell wall sources.

Conclusion

1. After 48 h of in vitro fermentation, maize straw showed higher 48 h gas production, theoretical maximum gas production, CH_4 production, IVDMD, and IVNDFD than the other four crop straws.
2. After 48 h of in vitro fermentation, maize straw fermentation fluid showed lower pH and NH_3-N concentration than the other four crop straws.
3. After 48 h of in vitro fermentation, maize straw produced higher yields of acetic acid, propionic acid, butyric acid, and total VFA than the other four crop straws.
4. Comprehensive analysis of in vitro gas production parameters, fermentation indices, and degradability revealed that maize straw exhibited the best in vitro fermentation effect and was more readily degraded and utilized by rumen microorganisms compared with other tested crop straws. However, maize straw also produced more CH_4 gas during fermentation, which could lead to energy waste in practical animal production. Therefore, various factors should be comprehensively considered in practical livestock production to maximize straw utilization efficiency.

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