

## Evaluation of the Feeding Value of Ramie by In Vitro Gas Production and Nylon Bag Methods (Postprint)

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### Abstract

This experiment evaluated the feeding value of ramie leaves, whole ramie plants, and defibered whole ramie plants using in vitro gas production and nylon bag methods. The in vitro gas production method was employed to determine the 72 h dynamic gas production of ramie leaves, whole ramie plants, and defibered whole ramie plants, and to calculate the gas production parameters. The nylon bag test was conducted using the aforementioned three materials to measure the degradation rates of dry matter (DM), crude protein (CP), neutral detergent fiber (NDF), and acid detergent fiber (ADF), and to derive the degradation parameters. The results demonstrated: 1) In the gas production test, the whole ramie plant exhibited the highest 72 h gas production and theoretical maximum gas production, which were significantly higher than those of the defibered whole ramie plant ( $P < 0.05$ ), while ramie leaves were intermediate, with no significant difference between ramie leaves and the other two treatments ( $P > 0.05$ ). 2) In the nylon bag test, the 72 h degradation rates of DM, CP, NDF, and ADF all followed the order of ramie leaves > whole ramie plant > defibered whole ramie plant. The effective degradation rates of DM, NDF, and ADF displayed the same pattern. However, the effective degradation rate of CP followed the order of whole ramie plant > ramie leaves > defibered whole ramie plant. Based on in vitro gas production and effective degradation rates, the feeding value of ramie leaves and whole ramie plants was superior to that of defibered whole ramie plants.

## Full Text

# Evaluation of the Feeding Value of Ramie Using In Vitro Gas Production and Nylon Bag Methods

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## Abstract

This study evaluated the feeding value of ramie leaf, whole-plant ramie, and defibered whole-plant ramie using in vitro gas production and nylon bag methods. The 72-hour dynamic gas production was measured via in vitro fermentation to determine gas production parameters, while nylon bag incubation was employed to measure degradation rates of dry matter (DM), crude protein (CP), neutral detergent fiber (NDF), and acid detergent fiber (ADF), from which degradation parameters were derived. The results showed: (1) In the gas production trial, whole-plant ramie exhibited the highest 72-hour and theoretical maximum gas production, significantly greater than defibered whole-plant ramie ( $P < 0.05$ ), with ramie leaf intermediate and not significantly different from the other two treatments ( $P > 0.05$ ). (2) In the nylon bag trial, 72-hour degradation rates of DM, CP, NDF, and ADF all followed the pattern: ramie leaf > whole-plant ramie > defibered whole-plant ramie. Effective degradation rates of DM, NDF, and ADF showed the same trend; however, CP effective degradation rate followed the order: whole-plant ramie > ramie leaf > defibered whole-plant ramie. Based on both gas production and effective degradation rates, ramie leaf and whole-plant ramie demonstrated superior feeding value compared to defibered whole-plant ramie.

**Keywords:** ramie; feeding value; in vitro gas production method; nylon bag method

## Introduction

Ramie [*Boehmeria nivea* (L.) Gaudich] is a perennial herbaceous plant belonging to the family Urticaceae and genus *Boehmeria* [1]. As a traditional fiber crop, ramie is widely cultivated in Chinese provinces such as Hunan, Hubei, Sichuan, and Jiangxi [2]. In recent years, ramie has attracted increasing attention as a novel high-protein forage resource [3-6]. Reports indicate that ramie is nutritionally rich and comparable to alfalfa, with high yield and good biological adaptability that allows it to thrive in southern China's rainy and hot seasonal conditions [7-8]. However, research on its application in animal production remains limited.

In vitro gas production and nylon bag methods are the two most commonly used techniques for evaluating feed nutritional value *in vitro*. The gas production method was established in 1979 by researchers including Menke at the

Institute of Animal Nutrition, University of Hohenheim, Germany, and has become an internationally recognized approach for assessing ruminant forage nutritional value. Gas production volume provides an accurate estimate of feed rumen organic matter digestibility. The nylon bag method effectively integrates feed with the animal, clearly presenting feed utilization in the rumen and enabling real-time measurement of rumen degradation rates, making it a rapid and efficient technique for evaluating the degradation speed and extent of feed nutrients in the rumen [9-10]. To thoroughly investigate the feeding value of ramie, our laboratory employed both methods to provide a theoretical basis for its utilization as a forage resource.

## Materials and Methods

### 1.1 Experimental Materials

Ramie samples were collected in June 2014 from the experimental base of the Hunan Institute of Bast Fiber Crops. The variety was Zhongzhu No. 1, a high-quality, high-yield, and multi-resistant cultivar selected from the hybrid offspring of *Boehmeria nivea* ‘Yuanyeqing’ × ‘Luzhuqing’. Samples were collected during the first growth stage at a location of 28°18’ N, 112°51’ E, with plant heights of 80-100 cm. Three types of samples were collected separately: ramie leaves (manually picked), whole-plant ramie (harvested with a sickle), and defibered whole-plant ramie (harvested whole and then defibered using a 4BM-260 ramie decorticator). After collection, samples were naturally dried, ground to pass a 1 mm sieve, and stored at room temperature in ziplock bags. The gas production system used was an ANKOM RFS gas production measurement device (Beijing Ankeborui Technology Co., Ltd.), and nylon bags measuring 8 cm × 12 cm (50 μm pore size) were used for the degradation trial.

#### 1.2.1 Nutrient Content Determination

Dry matter (DM), crude protein (CP), crude ash, ether extract (EE), and calcium (Ca) contents of ramie leaf, whole-plant ramie, and defibered whole-plant ramie were determined according to Zhang Liying [11]. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) contents were measured following Van Soest [12], with two replicates per sample. After nylon bag digestion, residues were analyzed for DM, CP, NDF, and ADF using the same methods.

#### 1.2.2 In Vitro Gas Production Trial

The ANKOM RFS gas production system (Beijing Ankeborui Technology Co., Ltd.) was used. All module batteries were charged for 16 hours before installation. The BASE transmitter was connected to the computer, and GPM software was launched to verify module-computer interaction and ensure all modules were recognized by the software.

The gas release valve was checked for proper function, with response time set

to 1 s and recording interval to 1 min. After clicking the record button, a valve cleaning tool was connected, and modules were pressurized to 41-69 kPa (note: not exceeding 69 kPa). After 6-10 min, data were observed to confirm no significant pressure drop (minor fluctuations are normal). The valve opening button was clicked to verify pressure reduction, and after confirming 0 kPa pressure, the module release valve was closed.

Samples of 0.5000 g each of ramie leaf, whole-plant ramie, and defibered whole-plant ramie were placed into 250 mL artificial rumen culture bottles, with five replicates per group and three blank controls (culture medium only, no substrate). All bottles were preheated in an electric thermostatic shaking water bath, and prepared microbial culture medium was preheated in a 39°C water bath.

Microbial culture medium was prepared according to Menke et al. [13]: 8.75 g  $\text{NaHCO}_3$ , 1.00 g  $\text{NH}_4\text{HCO}_3$ , 1.43 g  $\text{Na}_2\text{HPO}_4$ , 1.55 g  $\text{KH}_2\text{PO}_4$ , 0.15 g  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.52 g  $\text{Na}_2\text{S}$ , 0.015 g  $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ , 0.002 g  $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ , 0.012 g  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ , 0.017 g  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ , and 1.25 mg resazurin were dissolved in 1 L distilled water and gassed with  $\text{CO}_2$  until pH reached 6.8.

Rumen fluid was collected from three Dorper  $\times$  Hu crossbred sheep fitted with permanent rumen fistulas. Before morning feeding, 150 mL of rumen fluid was collected from each animal, filtered through four layers of cheesecloth, pooled in a thermos flask, and immediately transported to the laboratory while being gassed with  $\text{CO}_2$ .

The ANKOM RFS software interface was opened, connecting the BASE modem and USB cable to the computer and linking the reference module. Fifty milliliters of microbial culture medium and 25 mL of rumen fluid were added to each culture bottle, with direct  $\text{CO}_2$  gassing for approximately 5 s before module installation. After setting response time to 1 s, the valve opening button was clicked to open the module release valve, and  $\text{CO}_2$  was introduced through the inlet for over 15 s. When module pressure reached 0 kPa, the release valve was closed (note: pressure must not exceed 69 kPa).

The GPM software was configured by setting the recording interval, pressure units, and automatic file save path to determine file location. Recording was initiated, and fermentation gas production pressure was automatically recorded and saved for 72 hours by the computer.

### 1.2.3 Nylon Bag Trial

The procedure followed Wang Jiaqi [14]: precisely 3 g of ground sample was carefully placed into nylon bags (8 cm  $\times$  12 cm, 50  $\mu\text{m}$  pore size), which were sealed and attached to one end of a flexible plastic rod; the other end was tied to the rumen fistula. Nylon bags were incubated in the rumen for 6, 12, 24, 36, 48, and 72 h, with four replicates per sample and time point. Retrieved bags were gently rinsed under running water by hand until the rinse water ran clear,

then dried at 65°C to constant weight. Residues from bags of the same sample and time point were pooled for analysis.

### 1.3 Parameter Simulation and Statistical Analysis

Conversion of gas production pressure to volume [at 39°C, converting pressure (kPa) to volume (mL)]:

$$Vx = Vj \times PkPa \times 0.1450377 \times 0.06800408$$

where  $Vx$  is gas volume at 39°C (mL),  $Vj$  is the headspace volume above the liquid in the module bottle (mL), and  $PkPa$  is the cumulative pressure recorded by GMS software (kPa).

Based on DM content of test feeds, gas production data were converted to gas volume per unit DM mass and fitted using the following nonlinear equation to derive gas production parameters:

$$GPt = \frac{A}{1 + (C/t)^B}$$

where  $GPt$  is gas production at time  $t$  (h) (mL/g),  $A$  is the theoretical maximum gas production (mL),  $B$  is the curve inflection point parameter, and  $C$  is the time to reach half of the theoretical maximum gas production (h).

The degradation rate of feed nutrients in the rumen at different time points was calculated as:

$$A = 100 \times \frac{B - C}{B}$$

where  $A$  is the rumen degradation rate of the test feed nutrient (%),  $B$  is the nutrient content in the original sample (%), and  $C$  is the nutrient content in the residue (%).

Degradation parameters and effective degradation rates of nutrients were calculated according to Ørskov et al. [15]:

$$Deg(t) = a + b \times (1 - e^{-ct})$$

$$P = a + \frac{b \times c}{c + k}$$

where  $Deg(t)$  is nutrient degradation rate at time  $t$  (h) (%),  $a$  is the rapidly degradable fraction (%),  $b$  is the slowly degradable fraction (%),  $t$  is rumen retention time (h),  $c$  is the degradation rate of the slowly degradable fraction

(%/h),  $P$  is the effective degradation rate of the nutrient (%), and  $k$  is the rumen passage rate (%/h), calculated as  $k = 0.0364 + 0.614X$ .

#### 1.4 Data Processing and Analysis

Data were organized using Excel 2010, and one-way ANOVA was performed using SPSS 19.0. Significance was declared at  $P < 0.05$ .

### Results

#### 2.1 Nutrient Composition of Ramie Leaf, Whole-Plant Ramie, and Defibred Whole-Plant Ramie

The nutrient composition of the three ramie materials is presented in . Whole-plant ramie had the highest DM content, followed by ramie leaf, which was similar to defibred whole-plant ramie. Ramie leaf contained greater CP, EE, ash, and Ca contents than both whole-plant and defibred whole-plant ramie, while its NDF and ADF contents were lower. Due to similar harvest heights, whole-plant ramie and defibred whole-plant ramie showed minor differences in nutrient composition.

#### 2.2 In Vitro Gas Production Results for Ramie Leaf, Whole-Plant Ramie, and Defibred Whole-Plant Ramie

The dynamic gas production profiles during *in vitro* fermentation are shown in [Figure 1: see original paper]. Gas production increased sharply for all three materials during 0-12 h. For defibred whole-plant ramie, the increase gradually slowed after 12 h, with the gap widening compared to the other two materials. Ramie leaf and whole-plant ramie showed gradually slowing gas production after 20 h, with the difference between them increasing at this point.

[Figure 1: see original paper]

Gas production volumes and parameters from *in vitro* fermentation are presented in . At all time points, whole-plant ramie showed the highest gas production, followed by ramie leaf, with defibred whole-plant ramie the lowest. No significant differences were observed at 2 h ( $P > 0.05$ ), but differences became significant thereafter ( $P < 0.05$ ). Theoretical maximum gas production followed the same pattern: highest for whole-plant ramie (167.51 mL/g), intermediate for ramie leaf (153.69 mL/g), and lowest for defibred whole-plant ramie (145.07 mL/g), with a significant difference between whole-plant and defibred whole-plant ramie ( $P < 0.05$ ). No significant differences were detected among the three materials for time to reach half of theoretical maximum gas production or curve inflection point parameters ( $P > 0.05$ ).

### 2.3 Nylon Bag Trial Results for Ramie Leaf, Whole-Plant Ramie, and Defibred Whole-Plant Ramie

As shown in , DM degradation rates of all three materials increased with rumen incubation time. Ramie leaf degraded rapidly within 24 h, then gradually slowed; whole-plant ramie degraded slowly within 12 h, rapidly during 12-24 h, then gradually leveled off after 24 h; defibred whole-plant ramie degraded slowly within 12 h, rapidly during 12-36 h, then gradually slowed after 36 h. Significant differences in DM degradation rates among the three materials were observed at all time points ( $P < 0.05$ ), reaching 83.61%, 74.17%, and 63.87% at 72 h, respectively. The slowly degradable DM fraction was largest for ramie leaf, followed by whole-plant ramie, then defibred whole-plant ramie, with significant differences between ramie leaf/whole-plant and defibred whole-plant ramie ( $P < 0.05$ ). Effective DM degradation rate showed the same trend: 49.05%, 47.16%, and 36.07%, respectively. The rapidly degradable DM fraction was significantly higher in ramie leaf and defibred whole-plant ramie than in whole-plant ramie ( $P < 0.05$ ). The degradation rate of the slowly degradable DM fraction was highest for whole-plant ramie, followed by ramie leaf, then defibred whole-plant ramie, with significant differences among all three ( $P < 0.05$ ).

As presented in , CP degradation rates increased with incubation time for all materials. Ramie leaf CP degraded slowly before 12 h, rapidly during 12-36 h, then gradually slowed after 36 h. Whole-plant ramie CP degraded rapidly within 24 h, then slowly thereafter. Defibred whole-plant ramie CP degraded slowly within 24 h, rapidly during 24-36 h, then slowly after 36 h. Significant differences in CP degradation rates among treatments were observed at 6-48 h ( $P < 0.05$ ), but not at 72 h ( $P > 0.05$ ), with values of 79.91%, 78.27%, and 74.03%, respectively. The rapidly degradable CP fraction was significantly higher in ramie leaf than in the other two treatments ( $P < 0.05$ ). Slowly degradable CP fractions were 69.15%, 77.23%, and 85.26% for ramie leaf, whole-plant ramie, and defibred whole-plant ramie, respectively, without significant differences ( $P > 0.05$ ). The degradation rate of the slowly degradable CP fraction was highest in whole-plant ramie, significantly greater than in ramie leaf and defibred whole-plant ramie ( $P < 0.05$ ). Effective CP degradation rate followed the order: whole-plant ramie > defibred whole-plant ramie > ramie leaf, with whole-plant ramie significantly higher than the other two ( $P < 0.05$ ).

As shown in , NDF degradation rates increased with incubation time. Ramie leaf NDF degradation increased steadily; whole-plant ramie NDF degraded rapidly within 24 h, then slowly; defibred whole-plant ramie NDF degraded slowly within 12 h, rapidly during 12-36 h, then slowly after 36 h. No significant differences were observed at 6 h ( $P > 0.05$ ), but significant differences appeared at 12-72 h ( $P < 0.05$ ). At 72 h, ramie leaf showed the highest NDF degradation rate (77.63%), slightly higher than whole-plant ramie (66.01%) and significantly higher than defibred whole-plant ramie (51.31%) ( $P < 0.05$ ). No significant differences were found among treatments for rapidly degradable NDF fraction

or degradation rate of slowly degradable NDF fraction ( $P > 0.05$ ). The slowly degradable NDF fraction was highest for ramie leaf, followed by whole-plant ramie, then defibered whole-plant ramie, with ramie leaf significantly higher than defibered whole-plant ramie ( $P < 0.05$ ). Effective NDF degradation rates were numerically highest for ramie leaf, followed by whole-plant ramie, then defibered whole-plant ramie, but differences were not significant ( $P > 0.05$ ).

As indicated in , ADF degradation rates increased with incubation time. Ramie leaf ADF degraded rapidly and steadily throughout 72 h; whole-plant ramie ADF degraded rapidly within 24 h, then slowly; defibered whole-plant ramie ADF degraded slowly within 12 h, rapidly during 12-36 h, then slowly after 36 h. No significant differences were observed at 6 h ( $P > 0.05$ ), but significant differences appeared at 12-72 h ( $P < 0.05$ ). At 72 h, ramie leaf had the highest ADF degradation rate (79.16%), followed by whole-plant ramie (69.14%), with defibered whole-plant ramie the lowest (53.23%). No significant differences were detected among treatments for rapidly degradable ADF fraction or degradation rate of slowly degradable ADF fraction ( $P > 0.05$ ). The slowly degradable ADF fraction was highest for ramie leaf (73.57%), intermediate for whole-plant ramie (67.23%), and lowest for defibered whole-plant ramie (51.59%), with significant differences between ramie leaf/whole-plant and defibered whole-plant ramie ( $P < 0.05$ ). Effective ADF degradation rates were 41.97%, 40.25%, and 28.75% for ramie leaf, whole-plant ramie, and defibered whole-plant ramie, respectively, with ramie leaf and whole-plant ramie significantly higher than defibered whole-plant ramie ( $P < 0.05$ ).

## Discussion

### 3.1 Gas Production of Ramie Leaf, Whole-Plant Ramie, and Defibered Whole-Plant Ramie

The *in vitro* gas production method is widely used domestically and internationally to evaluate forage feeding value, as gas volume provides a reliable simulation of organic matter digestion in the rumen [16]. Menke et al. established in 1979 that gas production after 24 h of *in vitro* incubation with artificial rumen fluid correlates positively with *in vivo* digestibility measurements. In this study, whole-plant ramie showed the highest 72-hour and theoretical maximum gas production, significantly greater than defibered whole-plant ramie, while ramie leaf was intermediate. Although ramie leaf had the highest nutritional value, its gas production was lower than that of whole-plant ramie. For defibered whole-plant ramie, the removal of fiber from stems (considered poorly digestible for ruminants) was expected to improve utilization compared to whole-plant ramie, yet gas production was significantly lower.

### 3.2 Degradation Rates of Ramie Leaf, Whole-Plant Ramie, and Defibered Whole-Plant Ramie

Rumen DM degradation rate is a major factor affecting dry matter intake (DMI) and correlates positively with DMI. Higher DM degradation rates favor performance expression. Different feed ingredients exhibit varying effective protein degradation rates in the rumen, depending primarily on fermentation difficulty and rumen retention time. Longer rumen residence time yields higher degradation rates, while shorter times produce lower rates. Forage proteins exist mainly as nitrogenous compounds in cell contents, with degradation speed determined by plant cell wall cellulose structure. Typically, as plants mature and age, lignin content increases, affecting protein release and breakdown. The 72-hour DM degradation rates were 83.61%, 74.17%, and 63.87% for ramie leaf, whole-plant ramie, and defibered whole-plant ramie, respectively. Ramie leaf and whole-plant ramie showed higher 72-hour DM degradation rates than most alfalfa, perennial ryegrass, oat, corn silage, corn stover, and *Leymus chinensis* (Chinese wildrye), while defibered whole-plant ramie was lower than alfalfa and perennial ryegrass but similar to oat, corn silage, and corn stover, and higher than *Leymus chinensis* [17-24]. Effective DM degradation rates were 49.05%, 47.16%, and 36.07%, respectively, with ramie leaf and whole-plant ramie higher than corn silage, corn stover, and *Leymus chinensis*, but lower than alfalfa, and similar to corn silage, corn hay, and *Leymus chinensis* [17-25].

The 72-hour CP degradation rates were similar among treatments, slightly higher for ramie leaf than whole-plant ramie, which was slightly higher than defibered whole-plant ramie. These values were comparable to alfalfa and higher than other forages. Effective CP degradation rates were highest for whole-plant ramie (48.30%), intermediate for defibered whole-plant ramie (38.50%), and lowest for ramie leaf (35.97%), all lower than alfalfa but similar to most perennial ryegrass, oat, corn silage, and *Leymus chinensis* [17-24]. However, the rapidly degradable fractions of DM and CP in all three ramie materials were far lower than those in other forages.

Rumen NDF and ADF degradation rates are important indicators of roughage nutritional value, reflecting feed digestion difficulty. Improving their rumen degradation rates can increase DMI and performance in ruminants. For ramie leaf, 72-hour NDF degradation rate and effective NDF degradation rate were 77.63% and 35.72%; for whole-plant ramie, 66.01% and 35.24%; and for defibered whole-plant ramie, 51.31% and 17.66%. The 72-hour ADF degradation rates and effective ADF degradation rates were 79.16% and 41.97% for ramie leaf, 69.14% and 40.25% for whole-plant ramie, and 53.24% and 28.75% for defibered whole-plant ramie. Ramie leaf and whole-plant ramie showed higher 72-hour ADF and NDF degradation rates and effective degradation rates than alfalfa, oat, *Leymus chinensis*, and corn silage, while defibered whole-plant ramie values were similar to alfalfa and *Leymus chinensis* [18-25].

Overall, except for rapidly degradable DM and CP fractions, nylon bag param-

eters for ramie leaf and whole-plant ramie exceeded those of most forages such as *Leymus chinensis* and perennial ryegrass, and were comparable to alfalfa. In contrast, defibered whole-plant ramie parameters were lower than ramie leaf and whole-plant ramie, similar to *Leymus chinensis*.

Based on both gas production and effective degradation rates, ramie leaf and whole-plant ramie demonstrated superior feeding value compared to defibered whole-plant ramie. In conclusion, ramie exhibits good digestibility and utilization in the rumen of ruminants and can be developed as a forage resource.

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