Nutritional Value and In Vitro Gas Production Characteristics of Arundo donax at Different Growth Stages: Postprint

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Abstract

This experiment aimed to investigate the nutritional value and in vitro gas production characteristics of Arundo donax at different growth stages. Conventional analysis methods were employed to determine the nutrient contents of whole-plant Arundo donax, stems, and leaves at growth stages of 75, 90, 105, 120, and 135 days. The in vitro gas production method was used to measure fermentation broth pH, dry matter disappearance rate (DMD), neutral detergent fiber disappearance rate (NDFD), acid detergent fiber disappearance rate (ADFD), gas production (GP), and gas production dynamic parameters following 72 h of in vitro fermentation. The results showed: 1) As growth stages progressed, crude protein content in whole-plant Arundo donax gradually decreased, while neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) contents exhibited a wave-like pattern of increase-decrease-increase, with an overall upward trend; nutrient contents in stems and leaves fluctuated substantially. 2) DMD, NDFD, and ADFD gradually decreased with advancing growth stages, with no significant difference between 75 d and 90 d ($P>0.05$), while both were significantly or extremely significantly higher than at 105 d and 120 d ($P<0.05$ or $P<0.01$). 3) Maximum GP displayed a decreasing trend as growth stages advanced, with no significant difference between 75 d and 90 d ($P>0.05$); both were significantly or extremely significantly lower than other growth stages ($P<0.05$ or $P<0.01$), with maximum GP at 105, 120, and 135 d being 40.22%, 50.98%, and 51.53% lower than at 90 d, respectively; gas production rate showed a similar trend. In conclusion, the optimal growth stage for Arundo donax as forage is 90 d.

Full Text

Nutrition Values and in Vitro Gas Production Characteristics of Arundo donax in Different Growth Periods

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Abstract

This study investigated the nutritional values and in vitro gas production characteristics of Arundo donax at different growth periods. Whole plant, stem, and leaf samples of A. donax at growth periods of 75, 90, 105, 120, and 135 days were analyzed for nutrient composition using conventional methods. In vitro fermentation parameters including fermentation fluid pH, dry matter disappearance rate (DMD), neutral detergent fiber disappearance rate (NDFD), acid detergent fiber disappearance rate (ADFD), gas production (GP), and GP dynamic parameters were measured after 72 h of fermentation. The results showed: (1) With advancing growth period, crude protein content in whole plant A. donax gradually decreased, while neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) contents exhibited wave-like fluctuations (increase-decrease-increase) but showed an overall upward trend; nutrient contents in stems and leaves varied considerably. (2) DMD, NDFD, and ADFD decreased progressively with growth period, with no significant differences between 75 and 90 days ($P>0.05$), but both were significantly or extremely significantly higher than at 105 and 120 days ($P<0.05$ or $P<0.01$). (3) Maximum GP showed a decreasing trend with advancing growth period, with no significant difference between 75 and 90 days ($P>0.05$), but both were significantly or extremely significantly lower than other growth periods ($P<0.05$ or $P<0.01$). Specifically, maximum GP at 105, 120, and 135 days was 40.22%, 50.98%, and 51.53% lower than at 90 days, respectively; GP rate showed a similar trend. In conclusion, the optimal growth period for harvesting A. donax as forage is 90 days.

Keywords: Arundo donax; different growth periods; nutritional value; in vitro gas production method

Introduction

In recent years, with continuous economic development, animal husbandry has become increasingly important in the national economy. Given China' s large livestock production base, the country requires approximately 10 million tons

of hay annually, but domestic production capacity is only 2 million tons. Consequently, China must import at least 5 million tons of hay from international markets each year, with Japan and Korea alone importing over 200,000 tons annually [1]. This situation underscores the urgent need for forage development in China. Currently, with a severe shortage of high-protein forage products in China, exploring and utilizing high-yield, high-protein gramineous forages represents a timely and effective solution [2].

Arundo donax, a tall perennial gramineous plant in the genus Arundo, features erect stems and large, flat leaves, reaching heights of 2–6 m. It is widely distributed in China, particularly in the southern Jiangsu and Zhejiang regions. A. donax exhibits strong reproductive capacity and rapid growth, with 2-month-old plants reaching 1.8 m in height, leaves growing up to 20 cm long and 10 cm wide, and individual fresh weights reaching 490 g. Mature plants can yield 40 t/ha annually, increasing to 60–80 t/ha or even higher under irrigation and fertilization conditions. Historically in China, tender A. donax leaves have been used as forage for livestock. However, as gramineous plants mature, their lignin content increases substantially, with A. donax cellulose content rising rapidly to 30% and lignin content reaching 13%–20% after heading [3]. Increased lignin content inevitably affects feed palatability and nutrient absorption [4].

Due to its excellent stress resistance, high yield, high calorific value, and extremely high fiber content in later growth stages, A. donax is currently developed primarily as an energy crop for biogas production. Minimal research exists on lignin and other nutrient content changes during early growth periods, and no studies have reported on its rumen fermentation performance. This lack of knowledge leads to uncertainties regarding optimal harvest timing and appropriate feeding rates when using A. donax as forage. Therefore, investigating nutrient content variation patterns during early growth periods is necessary to provide a basis for forage resource development. If this high-yield energy crop can be developed as forage, it could help alleviate forage shortages in China, particularly in southern regions.

Materials and Methods

1.1 Sample Collection and Processing

Five-year-old A. donax plants receiving normal water and fertilizer supply were selected from the Miscanthus germplasm resource nursery at Hunan Agricultural University. Samples were collected at 15-day intervals from the end of the jointing stage to before heading: late May 2016 (75 days), early June (90 days), late June (105 days), early July (120 days), and late July (135 days). Plants were cut at 2 cm stubble height, and a small number of yellowed leaves near the ground were removed. Stems and leaves were separated, weighed, and immediately placed in a drying oven at (120±1)°C for 20 min to deactivate enzymes, then dried at 65°C, ground to pass through a 40-mesh sieve, and stored at room

temperature. Samples were taken using the quartering method for subsequent analysis.

1.2 In Vitro Gas Production Experiment

The experiment was conducted at the Institute of Subtropical Agriculture, Chinese Academy of Sciences. Three healthy Liuyang black goats with permanent rumen fistulas and similar body weights were used as rumen fluid donors, fed at 1.5 times maintenance requirements. Feed was reduced to half the normal amount the afternoon before sampling, with free access to water. Rumen fluid was collected from the three fistulated goats the next morning while fasting, mixed, transported in a thermos flask, and immediately filtered through four layers of cheesecloth in the laboratory.

Artificial saliva was prepared following Menke et al. [5]. Sodium bicarbonate (19.920 g), ammonium bicarbonate (2.280 g), disodium hydrogen phosphate dodecahydrate (8.168 g), potassium dihydrogen phosphate (3.516 g), magnesium sulfate heptahydrate (0.340 g), resazurin (0.792 mL), and trace element solution (0.288 mL) were dissolved in 2.4 L distilled water in a glass bottle. The solution was stirred with a magnetic stirrer at 39.5°C while continuously infusing CO . After 2 h of stirring, 5 mL of reducing agent was added, followed by 600 mL of filtered rumen fluid. After several minutes of stirring, 30 mL of the mixture was accurately dispensed into 100 mL syringes containing 200 mg of sample. Syringe needle ends were sealed with Vaseline. Each sample had three replicates (one syringe per replicate), with three blank syringes containing only fermentation fluid serving as controls to reduce experimental error. Syringes were incubated in a thermostatic shaking water bath at (39.2±0.1)°C.

1.3 Analytical Methods

1.3.1 Conventional Nutrient Content Analysis Conventional nutrient contents were analyzed according to *Feed Analysis and Detection* edited by He Jianhua [6]. Dry matter (DM) content was determined by direct drying; crude protein (CP) by the Kjeldahl method; crude ash by muffle furnace incineration; and neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) by the Van Soest fiber analysis method.

Neutral detergent solubles (NDS) were calculated as: NDS = 1 - ADF.

1.3.2 In Vitro Fermentation Indices Gas production was measured at 10 time points: 3, 6, 9, 12, 18, 24, 36, 48, 56, and 72 h of in vitro fermentation.

After 72 h of fermentation, syringes were immediately transferred to ice water to stop fermentation. Fermentation fluid pH was measured using a pH meter. Fermentation residues were collected by filtering the fluid through cheesecloth and rinsing the syringes with distilled water to determine DMD. ADFD and NDFD were calculated using the following formula:

Disappearance rate (%) = 100 - 100 × (post-fermentation content × DMD) / pre-fermentation content.

1.3.3 In Vitro Gas Production Indices GP was calculated as: GP at a given time (mL) = gas volume in sample syringe - gas volume in corresponding blank syringe.

Dynamic GP parameters were calculated using the model: y = B[1 - e^(-c(t-lag))].

Where: y = GP (mL) from 200 mg substrate at time t; B = maximum GP (mL) from 200 mg substrate; c = GP rate (mL/h); lag = GP lag phase (h).

1.4 Statistical Analysis

Data were analyzed using SPSS 13.0 software. One-way ANOVA was performed, and Duncan' s multiple comparison test was used. Data were expressed as mean ± standard deviation, with $P<0.05$ considered statistically significant.

Results

2.1 Nutrient Content of Arundo donax at Different Growth Periods

As shown in Table 1 , CP content in whole plant A. donax fluctuated significantly ($P<0.05$) with advancing growth period, being highest at 75 days and lowest at 120 days. A sharp CP decline occurred between 90 and 105 days, decreasing from 8.92% to 4.38% (a 50.90% reduction), with a slight recovery at 135 days. No significant differences in ether extract (EE) content were observed across growth periods ($P>0.05$). Overall, NDF, ADF, and ADL contents in whole plant A. donax increased with growth period, though a temporary decrease occurred at 120 days, followed by a rapid increase at 135 days. Notably, ADL content rose sharply to 8.29% at 135 days (a 50.73% increase), which would inevitably affect palatability.

Table 1 shows that A. donax leaves represent a relatively high-quality protein forage resource, with CP content exceeding 13.05% before 90 days and remaining at 6.73% even at its lowest point, recovering to 12.71% after 135 days. No significant differences were found among 75, 90, and 135 days ($P>0.05$), while other growth periods differed significantly ($P<0.05$). Although NDF, ADF, and ADL contents in leaves fluctuated considerably across growth periods, their absolute values remained relatively low.

Table 3 reveals that A. donax stems had low CP content, with a maximum of only 2.37%, while NDF, ADF, ADL, and NDS contents were relatively high.

Collectively, Tables 1, 2 , and 3 demonstrate that with advancing growth period, CP and EE contents in A. donax decreased while ADL, NDF, and ADF

contents increased. This pattern primarily reflects rapid lignification of stems with maturity.

2.2 In Vitro Fermentation Indices of Arundo donax at Different Growth Periods

As shown in Table 4 , fermentation fluid pH increased with growth period, being significantly higher at 105 days than at 75 and 90 days ($P<0.05$), and significantly higher at 120 and 135 days than at all other periods ($P<0.05$). DMD was highest at 75 days (71.12%), decreasing to 64.44% at 105 days. The decline from 105 to 120 days was relatively gradual and not significant ($P>0.05$), while DMD increased significantly from 120 to 135 days ($P<0.05$). Both ADFD and NDFD decreased with advancing growth period.

2.3 In Vitro Gas Production Indices of Arundo donax at Different Growth Periods

Figure 1 [Figure 1: see original paper] illustrates that in vitro GP varied among A. donax samples from different growth periods. GP increased rapidly within 18 h of fermentation, indicating high fermentation rate, then plateaued in later stages. GP from 105-day A. donax stabilized after 48 h, while GP from 75- and 90-day samples continued changing substantially between 24–72 h, showing another growth phase after 48 h and stabilizing after 56 h.

Table 5 shows substantial differences in maximum GP among growth periods. Maximum GP decreased progressively with growth period, with no significant difference between 75 and 90 days ($P>0.05$), but both were significantly lower than at 105 days ($P<0.05$) and extremely significantly lower than at 120 and 135 days ($P<0.01$). GP rate showed a similar trend.

Discussion

3.1 Nutrient Content of Arundo donax at Different Growth Periods

Forage nutrient content is influenced by species, growth period, environmental conditions, and genetic factors. Pei et al. [7] reported that CP and ash contents in forages gradually decrease while NDF and ADF contents increase with advancing growth period. Zhang et al. [8] evaluated the nutritional value of alfalfa, sweet clover, and vetch at different growth periods using in vitro gas production, finding that CP content decreased significantly while ADF and NDF contents increased with maturity. Huang [9] reported similar results. These findings consistently demonstrate that plant CP content declines while ADF and NDF contents increase with growth period.

All samples in this study were collected from the same planting site, so variations in nutrient content were primarily attributable to growth period. Our

results showed that whole plant CP content decreased rapidly with advancing growth period, consistent with previous research. This occurs because during the vegetative growth stage of gramineous plants, new leaf area expansion and enhanced photosynthesis promote rapid protein accumulation, while protein deposition slows in later growth stages as other substances accumulate, resulting in relatively lower CP content [10].

The temporary decrease in ADF, NDF, and ADL contents at 120 days may be attributed to A. donax being a C4 plant, where sudden increases in light intensity in early July stimulated temporary stem and leaf growth. However, the overall trend showed continuous increases in ADF, NDF, and ADL contents due to progressive lignification of gramineous plants with maturity, consistent with Zhang et al.' s [8] findings on six gramineous species. Nutrient contents in stems and leaves fluctuated considerably, particularly ash content, which generally decreased with growth period. This may be because mineral elements (the main ash components) can be mobilized within plants, typically translocating from old leaves to new leaves and from old tissues to new tissues, as reported by Yang et al. [11]. Thus, A. donax can deplete soil fertility, and our results showed relatively high ash content. However, we did not analyze trace mineral elements in the ash, so specific mineral composition could not be determined.

CP content is a primary indicator for evaluating forage nutritional value, with higher CP indicating higher nutritional value. Our study found A. donax CP content was relatively high before 70 days, then declined, consistent with Chen et al. [12]. While alfalfa, a common high-quality forage in China, contains 17.3%–21.2% CP, A. donax CP content of 8%–9% is comparable to corn silage (7%–10%) [13]. A. donax leaves, with CP content up to 15%, can serve as a high-quality protein forage resource. Fiber content is another critical indicator of forage quality, as NDF affects palatability and intake—higher NDF reduces palatability and intake. In this study, whole plant NDF content at 90 days was 65.38%, similar to flowering-stage Leymus chinensis (64.79%) and Avena fatua (65.35%) [14]. However, at 135 days, NDF content rose to 71.19% and ADL content reached 8.29%. Therefore, A. donax harvested before 90 days has comparable nutrient content to commonly used forages, with the added advantage of high yield (individual plant weight up to 400 g at this stage), demonstrating forage development potential. After 105 days, elevated NDF, ADF, and particularly ADL contents may compromise intake and digestibility, making it less suitable as forage.

3.2 In Vitro Fermentation Indices of Arundo donax at Different Growth Periods

DMD is an important indicator for evaluating ruminant feed nutritional value. Studies show that DMD in gramineous forages decreases with advancing growth period [15]. NDF and ADF are important energy sources for ruminants, fermented by rumen microorganisms to produce volatile fatty acids (VFA), methane, and ATP [16]. Therefore, NDFD and ADFD are primary references

for fiber digestibility in ruminants. In this study, DMD decreased from 71.12% at 75 days to 62.13% at 120 days, with significant differences, while NDFD and ADFD showed similar trends. This may be because increasing NDF, ADF, and ADL contents with growth period strengthened plant cell walls, making them less accessible to rumen microbial fermentation. pH is an important indicator of rumen metabolism, reflecting the production, absorption, and neutralization of organic acids [17]. Normal rumen pH ranges from 6–7; consistent pH below 5.5 can cause acidosis [18]. In this study, fermentation fluid pH ranged from 6.37–6.65 across all growth periods, indicating no risk of acidosis.

3.3 In Vitro Gas Production Indices of Arundo donax at Different Growth Periods

In vitro gas production is an efficient method for evaluating forage nutritional value by simulating rumen fermentation and measuring produced gases [19]. Rumen gas primarily consists of VFA, methane, and hydrogen produced from microbial fermentation of soluble carbohydrates and other nutrients [20]. Studies indicate that GP correlates positively with VFA production but negatively with microbial biomass [1], suggesting that A. donax before 90 days may produce higher VFA yields during rumen fermentation. Guo et al. [21] and Li et al. [22] evaluated the nutritional value of concentrates, cash crops, and bagasse using in vitro gas production, obtaining objective results. Our study showed that in vitro GP decreased with advancing growth period, with maximum GP declining from 34.64 mL at 75 days to 15.63 mL at 135 days (a 54.88% reduction). This correlates with decreasing CP and EE contents in gramineous plants with maturity. GP was positively correlated with DMD, consistent with Khazaal et al. [23] and Tuah et al. [24], possibly because fermentation of proteins and other nutrients in forages provides higher buffering capacity and produces less gas.

GP rate and lag phase are important parameters of rumen gas production dynamics, directly reflecting dynamic digestion in the rumen [25]. This study found substantial differences in GP rate and lag phase among growth periods, with higher rates in early growth stages and slower rates in later stages. Generally, GP from 75- and 90-day A. donax stabilized around 72 h, while GP from 105–135-day samples stabilized after 48 h, likely due to varying soluble and insoluble fractions at different growth stages, consistent with Tang et al. [26].

Conclusion

Based on the comprehensive evaluation of nutrient content and in vitro fermentation characteristics, the following conclusions can be drawn:

1. From post-jointing to pre-heading stages, nutrient contents in A. donax stems and leaves varied considerably, primarily due to rapid stem lignification with advancing growth period.

2. A. donax leaves have high CP content and low fiber content, making them a high-quality feed resource.
3. Considering CP content, GP, and other indices, A. donax should be harvested before 90 days of growth for optimal forage utilization.

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