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 composition of whole-plant Arundo donax, Arundo donax stems, and Arundo donax leaves at growth stages of 75, 90, 105, 120, and 135 days; the in vitro gas production method was used to determine the 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 after 72 h of in vitro fermentation of Arundo donax. The results showed that: 1) With the progression of growth stages, the crude protein content of whole-plant Arundo donax gradually decreased, while the contents of neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) exhibited wave-like fluctuations of increase-decrease-increase, but showed an overall upward trend; the nutrient composition in stems and leaves fluctuated considerably. 2) With the progression of growth stages, DMD, NDFD, and ADFD gradually decreased, with no significant difference between 75 d and 90 d ($P>0.05$), and these two were significantly or extremely significantly higher than 105 d and 120 d ($P<0.05$ or $P<0.01$). 3) With the progression of growth stages, the maximum GP showed a decreasing trend, with no significant difference between 75 d and 90 d ($P>0.05$), and both were significantly or extremely significantly higher than other growth stages ($P<0.05$ or $P<0.01$), with the maximum GP at 105, 120, and 135 d being 40.22%, 50.98%, and 51.53% lower than that at 90 d, respectively; the 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 plants, stems, and leaves 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 (up-down-up) but showed an overall increasing trend; nutrient contents in stems and leaves varied substantially. (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 decreased with growth period, with no significant difference between 75 and 90 days (P>0.05), but both were significantly or extremely significantly lower than other 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 similar trends. In conclusion, the optimal growth period for *A. donax* as forage is 90 days.

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

Introduction

With continuous economic development, livestock husbandry has become increasingly important in China' s national economy. Given the country' s massive livestock production base, annual demand for hay reaches approximately 10 million tons, while domestic production capacity is only 2 million tons, necessitating imports of at least 5 million tons annually. Japan and Korea alone import over 200,000 tons each year. This substantial deficit underscores the urgent need for forage development in China. Under current conditions of severe shortage in high-protein forage products, exploring and utilizing high-yield, high-protein gramineous forages represents a timely and effective solution.

Arundo donax, a tall perennial gramineous plant in the genus *Arundo*, features erect stems and large, flat leaves, reaching heights of 2-6 m. Widely distributed across China, particularly in southern Jiangsu and Zhejiang provinces, *A. donax* exhibits strong reproductive capacity and rapid growth. At two months of age, plants can reach 1.8 m in height with leaves up to 20 cm long and 10 cm wide, and individual fresh weights of 490 g. Mature stands yield 40 t/ha annually, increasing to 60-80 t/ha or higher under irrigation and fertilization. Historically in China, *A. donax* tender leaves have been used as livestock forage. However, lignin content in gramineous plants increases with maturity, and *A. donax* cellulose content rapidly rises to 30% after heading, with lignin reaching 13-20%. Elevated lignin adversely affects palatability and nutrient absorption. Due to its stress tolerance, high yield, high calorific value, and extremely high fiber content in later growth stages (making it suitable for biogas production), *A. donax* is currently developed primarily as an energy crop. 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 knowledge gap leads to unclear harvesting schedules and uncertain feeding rates when used as forage. Therefore, investigating nutrient content variation patterns during early growth periods is necessary to support forage resource development. If this high-yield energy crop can be developed as forage, it could alleviate forage shortages, particularly in southern China.

Materials and Methods

Sample Collection and Processing

Five-year-old *A. donax* receiving normal irrigation and fertilization was selected from the *Miscanthus* germplasm resource nursery at Hunan Agricultural University. Samples were collected at 15-day intervals from post-jointing to pre-heading stages: 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 yellowed lower leaves were removed. Stems and leaves were separated, weighed, immediately oven-dried at 120$±$1°C for 20 min for enzyme deactivation, then dried at 65°C, ground through a 40-mesh sieve, and stored at room temperature. Samples were taken using the quartering method for analysis.

In Vitro Gas Production Procedure

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 served as rumen fluid donors, fed at 1.5 times maintenance level. 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 following morning before feeding, mixed, transported in insulated bottles, filtered through four layers of cheesecloth, and held for use.

Artificial saliva was prepared according to 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_2. After 2 h, 5 mL of reducing agent was added, followed by 600 mL of filtered rumen fluid. After several minutes of stirring, 30 mL aliquots were precisely dispensed into 100 mL syringes containing 200 mg of sample. Syringe needle ends were sealed with Vaseline. Each sample had three replicates (one syringe each), with three blank syringes containing only fermentation fluid serving as controls to reduce experimental error. Syringes were incubated in a constant temperature shaking water bath at 39.2$±$0.1°C.

Analytical Methods

Conventional Nutrient Analysis Nutrient contents were analyzed according to *Feed Analysis and Detection* edited by He Jianhua [6]. Dry matter (DM) was determined by direct drying; crude protein (CP) by Kjeldahl method; crude ash by muffle furnace incineration; neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) by Van Soest fiber analysis. Neutral detergent solubles (NDS) were calculated as: NDS = 1 - ADF.

In Vitro Fermentation Indices Gas production was measured at 10 time points: 3, 6, 9, 12, 18, 24, 36, 48, 56, and 72 h. After 72 h of fermentation, syringes were immediately transferred to ice water to stop fermentation. Fermentation fluid pH was measured using a pH meter. Fluid was filtered through cheesecloth, and syringes were rinsed with distilled water to collect residues for DMD determination. ADFD and NDFD were calculated as:

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

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:

$$y = B[1 - e^{-c(t-\text{lag})}]$$

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

Statistical Analysis

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

Results

Nutrient Composition at Different Growth Periods

As shown in Table 1 , CP content in whole-plant *A. donax* varied significantly ($P<0.05$) with growth period, being highest at 75 days and lowest at 120 days. CP content dropped sharply between 90 and 105 days, decreasing from 8.92% to 4.38% (a 50.90% reduction), then increased slightly at 135 days. Ether extract (EE) content showed no significant differences among growth periods ($P>0.05$). Overall, NDF, ADF, and ADL contents increased with growth period, though a temporary decrease occurred at 120 days before rapid elevation at 135 days. ADL content increased sharply to 8.29% at 135 days (a 50.73% increase), which would adversely affect palatability.

Table 2 shows that *A. donax* leaves represent a relatively high-quality, high-protein forage resource, with CP content exceeding 13.05% before 90 days and remaining at 6.73% even at its lowest, rebounding to 12.71% after 135 days. No significant differences were observed among 75, 90, and 135 days ($P>0.05$), though other periods differed significantly ($P<0.05$). While NDF, ADF, and ADL contents in leaves fluctuated considerably, absolute values remained relatively low.

Table 3 reveals that stem CP content was comparatively low, peaking at only 2.37%, while NDF, ADF, ADL, and NDS contents were relatively high. Combined analysis of Tables 1–3 indicates that CP and EE contents decreased while ADL, NDF, and ADF contents increased with advancing growth period, primarily due to low stem CP content and rapid stem lignification.

In Vitro Fermentation Indices at Different Growth Periods

Table 4 shows that 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 peaked at 71.12% at 75 days, decreasing to 64.44% at 105 days; the decline was less pronounced between 105 and 120 days ($P>0.05$), but DMD increased significantly at 135 days compared to 120 days ($P<0.05$). Both ADFD and NDFD decreased with advancing growth period.

In Vitro Gas Production Indices at Different Growth Periods

Figure 1 [Figure 1: see original paper] demonstrates that GP varied among growth periods. GP increased rapidly within 18 h of fermentation, indicating high fermentation rates, before stabilizing. GP from *A. donax* at 105 days stabilized after 48 h, while GP from 75- and 90-day plants continued changing substantially between 24–72 h, with a second growth phase before 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 similar trends.

Discussion

Nutrient Composition at Different Growth Periods

Forage nutrient composition is influenced by variety, growth period, environment, and genetics. Pei et al. [7] reported that CP and ash contents decrease while NDF and ADF contents increase with advancing growth period. Zhang et al. [8] evaluated nutritional values 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 growth period. Huang [9] reported similar results. These findings align with our observation that CP content in whole-plant *A. donax* decreased rapidly with growth period while ADF, NDF, and ADL contents increased overall, consistent with progressive lignification in gramineous plants. The temporary decrease in ADF, NDF, and ADL at 120 days may be attributed to *A. donax* being a C4 plant experiencing sudden increased light intensity in early July, stimulating temporary stem and leaf growth.

Substantial fluctuations in stem and leaf nutrient contents were observed, particularly for ash, which generally decreased with growth period. This may be explained by the mobilization of mineral elements from older to newer tissues, consistent with Yang et al. [11]. While *A. donax* can deplete soil fertility based on its high ash content, this study did not analyze trace mineral composition.

CP content is a primary indicator of forage nutritional value. Our results show *A. donax* CP content was relatively high before 70 days, then declined, consistent with Chen et al. [12]. While *A. donax* CP content (8–9%) is lower than alfalfa (17.3–21.2%), it is comparable to corn silage (7–10%). *A. donax* leaves, with CP up to 15%, can serve as a high-quality protein forage. Fiber content also critically affects forage value; high NDF reduces palatability and intake. At 90 days, whole-plant NDF content (65.38%) was comparable to flowering-stage *Leymus chinensis* (64.79%) and wild oat (65.35%) [14]. However, at 135 days, NDF increased to 71.19% with ADL reaching 8.29%. Therefore, *A. donax* harvested before 90 days offers nutritional value comparable to common forages, with the added advantage of high yield (individual fresh weight reaching 400 g), making it a valuable forage resource. After 105 days, elevated NDF, ADF, and particularly ADL contents may compromise intake and digestibility, making it less suitable as forage.

In Vitro Fermentation Indices at Different Growth Periods

DMD is crucial for evaluating ruminant feed nutritional value. Studies show DMD in gramineous forages decreases with growth period [15]. NDF and ADF are important energy sources for ruminants, fermented by rumen microbes to produce volatile fatty acids (VFA), methane, and ATP [16]; thus, NDFD and ADFD indicate fiber digestibility. In this study, DMD decreased significantly from 71.12% at 75 days to 62.13% at 120 days, with NDFD and ADFD showing similar trends, likely due to increased NDF, ADF, and ADL contents hindering cell wall degradation by rumen microbes. Fermentation fluid pH reflects organic acid production, absorption, and neutralization [17]. Normal rumen pH ranges from 6–7; consistent pH below 5.5 causes acidosis [18]. All fermentation pH values in this study (6.37–6.65) remained within safe limits.

In Vitro Gas Production Indices at Different Growth Periods

In vitro gas production simulates rumen fermentation to evaluate forage nutritional value efficiently [19]. Rumen gas consists primarily of VFA, methane, and hydrogen from microbial fermentation of soluble carbohydrates and other nutrients [20]. Studies indicate GP correlates positively with VFA production but negatively with microbial biomass [1], suggesting that pre-90-day *A. donax* may produce higher VFA yields. Guo et al. [21] and Li et al. [22] successfully evaluated concentrates, cash crops, and bagasse using in vitro gas production. Our results show GP decreased with growth period, with maximum GP declining from 34.64 mL at 75 days to 15.63 mL at 135 days (a 54.88% reduction), correlating with decreased CP and EE contents. The positive correlation between GP and DMD aligns with Khazaal et al. [23] and Tuah et al. [24], possibly because protein fermentation yields higher buffering capacity and less gas.

GP rate and lag phase are important dynamic parameters reflecting rumen digestion kinetics [25]. This study found substantial differences among growth periods, with higher rates in early growth and slower rates later. GP from 75- and 90-day plants stabilized around 72 h, while 90–135-day plants stabilized after 48 h, likely reflecting differences in soluble and insoluble fractions among growth stages, consistent with Tang et al. [26].

Conclusion

Based on the comprehensive evaluation of nutrient composition and in vitro fermentation characteristics, *A. donax* shows significant potential as a forage resource when harvested at the appropriate growth stage. First, substantial differences in stem and leaf nutrient contents occur from post-jointing to pre-heading stages, primarily due to rapid stem lignification with advancing growth period. Second, *A. donax* leaves exhibit high CP content and low fiber content, making them suitable as a high-quality feed ingredient. Third, considering CP content, gas production, and other nutritional indices, the optimal harvest time for *A. donax* as forage is before 90 days of growth.

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