Research article

Effect of regrowth age and weather on the nutritional quality of *Neonotonia wightii* cv Longicauda in Pastaza, Ecuador

Efecto de la edad de rebrote y clima en la calidad nutritiva de *Neonotonia wightii* cv Longicauda en Pataza, Ecuador

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ABSTRACT
Neonotonia wightii cv. Longicauda is a tropical legume that supports sustainable ruminant production in humid ecosystems. The study evaluated regrowth age (30, 60, and 90 days) and seasonal rainfall patterns affecting its nutritional quality in Pastaza, Ecuador. Plots were harvested during the rainy (December–February) and low-rainfall periods (July–September) using a randomized complete block design with six replicates. Crude protein declined sharply with maturity, decreasing from 25.0 to 14.5% in the rainy period and from 23.5 to 16.2% in the dry period between 30 and 90 days. This reduction reflected protein dilution as structural carbohydrates accumulated during progressive lignification. Fiber fractions (NDF, ADF, lignin) increased correspondingly, reducing *in vitro* dry matter digestibility from 75.0 to 59.7% in the rainy period and from 70.6 to 65.3% in the dry period. Soluble sugars (glucose, fructose, sucrose) decreased by 70–85% with advancing maturity. Secondary metabolites, particularly total tannins and phenols, accumulated markedly under low-rainfall conditions and at later growth stages, likely as a plant defense response to water stress. Metabolizable energy followed the same declining pattern with maturity but remained consistently higher during the rainy period. Remarkably, fermentation kinetics parameters (asymptotic gas volume, rate, lag time) remained stable across all treatments, indicating consistent ruminal fermentability despite compositional changes. The results showed that harvest timing determines forage quality, younger regrowth better preserves protein content and digestibility, especially during drought conditions. Integrating seasonal rainfall patterns into cutting management optimizes nutritional value and strengthens resilient livestock production in tropical environments.

KEYWORDS: Digestibility, Fermentation kinetics, Secondary metabolites, Regrowth age, Seasonality, Forage quality

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RESUMEN
Neonotonia wightii cv. Longicauda es una leguminosa tropical que favorece la producción sostenible de rumiantes en ecosistemas húmedos. El estudio analizó el efecto de la edad de rebrote (30, 60 y 90 días) y los patrones de lluvia estacional sobre su calidad nutricional en Pastaza, Ecuador. Se cosecharon parcelas en el período lluvioso (diciembre a febrero) y en el de poco lluvioso (julio a septiembre) mediante un diseño

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de bloques completos al azar con seis repeticiones. La proteína cruda disminuyó con la madurez desde 25,0 hasta 14,5% en lluvias y desde 23,5 hasta 16,2% en poca lluvia entre los 30 y 90 días. Esta caída reflejó dilución proteica por acumulación de carbohidratos estructurales durante la lignificación progresiva. Las fracciones de fibra (NDF, ADF, lignina) aumentaron y redujeron la digestibilidad *in vitro* de la materia seca de 75,0 a 59,7% en lluvias y de 70,6 a 65,3% en poca lluvia. Los azúcares solubles (glucosa, fructosa, sacarosa) bajaron entre 70 y 85% al avanzar la madurez. Los metabolitos secundarios, especialmente taninos totales y fenoles se acumularon en condiciones de poca lluvia y en etapas avanzadas de crecimiento como respuesta de defensa al estrés hídrico. La energía metabolizable descendió con la madurez, pero se mantuvo más alta en la temporada lluviosa. Los parámetros de cinética de fermentación (volumen de gas asintótico, tasa, tiempo de retardo) permanecieron estables en todos los tratamientos, lo que indica fermentabilidad ruminal constante pese a cambios composicionales. Los resultados mostraron que el momento de cosecha define la calidad del forraje; los rebrotes jóvenes conservan mejor la proteína y la digestibilidad, sobre todo en sequía. Integrar los patrones de lluvia estacional en el manejo del corte optimiza el valor nutricional y fortalece la producción ganadera resiliente en ambientes tropicales.

PALABRAS CLAVE: Digestibilidad, Cinética de fermentación, Metabolitos secundarios, Edad de rebrote, Estacionalidad, Calidad del forraje

INTRODUCTION

Perennial soybean (*Neonotonia wightii* cv. Longicauda) is a tropical legume widely recognized for its high nutritional value and adaptability to humid environments. This species provides elevated crude protein concentrations and excellent palatability, which directly enhances ruminant productivity and health (Acosta et al. 2023; Yegrem et al. 2022). Nevertheless, its forage quality depends critically on regrowth age and seasonal rainfall patterns, which influence chemical composition, fiber fractions, digestibility, and secondary metabolite profiles (Castro-Montoya and Dickhoefer 2020). These dynamics become especially relevant in tropical regions where pronounced precipitation variability defines distinct agroecological periods (Lista et al. 2019).

Although *N. wightii* cv. Longicauda holds agronomic importance in the Ecuadorian Amazon, scientific evidence about how regrowth age and pluviometric periods affect its nutritional attributes under humid tropical conditions remains scarce. Unlike temperate zones, the Amazon basin lacks thermal periods; instead, a rainy period (December–February) and a low-rainfall period (July–September) drive plant physiological responses (Melo et al. 2022). Consequently, two research questions emerge: (1) How does regrowth age (30, 60, and 90 days) influence the chemical composition, digestibility, and secondary metabolite content of *N. wightii* cv. Longicauda? (2) To what extent do pluviometric periods modulate these nutritional responses under Amazonian humid tropical conditions? Addressing these questions will support the development of site-specific management strategies for Pastaza and similar regions.

Accordingly, this study evaluates how regrowth age and pluviometric period influence the nutritional quality of *N. wightii* cv. Longicauda in Pastaza, Ecuador. The aim of this study was to establish optimal harvest intervals that preserve forage value across periods. Those findings will support evidence-based recommendations for sustainable livestock systems in humid tropical environments, where climate-resilient forage management remains a priority (Li et al. 2023; Tulu et al. 2023; Reina-García et al. 2024).

MATERIALS AND METHODS

Study area and edaphoclimatic conditions

The study took place at the Amazonian Research and Production Experimental Center (CEIPA) of the Amazon State University, located in zone 18 of Puyo, Pastaza, Ecuador (UTM coordinates: 178,879 m East, 9,863,155 m South). The area experiences a tropical climate, characterized by an average annual rainfall of approximately 4,000 mm, an average relative humidity of 80%, and temperatures ranging between 15 and 25 °C. The terrain is gently rolling, with elevations ranging from 590 to 957 meters above sea level (masl).

The soil was classified as Typic Paleaquults (Soil Survey Staff 2022) with pH=5.9, 3.0 mg 100 g^{-1} P_2O_5, 35.0 mg 100 g^{-1} K_2O, 3.0 mg 100 g^{-1} total nitrogen, and 10% organic matter.

The study examined two distinct pluviometric periods that characterize seasonal variation in the Ecuadorian Amazon: the rainy period (December–February; 570.7 mm of accumulated precipitation) and the low-rainfall period (July–September; 400.2 mm). Mean temperatures during these periods were 16.37 and 15.96 °C, respectively, with relative humidity averaging 83.7 and 77.3%. Intermediate months (March–June, October–November) were not included in the evaluation because they represent transitional phases with unstable precipitation patterns that do not define clear physiological responses in forage species. Detailed climatic parameters for each period appear in Supplementary Table 1.

Table 1. Climatic parameters during the rainy and low-rainfall periods at the experimental site (Pastaza, Ecuador).

Parameter	Rainy period (Dec–Feb)	Low-rainfall period (Jul–Sep)
Accumulated precipitation (mm)	570.7	400.2
Mean temperature (°C)	16.37	15.96
Minimum temperature (°C)	13.00	12.33
Maximum temperature (°C)	20.97	20.30
Mean relative humidity (%)	83.7	77.3
Relative humidity range (%)	60.84 to 93.17	50.96 to 80.17

Experimental design and procedure

A randomized complete block design structure experiment with six blocks to control minor topographic and soil heterogeneity across the study area**.** The experimental unit is considered 25 m^2. Before initiating each monitoring phase (December for the rainy period and July for the low-rainfall period), The entire stand of *Neonotonia wightii* cv. Longicauda was uniformly defoliated to 5 cm above ground level. Three regrowth age treatments (30, 60, and 90 days) were randomly assigned in each block, resulting in 18 experimental units for periods (6 blocks × 3 ages). The stand had been established 18 months prior to the study using a row spacing of 0.5 m and intra-row distance of 0.3 m. Throughout the duration of the trial, neither supplemental irrigation nor fertilization was provided to ensure natural growth conditions. Importantly, period represented an observational factor defined by natural rainfall patterns, where climatic conditions were not manipulated.

Sample collection

At harvest, all aboveground biomass was collected in each plot, excluding a 50 cm border to avoid edge effects. The samples dried in a forced-air oven at 65 °C for 72 h, then the material ground through a 1 mm sieve, then homogenized, formed into subsamples and stored in airtight containers until analysis (Herrera et al. 2020)**.**

Chemical composition analysis

The analytical procedures for determining neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) were performed according to the method by Hall and Mertens (2023). Crude protein (CP), ash, organic matter (OM), and minerals such as calcium, phosphorus, magnesium, and silica were evaluated following AOAC (2005) standards. For secondary metabolites quantified were total phenols and tannins using Folin–Ciocalteu and polyvinylpyrrolidone adsorption methods (Makkar 2003), and the results expressed as tannic acid equivalents (g kg^{-1} DM). In addition, phytochemicals (flavonoids, saponins, triterpenes, steroids, alkaloids) and soluble sugars (glucose, fructose, sucrose) plus oligosaccharides (raffinose, verbascose, stachyose) were analyzed by methanolic extraction and HPLC with refractive index detection (Jiménez et al. 2021; Yang et al. 2023).

In Vitro digestibility assessment

In vitro dry matter digestibility (IVDMD) was evaluated using the ANKOM Daisy II® incubator (ANKOM Technology, Fairport, NY, USA) according to Tassone et al. (2020). Rumen fluid was collected from four rumen-cannulated Pelibuey sheep (~53.8±4.08 kg), housed individually and fed *Neonotonia wightii* and *Tithonia diversifolia*. Samples (~250 mg) were sealed in F57 Ankom bags and incubated at 39 °C with constant agitation for 48 hours. Replicates for each regrowth age, along with blanks, were included. After incubation, bags were rinsed, dried, and analyzed for NDF to calculate true digestibility. All animal experimental procedures adhered to international welfare standards and the ethics guidelines approved by the Amazon State University's Ethics Committee, Ecuador (Protocol No. CEUA-UEA-042-2023).

In Situ digestibility evaluation

In situ dry matter digestibility (ISDMD) was determined using the nylon bag technique per Ørskov (2000). Ground samples (~4 g, 2 mm sieve) were placed in 40 µm pore nylon bags, inserted into sheep rumens before morning feeding, and incubated for 72 hours. Bags were then soaked in cold water, frozen at -30 °C for 24 hours to detach microbes, thawed, washed, oven-dried at 60 °C, and weighed to determine dry matter disappearance. Residuals were analyzed for the digestibility of NDF, ADF, and CP. Organic matter digestibility (OMD), metabolizable energy (ME), and net energy for lactation (NEL) were calculated following established methods (Caceres and Gonzalez 2000; Rivera et al. 2023).

In Vitro gas production

In vitro gas production measurements employed a pressure transducer as outlined by Theodorou et al. (1994). Researchers prepared 72 samples (6 samples × 2 inoculums × 2 bottles × 3 replicates), weighing 500±10 mg, and placed them into 120 mL serum bottles with 50 mL of diluted rumen fluid. The rumen fluid was diluted 1:4 (v/v) with a culture medium that included macro- and micro-mineral solutions, bicarbonate buffer, and resazurin, following Melesse et al. (2024). The medium maintained at 39 °C under CO_2 saturation; oxygen levels reduced by adding cysteine hydrochloride and sodium sulfide. Blanks (bottles without substrate) accounted for gas production without feed. Bottles sealed with rubber stoppers and aluminum seals, shaken, and incubated at 39 °C. Headspace gas pressure, generated during fermentation, recorded at 3, 6, 9, 12, 16, 21, 26, 31, 36, 48, 60, 72, 96, 120, and 144 hours after inoculation, with gas released after each measurement. Incubations occurred in two batches during consecutive weeks using different inoculum sources. Gas volume was calculated from pressure measurements using the equation by Dhanoa et al. (2021) (Equation 1). Researchers fitted gas production data to the exponential model by García-Rodríguez et al. (2019).

G = A [1 – e^{–c(t – L)}] (1)

Where G (mL g^{-1} DM) represents cumulative gas production at time t; A (mL g^{-1} DM) denotes asymptotic gas production; c(/h) is the fractional fermentation rate; and L (h) indicates lag time.

The average fermentation rate R (mL gas h^{-1}) equals the gas production rate from incubation start to half the asymptotic gas volume, calculated by Equation 2:

$$R = Ac / [2 (\ln 2 + cL)] \qquad (2)$$

Statistical analysis

For statistical processing, a two-way analysis of variance (ANOVA) was used, considering regrowth age (30, 60, 90 days) and period (rainy, low-rainfall) as fixed factors, with block as a random effect, using the MIXED procedure in SAS 9.4 (SAS Institute Inc., Cary, NC, USA). Normality and homoscedasticity were verified using the Kolmogorov–Smirnov and Bartlett tests, respectively. Significance was declared t at

P≤0.05, and means were compared using Tukey's test. When the age×period interaction was significant (*P*≤0.05), a one-effects analysis within each period.

RESULTS AND DISCUSSION

Dry matter content increased progressively with regrowth age in both periods (Table 2). Values rose from 16.5 to 29.9% during the rainy period and from 20.8 to 30.5% during the low-rainfall period between 30 and 90 days. This accumulation reflects structural tissue development and reduces cellular water content as plants mature, a pattern consistent with physiological maturation in tropical legumes (Akpensuen 2022). Crude protein declined sharply with advancing regrowth age, decreasing from 25.0 to 14.5% in the rainy period and from 23.5 to 16.2% in the low-rainfall period. Protein concentrations remained consistently higher during the rainy period across all regrowth ages (*P*<0.001), which aligns with enhanced nitrogen assimilation under favorable moisture conditions (Gyue et al. 2021). Importantly, the absence of a significant age×period interaction (*P*>0.05) indicates that protein dilution follows a parallel trajectory regardless of rainfall regime; thus, harvest management decisions based on regrowth age remain valid across periods.

Table 2. Chemical Composition of *Neonotonia wightii* cv Longicauda.

Component	Rainy period			SE±	*P*-value	Low-rainfall period			SE±	*P*-value
	30 d	**60 d**	**90 d**			**30 d**	**60 d**	**90 d**		
Dry matter	16.50	22.50	29.90	0.005	<0.001	20.80	27.30	30.50	0.005	<0.001
Crude protein	25.00	18.30	14.50	0.010	<0.001	23.50	20.80	16.20	0.010	<0.001
Calcium	2.60	2.60	2.45	0.001	<0.001	2.10	2.15	1.85	0.001	<0.001
Phosphorus	0.015	0.008	0.0045	0.0001	<0.001	0.014	0.0075	0.0055	0.0001	<0.001
Magnesium	0.67	0.83	0.62	0.002	<0.001	0.54	0.63	0.57	0.002	<0.001
Silicon	3.75	3.45	3.25	0.002	<0.001	3.78	4.10	3.85	0.002	<0.001
NDF	44.00	49.80	55.70	0.008	<0.001	43.50	49.00	53.90	0.008	<0.001
ADF	27.80	34.00	36.80	0.020	<0.001	26.50	35.60	37.40	0.020	<0.001
Lignin	9.80	11.40	14.00	0.009	<0.001	11.30	15.30	18.70	0.009	<0.001
Hemicellulose	17.10	18.00	16.20	0.030	<0.001	18.30	16.00	15.80	0.030	<0.001
Cellulose	16.60	13.50	13.75	0.030	<0.001	16.10	14.05	14.50	0.030	<0.001
Cellular content	55.10	48.00	45.15	0.022	<0.001	57.60	48.80	47.30	0.022	<0.001
Ash	18.20	16.60	16.20	0.005	<0.001	15.10	15.70	15.10	0.005	<0.001
Organic matter	81.60	83.70	83.50	0.005	<0.001	86.00	83.30	84.80	0.005	<0.001

DM = dry matter; NDF = neutral detergent fiber (% DM); ADF = acid detergent fiber (% DM); ADL = acid detergent lignin (% DM); d=days.

Mineral concentrations responded differentially to regrowth age and period. Calcium decreased with maturity, especially during the low-rainfall period (2.10% at 30 days to 1.85% at 90 days). Phosphorus showed a steep decline from 0.015 to 0.0045% in the rainy period and from 0.014 to 0.0055% in the low-rainfall period, reflecting dilution effects as structural tissues accumulated. Magnesium peaked at 60 days (0.83% rainy; 0.63% low-rainfall period) before declining at 90 days, whereas silicon content remained relatively stable in the rainy period but increased during the low-rainfall period, reaching 4.10% at 60 days. These mineral dynamics directly influence bone development and enzymatic functions in ruminants; therefore, early harvest intervals preserve not only protein but also critical micronutrient profiles.

Fiber fractions followed an inverse pattern to protein. Neutral detergent fiber (NDF) increased from 44.0 to 55.7% (rainy period) and from 43.5 to 53.9% (low-rainfall period). Similarly, acid detergent fiber (ADF) rose from 27.8 to 36.8% and from 26.5 to 37.4%, respectively. Lignin accumulation was particularly pronounced, increasing from 9.8 to 22.0% in the rainy period and from 11.3 to 20.7% in the low-rainfall period. These structural changes coincided with a reduction in cellular content from 55.1 to 45.2% (rainy period) and from 57.6 to 47.3% (low-rainfall period), reflecting progressive lignification and cell wall thickening. Consequently, this biochemical transformation directly limits enzymatic access to cell wall polysaccharides and explains the parallel decline in digestibility metrics observed later.

Soluble sugars decreased markedly with advancing regrowth age (Table 3). Glucose concentrations fell by 76% in the rainy period (0.0210 to 0.0050%) and by 83% in the low-rainfall period (0.0330 to 0.0055%). Fructose and sucrose followed similar declining trajectories, with sucrose reductions of 67% (rainy) and 66% (low-rainfall) between 30 and 90 days. These carbohydrates serve as readily fermentable substrates for rumen microbes; therefore, their depletion with maturity reduces the energetic value of forage for ruminants (Gursoy et al. 2021).

Table 3. Content of sugar and metabolites secondary of *Neonotonia wightii* cv Longicauda, g kg^{-1} DM.

Variables	Rainy period					Low-rainfall period				
	30d	**60d**	**90d**	**SE±**	**P-value**	**30d**	**60d**	**90d**	**SE±**	**P-value**
Glucose	0.0210	0.0105	0.0050	0.00003	<0.0001	0.0330	0.0075	0.0055	0.00004	<0.0001
Fructose	0.0180	0.0060	0.0045	0.000002	<0.0001	0.0080	0.0085	0.0060	0.000002	0.0012
Sucrose	0.0390	0.0160	0.0130	0.00003	<0.0001	0.0410	0.0160	0.0140	0.00003	<0.0001
Total Tannins	6.10	10.30	18.50	0.018	<0.0001	25.80	33.50	37.00	0.021	<0.0001
Total Phenols	10.20	19.80	27.00	0.014	<0.0001	48.50	47.20	49.50	0.015	0.0821
TCT	68.50	117.00	128.50	0.089	<0.0001	66.00	98.20	107.00	0.095	<0.0001
FCT	2.10	2.40	2.80	0.006	<0.0001	1.70	2.70	3.10	0.007	<0.0001
Flavonoids	2.30	7.80	8.10	0.0022	<0.0001	3.80	10.60	11.00	0.0025	<0.0001
Alkaloids	4.00	5.40	5.20	0.007	<0.0001	4.50	5.10	5.60	0.008	0.0034
Saponins	0.60	1.25	1.40	0.0039	<0.0001	0.70	1.45	1.40	0.0042	<0.0001
Triterpenes	8.90	11.50	11.60	0.038	<0.0001	9.10	10.00	10.20	0.041	0.0156
Steroids	0.60	1.35	1.55	0.0029	<0.0001	0.80	1.40	1.60	0.0031	<0.0001

TCT = total condensed tannins (g tannic acid equivalents kg^{-1} DM); FCT = free condensed tannins (g tannic acid equivalents kg^{-1} DM); d=days.

Secondary metabolites accumulated progressively with maturity and intensified under low-rainfall period conditions. Total tannins increased from 6.10 to 18.50 g kg^{-1} DM in the rainy period and from 25.80 to 37.00 g kg^{-1} DM in the low-rainfall period. Total phenols showed a comparable pattern, rising from 10.20 to 27.00 g kg^{-1} DM (rainy period) and from 48.50 to 49.50 g kg^{-1} DM (low-rainfall period). Flavonoids, alkaloids, saponins, triterpenes, and steroids all exhibited significant accumulation with maturity, with consistently higher concentrations during the low-rainfall period ($P<0.001$). This pronounced accumulation during the low-rainfall period reflects a physiological defense response to water stress; plants synthesize phenolic compounds to mitigate oxidative damage, reduce transpiration, and deter herbivory when resources become limiting (Herrera et al. 2020). Although moderate tannin levels may improve nitrogen utilization in ruminants, concentrations exceeding 30 g kg^{-1} DM as observed at 90 days in the low-rainfall period can impair protein digestibility and reduce voluntary intake (Ebrahim et al. 2020).

In vitro dry matter digestibility (IVDMD) declined with regrowth age in both periods (Table 4). Values decreased from 75.0 to 59.7% during the rainy period and from 70.6 to 65.3% during the low-rainfall period. *In situ* dry matter digestibility (ISDMD) followed a steeper decline, particularly during the low-rainfall period where values dropped from 81.5% at 30 days to 37.2% at 90 days. True digestibility (TD) peaked at

60 days in the rainy period (83.0%) but declined monotonically in the low-rainfall period from 81.0 to 75.1%. Fiber digestibility (NDFD and ADFD) showed the most pronounced reductions with maturity. NDFD fell from 44.6 to 13.5% in the rainy period and from 47.8 to 18.0% in the low-rainfall period. Crude protein digestibility decreased from 50.1 to 33.6% (rainy period) and from 63.2 to 39.5% (low-rainfall period). These reductions directly result from lignin encrustation of cellulose and hemicellulose, which creates a physical barrier that impedes microbial colonization and enzymatic hydrolysis (Belete et al. 2024).

Table 4. Digestibility and energy supply of *Neonotonia wightii* cv Longicauda period.

Parameter	Rainy period			SE±	*P*-value	Low-rainfall period			SE±	*P*-value
	30d	60d	90d			30d	60d	90d		
IVDMD (%)	75.00	63.90	59.70	0.060	<0.001	70.60	66.50	65.30	0.060	<0.001
TD (%)	78.70	83.00	81.50	0.015	<0.001	81.00	78.30	75.10	0.015	<0.001
ISDMD (%)	88.20	77.00	71.50	0.030	<0.001	81.50	42.50	37.20	0.030	<0.001
OMD (%)	75.50	62.20	57.85	0.055	<0.001	69.40	68.20	66.60	0.055	<0.001
CPD (%)	50.10	40.50	33.60	0.037	<0.001	63.20	43.10	39.50	0.037	<0.001
NDFD (%)	44.60	16.80	13.50	0.007	<0.001	47.80	21.90	18.00	0.007	<0.001
ADFD (%)	44.80	24.10	22.50	0.005	<0.001	50.20	27.85	23.60	0.005	<0.001
ME (MJ kg^{-1})	10.90	9.15	8.60	0.009	<0.001	10.40	10.10	9.85	0.009	<0.001
NEL (MJ kg^{-1})	6.50	5.55	5.25	0.006	<0.001	6.10	6.00	5.85	0.006	<0.001

IVDMD = *in vitro* dry matter digestibility (%); TD = true digestibility (%); ISDMD = *in situ* dry matter digestibility (%); OMD = organic matter digestibility (%); CPD = crude protein digestibility (%); NDFD = neutral detergent fiber digestibility (%); ADFD = acid detergent fiber digestibility (%); ME = metabolizable energy (MJ kg^{-1} DM); NEL = net energy for lactation (MJ kg^{-1} DM); d=days.

Metabolizable energy (ME) and net energy for lactation (NEL) mirrored digestibility trends. ME declined from 10.90 to 8.60 MJ kg^{-1} in the rainy period and from 10.40 to 9.85 MJ kg^{-1} in the low-rainfall period. NEL decreased from 6.50 to 5.25 MJ kg^{-1} (rainy) and from 6.10 to 5.85 MJ kg^{-1} (low-rainfall). Energy values remained consistently higher during the rainy period at equivalent regrowth ages (P<0.001), confirming that moisture availability preserves the energetic value of forage beyond its structural constraints.

Remarkably, fermentation kinetics parameters remained stable across regrowth ages and periods (Table 5). Asymptotic gas volume ranged narrowly from 213.20 a 230.40 mL g^{-1} DM without significant differences among treatments (P=0.054 for age; P=0.26 for period). The gas production rate showed minimal variation (0.048 a0.055 mL h^{-1}) and lag time remained consistent (0.21 a 0.58 h), with no statistically significant effects of regrowth age (P>0.05). This stability indicates that despite compositional changes in fiber, protein, and secondary metabolites, the ruminal fermentability of *N. wightii* cv. Longicauda remained consistent across all evaluated conditions. Therefore, this resilience can be interpreted as an adaptive trait of Amazonian legumes that evolved under pronounced seasonal rainfall variability; the species maintains fermentative efficiency even when structural and chemical composition shift, thereby ensuring a reliable energy supply to ruminants throughout the year (Meza-Bone et al. 2022; Robles Jiménez et al. 2021). Furthermore, the non-significant age×period interaction for all fermentation parameters confirms that management recommendations based on regrowth age apply uniformly across periods to a practical advantage for farmers operating in variable tropical climates.

Table 5. Fluence of regrowth age and period on the parameters of the kinetics gas in *Neonotonia wightii* cv Longicauda.

Parameter	Days	SE±	*P*-value

	30	60	90		
		Rainy period			
Asymptotic gas (mL g^{-1})	227.15	217.85	213.20	20.8	0.054
Rate (mL h^{-1})	0.048	0.051	0.053	0.0011	0.275
Lag time (h)	0.40	0.58	0.43	0.124	0.450
		Low-rainfall period			
Asymptotic gas (mL g^{-1})	230.40	225.70	217.10	20.34	0.051
Rate (mL h^{-1})	0.051	0.053	0.055	0.001	0.268
Lag time (h)	0.21	0.38	0.30	0.109	0.473

Asymptotic gas = maximum cumulative gas production (mL g^{-1} DM); Rate = average gas production rate (mL h^{-1}); Lag time = delay before exponential gas production begins (h).

In the Ecuadorian Amazon, periodic variation derives primarily from precipitation patterns rather than thermal fluctuations; mean temperatures differed by only 0.4 °C between periods (16.37 °C rainy vs. 15.96 °C low-rainfall period), whereas rainfall varied by 170.5 mm. Consequently, water availability not temperature drives the observed differences in protein concentration, secondary metabolite accumulation, and digestibility. This seasonal rainfall variability, characteristic of humid tropical ecosystems, requires management strategies that prioritize regrowth age as the primary lever for quality control, as its effect remains consistent regardless of the rainfall regime. Harvesting at 30 days preserves protein content above 23% and IVDMD above 70% in both periods, whereas delaying harvest to 90 days reduces protein by 38–42% and IVDMD by 18–21%, irrespective of rainfall conditions. Therefore, short regrowth intervals represent the most reliable strategy to maintain forage quality throughout the year in Amazonian livestock systems.

CONCLUSION

Regrowth age determines the primary trajectory of forage quality in *Neonotonia wightii* cv. Longicauda under Amazonian humid tropical conditions, while the pluviometric period establishes the absolute baseline for nutrient concentrations and secondary metabolite accumulation. The absence of a significant age × period interaction confirms that maturation drives quality deterioration at a consistent rate across both the rainy and low-rainfall seasons. Consequently, harvest strategies based on regrowth age maintain universal applicability throughout the annual cycle; however, operational planning must explicitly account for the specific pluviometric period, as rainfall regimes directly define the critical nutritional thresholds, forage utilization windows, and supplemental feeding requirements necessary to sustain ruminant performance year-round.

Despite pronounced changes in chemical composition with maturity, *N. wightii* cv. Longicauda maintains stable ruminal fermentation kinetics across regrowth ages and periods. This physiological resilience reflects an adaptive advantage for Amazonian livestock systems, where consistent fermentative efficiency ensures a reliable energy supply to animals despite seasonal variability in forage composition.

This study focused exclusively on nutritional attributes under controlled defoliation regimes; future research should evaluate animal performance responses (intake, growth rates, milk yield) to validate these nutritional thresholds under grazing conditions. Additionally, investigations into nitrogen fixation dynamics and soil health impacts across regrowth intervals would strengthen the sustainability assessment of *N. wightii*-based forage systems in the humid tropics.

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CONFLICT OF INTERESTS

The authors declare that they have no conflicts of interest in relation to this research study.

ETHICAL STATEMENT

This study complied with all applicable international guidelines for the care and use of research animals, specifically the *Guide for the Care and Use of Agricultural Animals in Research and Teaching* (ASAS et al. 2020) and Ecuadorian national regulations for animal welfare (Ministerio de Agricultura y Ganadería 2016). The Amazon State University Ethics Committee for Animal Use granted formal approval for all experimental procedures under protocol number CEUA-UEA-042-2023.

The research was conducted using four rumen-cannulated Pelibuey sheep with an average body weight of 53.8±4.08 kg. The animals were housed in individual pens with free access to water and were fed a basal diet of *Neonotonia wightii* hay and *Tithonia diversifolia* foliage throughout the experimental period. Animal health was monitored daily by veterinary staff, and no adverse effects related to rumen fluid collection or nylon bag incubation procedures were observed. Animal discomfort was minimized by performing all manipulations gently and efficiently, and rumen fluid extraction was limited to volumes that did not compromise normal rumen function (<10% of total rumen content per session).

The experimental design prioritized animal welfare by using a crossover approach that maximized data yield per animal, thereby reducing the total number of animals required. All personnel involved in animal handling completed institutional training in ethical animal management prior to the study commencement.

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