

In Vivo Digestibility, In Vitro Gas Fermentation and Enteric Methane Values of Sunn Hemp's (*Crotalaria juncea* L.) as Affected by Cutting Stages

Muazzez Cömert Acar

muazzez.comert@ege.edu.tr

Ege University: Ege Üniversitesi <https://orcid.org/0000-0002-1742-8076>

FATMA ATLI

Ege University: Ege Üniversitesi

GULCAN DEMIROGLU TOPCU

Ege University: Ege Üniversitesi

ŞÜKRÜ SEZGİ OZKAN

Ege Üniversitesi

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Abstract

This study evaluated the effects of four cutting stages on the crude nutrient composition, *in vivo* digestibility, *in vitro* gas production, and enteric methane (CH₄) production of Sunn Hemp's (*Crotalaria juncea* L.). As vegetation progressed, crude protein (CP) decreased from 16.27% to 13.05%, while neutral detergent fiber (NDF) rose from 63.90% to 84.01%. *In vivo* trials with a year old wethers showed that dry matter (DM) and organic matter digestibility (OMD) peaked in the first two cuts (~ 65–71% and 71%, respectively) before falling significantly to 50–55%. Concurrently, *in vivo* metabolizable energy (ME) decreased from 10.67 to 7.07 MJ/kg DM ($P < 0.05$). *In vitro* results showed a higher early fermentation rate (3–6 h) for the 1st cut, though 96-h total gas production remained stable (49.75–55.00 mL) across stages. Critically, advanced maturity shifted fermentation end-products, increasing acetic acid (AA) from 1.06 to 1.45 mg/mL and triggering enteric methanogenesis. Enteric CH₄ emissions rose from 9.99 mol/mol VFA (1st cut) to 13.57 mol/mol VFA (4th cut) ($P = 0.014$). In conclusion, Sunn Hemp's provides high-quality forage during the first and second cutting stages due to its superior protein and energy content.

INTRODUCTION

The increasing global demand for sustainable animal production has intensified the search for alternative forage sources that provide high nutritional value while minimizing environmental impacts. Sunn Hemp's (*Crotalaria juncea* L.), a tropical legume traditionally utilized as green manure or for industrial fiber applications, has gained substantial prominence as a multi-purpose crop due to its exceptionally rapid biomass production and outstanding atmospheric nitrogen-fixing capacity (Heuzé et al., 2017). Beyond these conventional uses, it serves as a strategic and high-quality alternative forage option to meet the protein requirements of livestock, particularly in tropical and subtropical areas—as well as specific ecological zones like Türkiye—where traditional forage production is severely constrained by seasonal droughts, high feed costs, and poor soil fertility (Diniz et al., 2017; Barbosa et al., 2020). However, utilizing this species effectively requires a comprehensive evaluation of its degradability and fermentation dynamics across different growth stages. The feed value of forages is primarily determined by their crude nutrient composition, which undergoes significant transformations during the vegetative process. As plants mature, there is a characteristic decline in crude protein (CP) content and a concomitant increase in structural carbohydrates, particularly neutral detergent fiber (NDF) and lignin (Minson, 2012). According to the nutritional ecology principles defined by Van Soest (1994), the formation of lignocellulose complexes during advanced maturity stages physically obstructs the access of fibrolytic bacteria to fermentable substrates. This structural shift not only reduces DM and organic matter (OM) digestibility but also alters the end-products of ruminal fermentation. One of the most critical environmental concerns in ruminant nutrition is the production of enteric methane (CH₄), a potent greenhouse gas (Moss et al., 2000). The composition of volatile fatty acids (VFA) serves as a primary indicator of methanogenesis; specifically, the degradation of fiber-rich mature forages promotes an acetate-dominant fermentation, which releases excess hydrogen (H₂). As noted by Moss et al. (2000),

this free hydrogen is subsequently utilized by methanogenic archaea to reduce carbon dioxide into CH₄. Consequently, identifying the optimal cutting time is vital to balance the need for biomass yield with the goals of high energy efficiency and low environmental pollution (Minson, 2012). Although Mosjidis and Wang (2011) suggest cutting Sunn Hemp's at the early flowering stage for general purposes, the specific shifts in *in vitro* gas production and CH₄ emissions across early vegetative stages remain under-documented. Therefore, this study aims to determine the effect of different cutting stages on the crude nutrient composition, *in vivo* digestibility, and *in vitro* fermentation parameters of Sunn Hemp's produced under the ecological conditions of Izmir, Türkiye.

MATERIALS AND METHODS

Materials

The study was conducted during the 2025 summer growing season in Bornova, Izmir, Türkiye, under Mediterranean climatic conditions. Sunn Hemp's (*Crotalaria juncea* L.) was cultivated at the experimental fields of Ege University, Faculty of Agriculture, Department of Field Crops, using a randomized complete block design with four replicates. The animal material comprised three 1-year-old wethers for *in vivo* and *in vitro* studies. All experimental procedures were approved by the Ege University Faculty of Agriculture Local Ethics Committee for Animal Experiments (Approval No: 2024/04; Date: 15.11.2024).

Methods

Crude nutrient Composition and Van Soest Analysis

Sunn Hemp's samples were harvested at four different vegetative stages (1st Cut: Early bud, 2nd Cut: 50% bloom, 3rd Cut: Full bloom, 4th Cut: Early pod-setting). Dry matter (DM) was determined by drying at 105°C overnight, and crude ash (CA) by incineration at 550°C overnight (AOAC, 2005). Organic matter (OM) was calculated as the difference between DM and CA. Crude protein (CP), ether extract (EE), and crude fiber (CF) were determined using standard Weende methods (AOAC, 2005), and nitrogen-free extract (NFE) was calculated by difference. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) concentrations were determined via the Fiber Bag System following the procedures of Goering and Van Soest (1970). All analyses replicated 3 times.

Classical Digestion Trial (In Vivo Digestibility)

The *in vivo* digestion trial was conducted between September and December 2025 at the Biological Analysis Laboratories of the Department of Animal Nutrition and Feed Science, Ege University, utilizing 3 wethers (n = 3) in a randomized complete block design to determine digestibility coefficients (DC) and digestible crude nutrient (DCN) contents (Menke and Huss, 1975; GfE, 1991). Following health checks and parasite treatments, the trial consisted of a 15-day preliminary adaptation period—where animals

received experimental feed at 1.2–1.5 times maintenance requirements to establish intake levels—and a 7-day main collection period for each cutting stage, separated by 1-week rest intervals. During the main period, predetermined daily feed amounts were provided in two equal meals (08:30 and 16:30 h), and fecal collection bags were utilized. Approximately 10% of the daily collected feces were sampled, preserved with 3–5 mL of chloroform, and refrigerated. Nutrient compositions of feed and fecal samples were analyzed via standard methods, and DCs were calculated by subtracting fecal excretion from total intake. *In vivo* metabolizable energy (ME) values were calculated using the following regression equation based on the obtained g/kg DM values of digestible crude protein (DCP), digestible crude fat (DEE), digestible crude fiber (DCF), and digestible nitrogen-free extract (DNFE) (Alderman, 1984):

$$\text{ME, MJ/kg KM} = 0.0152 \times \text{DCP} + 0.0342 \times \text{DEE} + 0.0128 \times \text{DCF} + 0.0159 \times \text{DNFE}$$

Gas Production Technique (In Vitro)

In vitro gas production (GP) was evaluated using the Hohenheim Feed Test at incubation intervals of 3, 6, 9, 12, 24, 48, 72, and 96 h, utilizing 6 replicates across 5 independent runs ($n = 5$) for each cutting stage. Rumen fluid was collected immediately post-slaughter from three wethers (fed ad libitum forage and 1–1.5% body weight concentrate) at a slaughterhouse in Kemalpaşa, Izmir, and transported within 30 min in a preheated thermos (38–40°C); pH values ranged between 6.54 and 7.11 for runs. Dried, ground samples (200 mg, 1 mm sieve) were transferred into 100 mL calibrated glass syringes. Under continuous CO₂ infusion and constant stirring at 39°C, a mixture containing 474.5 mL distilled water, 0.12 mL trace element solution, 237.23 mL buffer solution, 237.23 mL main element solution, 1.22 mL resazurin, and reduction solution was combined with 500 mL filtered rumen fluid. Syringes were filled with 30 mL of this mixture, cleared of air bubbles, and incubated at 39°C. Net GP was recorded at designated hours and calculated according to Menke and Steingass (1988). Forage and concentrate standards were included to ensure analytical accuracy.

Calculation of OMD, ME, and NEL Values by Gas Production Technique

In vitro organic matter digestibility (OMD), metabolizable energy (ME), and net energy for lactation (NEL) values were calculated based on 24-h GP values and crude nutrient composition (AOAC, 1995) using the equations described by Menke and Steingass (1988):

$$\text{ME (MJ/kg DM)} = 1.2 + (0.1456 \times \text{GP}) + (0.007675 \times \text{CP}) + (0.01642 \times \text{EE})$$

$$\text{NEL (MJ/kg DM)} = (0.101 \times \text{GP}) + (0.0051 \times \text{CP}) + (0.011 \times \text{EE}) + (0.002 \times \text{CA}) - 0.36$$

$$\text{OMD (\%)} = 14.88 + (0.8893 \times \text{GP}) + (0.0448 \times \text{CP}) + (0.0651 \times \text{CA})$$

ME: Metabolizable Energy (MJ/kg DM), NEL: Net Energy Lactation (MJ/kg DM, OMD: *In vitro* Organic Matter Digestibility (%), GP: Net gas production (ml/200 mg DM) of 200 mg dry feed sample at the end of

a 24-hour incubation period, CP: Crude Protein, EE: Crude Fat, CA: Crude Ash, Note: The amounts of CP, EE, and GP are expressed in g/kg DM within the equations.

Volatile Fatty Acids (VFA) and Enteric Methane Analysis

At the end of the 48-h incubation period, fluid samples (5–7 mL) were filtered through cheesecloth, centrifuged at 6000 rpm for 10 min, mixed with 1 mL of 25% metaphosphoric acid, and left to stand for 30 min. After a final centrifugation at 3000 rpm, the supernatant was stored at -20°C. Volatile fatty acids (acetic acid [AA], propionic acid [PA], and butyric acid [BA]) were quantified using an Agilent 1260 Infinity HPLC system equipped with a DAD detector (210 nm) and an Agilent Hi-Plex H column (8 µm, 300 x 7.7 mm) at 45°C, with a mobile phase of 10 mM H₂SO₄ at a flow rate of 0.8 mL/min (Erwin et al., 1961). Calibration curves ranged from 0.1 mg/mL to 10 mg/mL. *In vitro* CH₄ (mol/mol VFA) was modeled based on Wolin (1960) using the equation from Chalupa (1977):

CH₄ VFA (Wolin, 1960), (mol/mol VFA): $(0.5 \times \text{acetate} - 0.25 \times \text{propionate} + 0.5 \times \text{butyrate})$

Statistical Analysis

Data were analyzed using Analysis of Variance (ANOVA) via the SPSS (2006) software package. Duncan’s Multiple Range Test was applied to define significant differences among the cutting stages. Statistical significance was declared at $P < 0.05$.

RESULTS

Sunn Hemp’s Crude Nutrient Composition

Table 1 details the crude nutrient composition of Sunn Hemp’s across the different cutting stages investigated.

Table 1

The crude nutrient composition of Sunn Hemp's according to different cutting stages (% in DM)

FORAGE	1st Cut	2nd Cut	3rd Cut	4th Cut	SEM	p-value
DM, %	93.91 a	93.29 ab	92.31 b	90.78 c	0.46	0.004
CA	7.29 a	5.82 b	5.10 b	5.30 b	0.33	0.005
OM	92.71 a	94.18 b	94.90 b	94.70 b	0.33	0.005
CP	16.27 a	14.93 b	13.51 c	13.05 c	0.49	0.002
EE	0.86 bc	0.76 c	1.42 ab	1.47 a	0.13	0.051
CF	53.01 a	55.72 b	58.01 c	60.67 d	1.09	0.003
NFE	22.56 a	22.77 a	21.96 ab	19.50 b	0.55	0.063
NDF	63.90 c	65.85 c	77.02 b	84.01 a	3.16	0.002
ADF	53.70 c	56.01 bc	60.37 ab	63.71 a	1.53	0.013
1st Cut: Early bud stage; 2nd Cut: 50% bloom stage; 3rd Cut: Full bloom stage; 4th Cut: Early pod-setting stage. SEM: Standard Error of the Mean; P-value: Probability value; DM: dry matter; CA: crude ash; OM: organic matter; CP: crude protein; EE: crude fat; CF: crude fiber; NFE: nitrogen-free extract; NDF: neutral detergent fiber, ADF: Acid detergent fiber						

a–d Means within the same column with different superscripts are significantly different ($P < 0.05$)

The first cut had the highest DM content (93.91%); as the cutting stage progressed, this ratio fell to 90.78% in the fourth cut. The CA concentration decreased significantly after peaking at 7.29% in the first cut. Accordingly, the OM content was lowest (92.71%) in the first cut and increased in succeeding cuttings. CP concentration decreased from 16.27% in the first cut to 13.05% in the fourth cut ($P < 0.05$). The crude fiber ratio increased with each cutting phase, from 53.01% in the first cut to 60.67% in the fourth. Similarly, NDF values rose from 63.90% to 84.01%, ADF values rose from 53.70% to 63.71% reaching the maximum level in the final cut ($P < 0.05$). The fourth cut (1.47%) had the highest EE content, while the second cut (0.76%) had the lowest ($P = 0.05$). NFE values did not differ statistically significantly between the cuts ($P > 0.05$).

In Vivo Digestibility and Metabolizable Energy Values

Table 2 shows the *in vivo* nutrient digestibility coefficients, DCN contents, and ME values derived from the nutrient composition of Sunn Hemp's across different cutting stages.

Table 2

In vivo nutrient digestibility coefficients (%), digestible nutrients (g/kg DM), and metabolizable energy (MJ/kg DM) values of Sunn Hemp's at different cutting stages (n = 3)

Parameter	1st Cut	2nd Cut	3rd Cut	4th Cut	SEM	p-value
Digestibility coefficients, %						
DM	71.02 a	65.27 a	50.29 b	53.61 b	2.81	0.002
CA	40.34	43.15	40.99	33.38	2.76	0.688
OM	71.06 a	67.04 ab	57.40 b	55.22 b	2.51	0.041
CP	82.55	80.33	78.91	77.97	0.88	0.269
EE	18.90 b	14.66 b	47.50 a	40.55 a	4.77	0.004
CF	63.15	51.88	51.82	53.71	2.57	0.385
NFE	83.76 a	87.36 a	44.09 b	30.69 b	11.21	0.003
NDF	57.19	53.95	52.96	56.06	2.72	0.961
Digestible nutrients, (g/kg DM)						
DOM	752.68 a	630.43 ab	507.44 b	525.30 b	34.28	0.029
DCP	154.83 a	132.66 b	121.60 bc	115.12 c	4.78	0.000
DEE	7.30 b	6.01 b	11.75 a	11.56 a	0.82	0.000
DCF	412.48 a	320.20ab	257.66 b	302.27 b	21.82	0.049
DNFE	128.86	171.56	116.43	96.35	10.93	0.064
DNDF	448.07	384.96	384.21	445.38	20.2	0.562
ME	10.67 a	9.05 ab	7.40 b	7.07 b	0.52	0.029

1st Cut: Early bud stage; 2nd Cut: 50% bloom stage; 3rd Cut: Full bloom stage; 4th Cut: Early pod-setting stage. SEM: Standard Error of the Mean; P-value: Probability value; DM: dry matter; CA: crude ash; OM: organic matter; CP: crude protein; EE: crude fat/ether extract; CF: crude fiber; NFE: nitrogen-free extract; NDF: neutral detergent fiber; DOM: digestible organic matter; DCP: digestible crude protein; DEE: digestible ether extract; DCF: digestible crude fiber; DNFE: digestible nitrogen-free extract; DNDF: digestible neutral detergent fiber; ME: metabolizable energy. a–c Means within the same column with different superscripts are significantly different ($P < 0.05$).

Overall digestibility levels significantly decreased as the plant grew. DM digestibility was statistically equivalent in the first two cuts (71.02% and 65.27%) but fell to 50–53% in the 3rd and 4th cuts ($P = 0.002$). OM digestibility followed a similar pattern, reaching its highest level (71.06%) in the first cut and

its lowest (55.22%) in the fourth cut ($P = 0.041$). CP, CF, and NDF digestibility did not show a statistically significant impact of the cutting time ($P > 0.05$). NFE digestibility was high in the 1st and 2nd cuts (83.76% and 87.36%) and declined to 30.69% in the 4th cut ($P = 0.003$). Conversely, EE digestibility was statistically greater in the 3rd and 4th cuts (47.50% and 40.55%) ($P = 0.004$). No variation was observed in CA digestibility between cutting periods ($P = 0.688$). As the plant matured, both DOM and ME levels showed a decline. The first cut yielded the greatest DOM (752.68 g/kg DM) and ME (10.67 MJ/kg DM) values, which dropped in the third and fourth cuts ($P < 0.05$). There was a significant drop in the amount of DCP, which decreased from 154.83 g/kg DM in the first cut to 115.12 g/kg by the fourth cut ($P = 0.000$). DCF peaked in the first cut (412.48 g/kg DM) and reached its lowest level in the third cut ($P = 0.049$). DEE values were lower in the first two cuts but increased significantly in the third and fourth cuts to approximately 11 g/kg DM ($P = 0.000$). DNFE and DNDF values showed no statistically significant impact of cutting time ($P > 0.05$).

In Vitro Digestibility, Metabolizable Energy, Net Energy for Lactation, and OMD Values

Figure 1 illustrates the *in vitro* gas production dynamics of Sunn Hemp's across different cutting stages and incubation periods ranging from 3 to 96 hours.

Examining the GP values revealed that the cutting stage had a statistically significant impact during the first six hours of incubation ($P < 0.01$). At the 3rd hour (17.00 ml) and the 6th hour (24.78 ml), the first cut produced the highest gas volume. GP values decreased in the third and fourth cuts during this early period. The difference between cutting stages was statistically negligible during the medium and late incubation periods from the 9th to the 96th hours ($P > 0.05$). By the end of the 96th hour, the total GP was 55.00 ml for the first cut and 49.75 ml for the fourth cut (Table 3, Fig. 1). Higher NDF and CF content associated with advanced vegetation stages resulted in a slower fermentation rate in the early hours, while the total GP over the long run (48–96 hours) remained comparable across stages.

Table 3

In vitro gas production amounts of Sunn Hemp's according to different cutting stages and incubation periods (ml/200 mg DM, n = 5)

Cutting stages	Incubation periods, hours							
	3	6	9	12	24	48	72	96
1st Cut	17.00 a	24.78 a	27.67	31.61	38.75	50.00	53.75	55.00
2nd Cut	13.94 ab	20.61 ab	25.61	30.33	37.80	49.10	51.50	52.83
3rd Cut	13.25 b	19.38 b	23.88	29.25	35.50	47.43	47.98	49.20
4th Cut	12.39 b	16.95 b	22.89	25.39	31.80	45.30	47.17	49.75
SEM	0.60	0.94	1.32	1.65	1.07	1.70	1.45	1.28
P-value	0.01	0.01	0.58	0.53	0.09	0.84	0.34	0.31
1st Cut: Early bud stage; 2nd Cut: 50% bloom stage; 3rd Cut: Full bloom stage; 4th Cut: Early pod-setting stage. P-value: Probability value; SEM: Standard Error of the Mean;								

a–b Means within the same column with different superscripts are significantly different ($P < 0.05$).

Findings Regarding AA (mg/mL), PA (mg/mL), BA (mg/mL), Total VFA (mg/mL) values of Sunn Hemp's and Enteric CH₄ (mol/mol VFA) Values

Table 4 presents the combined data for ruminal VFA profiles, calculated enteric CH₄ emissions, and the energy values (ME, NEL, and OMD) of Sunn Hemp's derived from 24-hour *in vitro* GP across different cutting stages.

Table 4
Effects of different cutting stages on the ruminal VFA profiles (AA, PA, BA, TVFA; mg/mL), calculated enteric CH₄ emissions (mol/mol VFA), and the nutritional energy values (OMD (%), ME and NEL (MJ/kg DM)) of Sunn Hemp's .

Parameter	1st Cut	2nd Cut	3rd Cut	4th Cut	SEM	p-value
OMD	61.38 a	58.97 ab	55.82 b	53.71 b	0.98	0.022
ME	8.23	7.97	7.64	7.28	0.15	0.130
NEL	4.62	4.42	4.17	3.93	0.11	0.103
AA	1.06 b	1.20 b	1.25 ab	1.45 a	0.05	0.013
PA	0.25 b	0.34 a	0.33 a	0.34 a	0.01	0.017
BA	0.33 b	0.44 a	0.45 a	0.43 a	0.01	0.002
TVFA	1.63 b	1.98 a	2.03 a	2.22 a	0.06	0.009
Enteric CH ₄	9.99 b	11.52 ab	12.01 ab	13.57 a	0.42	0.014

1st Cut: Early bud stage; 2nd Cut: 50% bloom stage; 3rd Cut: Full bloom stage; 4th Cut: Early pod-setting stage. SEM: Standard Error of the Mean; P-value: Probability value; OMD: *in vitro* organic matter digestibility; ME: metabolizable energy; DM: dry matter; NEL: net energy for lactation; AA: acetic acid; PA: propionic acid; BA: butyric acid; TVFA: total volatile fatty acids; Enteric CH₄: calculated enteric methane.

a–b Means within the same column with different superscripts are significantly different (P < 0.05).

The changes in rumen fermentation parameters of Sunn Hemp's depending on its vegetation periods were statistically significant (P < 0.05). VFA (AA, PA, BA) generally showed an increasing trend as the plant matured. Specifically, the AA concentration was lowest in the 1st cutting (1.06 mg/mL) and reached its peak (1.45 mg/mL) in the 4th cutting (P = 0.013). PA and BA values increased after the 1st cutting and remained at statistically similar levels throughout the remaining periods. TVFA increased significantly with the progression of vegetation (P = 0.009), rising from 1.63 mg/mL in the 1st cutting to 2.22 mg/mL in the 4th cutting. Enteric CH₄ production increased in parallel with the VFA composition (P = 0.014). The lowest CH₄ production was recorded in the 1st cutting (9.99 mol/mol VFA), while the highest was in the 4th cutting (13.57 mol/mol VFA). OMD significantly declined as the cutting stage progressed (P = 0.022), dropping from 61.38% in the first cut to 53.71% in the fourth cut. The difference between the first and second cuts was negligible. While there was a numerical decrease in ME (from 8.23 to 7.28 MJ/kg DM) and NEL (from 4.62 to 3.93 MJ/kg DM) values as cutting time increased, these differences were not statistically significant (P > 0.05). The amount of digestible OM decreased by approximately 8% by the fourth cut.

DISCUSSION AND CONCLUSION

The bulk of research on Sunn Hemp's has focused on agronomic traits, green manuring potential, and crude nutrient composition. However, there is limited information regarding how its vegetative stages affect GP, OMD, and particularly enteric CH₄ emissions. This study seeks to close this gap by evaluating these factors under Türkiye's ecological conditions. Given the chronic shortage of quality forage supply and high feed costs in Türkiye, investigating alternative and sustainable crops has become a matter of paramount importance for the livestock sector. In this context, evaluating climate-resilient summer legumes like Sunn Hemp's under Mediterranean ecological conditions offers a strategic solution to bridge the gap where alternative high-quality forage materials are urgently required to support high-yielding ruminants.

The study found notable changes in nutritional composition and fodder quality as the plant matured. As vegetation progressed, CP content decreased from 16.27% to 13.05%, while the NDF ratio increased from 63.90% to 84.01%. These results align with the theory that fibrous structures increase while the stem-to-leaf ratio rises and protein content falls due to lignification as leguminous forage crops grow (Minson, 2012). Although a CP ratio above the 7% threshold is sufficient for microbial protein synthesis in tropical legumes, the 16% protein content found in the first cut offers a more optimal nitrogen source for high-yielding ruminants. The increase in the EE ratio in the final cut is attributed to the seed-setting stage and fatty acid accumulation in the seeds. The 16.27% CP ratio in our first cut exceeded the ranges of 12.5%–15.6% reported by Barbosa et al. (2020) and 14.75%–15.20% reported by Diniz et al. (2017). This demonstrates that, under Türkiye's ecological conditions, the plant performs strong nitrogen fixation and protein accumulation during the early vegetative period.

Reports indicate that during the maturity of legumes, protein and soluble carbohydrates within the cell contents are obscured by the buildup of cellulose and lignin, significantly hampering digestibility. As the plant ages, photosynthetic activity shifts from leaf production toward the development of the stem as support tissue. During this process, hemicellulose and cellulose create cross-links with lignin, forming a lignocellulose complex that physically blocks rumen bacteria from accessing fermentable plant tissues. Mosjidis and Wang (2011) highlight that as plants mature, the lignin ratio in stems rises, leading to decreased overall digestibility. The increases in CF (from 53.01% to 60.67%) and NDF (from 63.90% to 84.01%) found in our study align with this hypothesis. These high NDF and CF values in the late vegetative stages are structurally explained by the high cellulose ratios (60–88%) reported in research for industrial applications. When examining digestibility, late cuttings (3rd and 4th cuts) showed significantly lower OM and DM digestibility. The drop in digestibility from approximately 70% to the 50–55% range confirms that higher fiber content limits microbial access to the cell wall. Furthermore, the fall in NFE digestibility to 30.69% in the fourth cut ($P < 0.05$) suggests that easily fermentable carbohydrates are replaced by difficult-to-digest structural polysaccharides. Similarly, ME values dropped from 10.67 MJ/kg DM to 7.07 MJ/kg DM ($P < 0.05$), demonstrating a nearly 30% reduction in net energy density as the plant ages.

As the plant ages, the fermentation rate during the first 6 hours slows down, and the total OMD drops from 61.38% to 53.71% ($P < 0.05$), according to *in vitro* GP parameters. The high concentration of water-

soluble carbohydrates in young plants accounts for this, while the high cell content and low lignin content are directly linked to the first cut's superior OMD. This aligns with McDonald et al. (2011), who state that digestible OM is the primary factor influencing energy value. The plant undergoes a "loss of nutritional value" as vegetation advances. By increasing rumen fill, this condition may also reduce overall feed intake (Allen, 1996). High NDF in mature forages produces "physical fill" by reducing the velocity of ruminal transit. According to Allen (1996), high-fiber diets restrict total energy intake but enhance saliva secretion through lengthened chewing and rumination times. This explains why total gas volumes at the end of the 96-hour incubation were comparable across stages. Conversely, the rapid onset of GP in the first cut suggests that rumen bacteria quickly access fermentable carbohydrates such as sugars and starch. According to Getachew et al. (1998), the gas production technique represents the contribution of feeds to microbial protein synthesis as well as overall digestibility. The prolonged attachment time of the microbial population—particularly *Ruminococcus albus* and *Fibrobacter succinogenes*—to the substrate (lag phase) is the cause of the lower initial GP in mature cuts. This lack of early-stage statistical variance also clarifies why the *in vitro* ME calculation showed only numerical rather than statistically significant differences ($P = 0.130$). Since the *in vitro* ME formula relies heavily on the 24-h net GP values, and given that the cumulative GP stabilized and remained statistically comparable across all cutting stages in the medium to long term (Table 3), the resulting mathematical estimation for *in vitro* energy content naturally reflected a similar statistical uniformity.

Upon examining the end products of rumen fermentation, it was observed that AA production and, consequently, enteric CH_4 emissions (13.57 mol/mol VFA) increased in mature plants (4th cut). The literature states that the degradation of structural carbohydrates in forages increases the acetate/propionate ratio, triggering methane production through hydrogen release (Moss et al., 2000). As AA production increases during rumen fermentation, H_2 is released as a byproduct. Methanogenic archaea combine this free hydrogen with CO_2 to produce CH_4 gas. The rise in methane production to 13.57 mol/mol VFA in the final cutting demonstrates that as forage quality decreases, the release of greenhouse gases increases. Conversely, the high protein content and balanced carbohydrate structure in early cuttings offer a more environmentally sustainable feeding model by suppressing CH_4 production. Furthermore, the significant rise in TVFA concentration from 1.63 to 2.22 mg/mL with advancing maturity ($P = 0.009$) should not be misinterpreted as an increase in initial fermentation velocity. Instead, this elevation represents the cumulative accumulation of acids—predominantly acetic acid—resulting from the extended incubation period required by cellulolytic microbes to systematically break down the rigid, highly lignified structural carbohydrates found in the later cutting stages. This research fills a significant gap by presenting GP and enteric CH_4 emission data according to vegetation stages, which is limited in current literature (Mahur et al., 2023; Pavan et al., 2024). While Mosjidis and Wang (2011) recommend "cutting at early flowering," our findings regarding energy efficiency, amino acid balance and low methane emissions suggest that shifting the cutting to the early vegetative stage (1st and 2nd cuts) is more rational for ruminant nutrition.

In conclusion, the findings of this study demonstrate that Sunn Hemp's exhibits remarkable nutritional dynamics and favorable fermentation profiles, particularly during its early vegetative stages. These results generate critical evidence supporting the wider adoption and integration of this climate-resilient legume as an alternative forage source, contributing significantly to sustainable animal production strategies not only in Türkiye but also in similar agro-ecological zones worldwide. Sunn Hemp's has the potential to serve as high-quality fodder during the 1st and 2nd cutting periods due to its outstanding protein quality and high ME content. However, advancing vegetation (3rd and 4th cuts) raises methane emissions, lowers digestibility and energy content, and decreases protein levels, increasing both financial and environmental costs. This shift is explained by the transition to flowering and seed-setting stages, which strengthens the lignocellulose barrier and shifts ruminal fermentation toward AA. Based on these results, it is advised that producers seeking to maximize live weight gain or milk yield prioritize the first cut. Due to their fibrous texture, the third or fourth cuts may be utilized as "fillers" for dry animals or low-yielding herds. Consequently, future studies should physiologically investigate the effects of this forage on fattening performance, meat, and milk quality of ruminants.

Declarations

Data Availability Statement

The raw data and datasets generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Competing Interest Declaration

The authors declare no competing interests.

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Figures

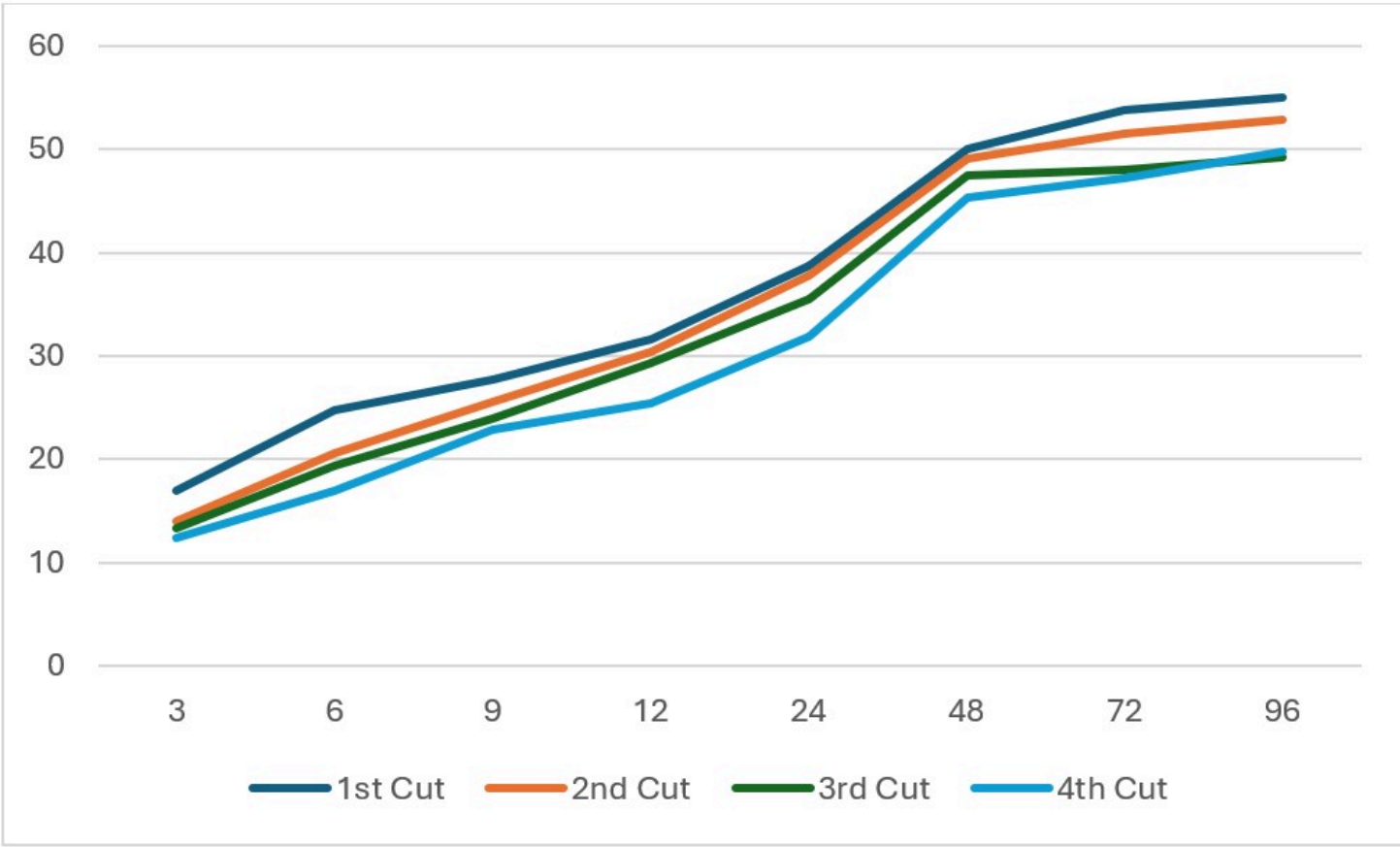


Figure 1

The *in vitro* gas production dynamics of Sunn Hemp's across different cutting stages (ml/200 mg DM)