NUTRIENT COMPOSITION, RUMEN DEGRADATION CHARACTERISTICS AND FEEDING VALUE OF CHANHLUONG GRASS (*Leptocarpusdisjunctus* Mast.) - A DROUGHT TOLERANT GRASS IN VIETNAM

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Abstract:
This study aimed to investigate the chemical composition, rumen degradation characteristics and feeding value of Chanhluong grass – a drought-tolerant grass at three harvest times as ruminant feed, including pre-flowering, flowering and post-flowering stages. The gas production technique was used for the estimation of the degradation of dry matter (DM), organic matter (OM) and metabolic energy (ME). The nylon bag method was used to investigate the degradability of DM and OM. The feeding values were evaluated. Results showed that the DM and neutral detergent fiber (NDF) content of the pre-flowering stage (39.57 and 68.50%) were lower than flowering stage (40.38 and 70.14%) and post-flowering stage (45.23 and 71.66%) ($P<0.001$). The crude protein (CP) content of the pre-flowering stage (6.57%) was higher, (6.04%) and higher than the post-flowering stage (5.04%) ($P<0.001$). The ADF content of pre-flowering (40.34%) and flowering stages (41.36%) was similar and lower than that of the post-flowering stage (43.78%). The estimated ME decreased by stages (pre-flowering > flowering > post-flowering stage), and this difference was statistically significant ($P = 0.001$). The grass's rumen DM and OM degradation at the pre-flowering stage was significantly ($P<0.05$) higher than the flowering and post-flowering stage. The comprehensive analysis of our current study showed that Chanhluong grass at the pre-flowering stage had the highest ruminal degradability and feeding value, therefore, a promising alternative to utilise for ruminant feeding, especially in arid regions.

Keywords: Drought-tolerant grass, Nylon bag, Gas production, Feeding value

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INTRODUCTION

Forage crops for livestock are essential for ruminant production, with grazing land accounting for approximately 60% of global agriculture (FAO 1997). Continued population growth is predicted to grow to 9.7 billion by 2050, which will lead to an increase in demand for animal products coupled with pressure to reduce polluting output. Ruminant production increasing has been achieved through continual improvement of forage genetics, with a major focus on traits such as performance and digestibility (Kingston *et al.* 2013). However, the lack of forage sources for ruminants still occurs in many countries around the world. Similarly, in Vietnam, beef cattle production has been a traditional and important component of the smallholder farm system but feeding these livestock has been a major challenge (Ba *et al.* 2013). Especially in the context of climate change, high temperature in the summer season, long rain in the winters season, very few grass varieties can survive these extreme periods. Therefore, the shortage of forage for ruminants occurs in the whole country and is becoming more and more serious. So, finding forage plants that can adapt to hot climates and high temperatures is extremely necessary (Elizabeth *et al.* 2022).

In Vietnam, Chanhluong grass (*Leptocarpus disjunctus* Mast.) also known as Ruoi grass (Fig. 1), is a grass with strong vitality, it can withstand water logging for 2-3 months in the rainy season, on the contrary, it can withstand the scorching heat burning on white sand in summer. Plants with underground leaves are also stiff tubular branches, usually about 1m high, about 3mm in diameter. In the summer, the Chanhluong grass is an important source of green fodder for ruminants in the coastal sand region of Vietnam. However, up to now, there is no information about the chemical composition as well as the feeding value of this species for ruminants.

The feed quality of grass is determined by the quality of the animal products (beef, milk,..) and its content. Gürsoy (2021) reported that the maturity or growth stage is the main factor responsible for the decrease in the nutritional value of a feed, and in case the feed is too mature, its fiber content increases and the factors such as digestibility and CP in feed tissue decrease. Therefore, the time of grass harvesting is also very important to ensure the nutritional value. Furthermore, Kaur *et al*. (2011) recommended that a study has documented that the chemical composition and the degradation characteristics of the forage in the rumen are important indicator for evaluating the nutritional value. However, very limited data is available on the chemical composition and rumen degradability characteristic of low quality forage, especially of Chanhluong grass. Therefore, the aim of present study to investigate the chemical composition, rumen degradation characteristics and feeding value of Chanhluong grass *Leptocarpus disjunctus* Mast.) – a drought tolerant grass in Vietnam.

MATERIALS AND METHOD

Grass was harvested at the time of pre-flowering, flowering and post-flowering in the dry season. Samples of grass were collected and brought to the laboratory to be dried at 60°C, then finely ground to a size of 1 mm and analyzed for chemical composition including dry matter (DM), organic matter (OM), crude protein (CP), Ether extract (EE), Ash, crude fibre (CF). The chemical composition was analyzed according to the method of AOAC (1990). Acid detergent fibre (ADF) and neutral detergent fibre (NDF) were determined according to the method of Van Soest*et al.* (1991).

*In vitro gas production:*Rumen fluid was collected before morning feeding from 4 fistulated beef cattle fed on 70% of roughage feed and 30% of concentrate. The rumen fluid was immediately transferred to the laboratory in a warmed thermos flask (39 ±0.5^{0}C), and then filtered through 4 layers of cheesecloth to eliminate feed particles and then mixed with the buffer mineral solution in a 1 to 9 ratio. All operations were made under anaerobic conditions by flushing with carbon dioxide. Buffer mineral solution according to Theodorou*et al.* (1994) was preheated in a water bath at 39 ^{0}C and purged continuously with $CO_2$for 30 minutes.

Three feeds were investigated, including three harvest times (pre-flowering, flowering and post-flowering). Weighing 250 mg of the air-dried substrate was incubated in 120 ml bottles containing 30 ml of mixed rumen fluid and buffer mineral solution (Menke and Staingass, 1988). The total gas production was measured at 6, 12, 18, 24, 36, 48 and 72 hours during the incubation using a manual pressure transducer (Digitron 2023P, Digitron, Torquay, Devon, UK) combined with a syringe. Digestibility of DM, OM and the pH value was measured at 24 hour of incubation, the pH was measured by a pH meter (Model HI8314, Hana, Rumania). The metabolic energy (ME) was estimated by equation: ME (MJ/kg DM) = 2.2 + 0.136 Gas_{24} + 0.057CP + 0.029CF.

In situ nutrient degradability: The nylon bag technology was used to determine the rumen degradation characteristic of DM and OM of three kinds of feed. The bag size is 8 x 12 cm with a pore size of 50 um (Ruitong Biotech Co., Ltd., Xinjiang, China). The pre-flowering, flowering and post-flowering samples were milled through a 2.5 mm sieve. Three grams of each sample was weighed and sealed in each nylon bag. Consequently, a polyethylene plastic tube with a length of approximately 70 cm was prepared with gaps of approximately 1 cm cut at a distance of 3 cm from the port and a small hole drilled at the other end and the nylon bag containing the samples was fixed on the soft rubber stopper with rubber bands. The other end of the tube was tied with a nylon rope, approximately 20 cm in length, through a small hole. The polyethylene plastic tube with the bag fixed was fed into the rumen one hour before the morning feeding. Each forage sample was put into the rumen of 2 cattle(each cow had two parallel bags of each forage at a given time point). All the samples were incubated in the rumen for 4h, 8h, 12h, 24h, 48h and 72h. There were 42 bags in the rumen of each cattle for all time points. The two bags of each feed sample from the rumen were taken out at each time point; that is, 8 bags were taken out from the rumen of each cattle at each time point, and were continued till 72 h. After removal from the rumen, the nylon bags were rinsed quickly under the water to prevent fermentation. Consequently, the nylon bags were washed with tap water till the water ran clear. Furthermore, the nylon bags were oven-dried at 60^{o}C for 48h to constant weight and regain moisture for 24 h and then ground using a ball mill to pass through a 1 mm screen. Then, they were thoroughly mixed, and the DM and Ash of samples were analyzed using the method previously described. The degradability value at time 0 was obtained by rinsing four bags per sample

Feeding value evaluation: The feeding value of grass was measured based on the description of Ma *et al* (2021). The quality and expected feed intake of roughage: RFV% = DMI × DDM/ 1.29 was measured according to the relative value of roughage (Rohweder *et al.* 1983). The unit for DMI is % of BW (bodyweight) and was only calculated according to the weight of each animal. Relative feed value (RFV) was proposed by the American Pasture and Grassland Council (Rohweder *et al.* 1983). The other indicators examined in our study were: relative forage quality (RFQ): RFQ% = DMI × TDN/1.23 (Wang *et al.* 1997); total digestible nutrients (TDN): TDN% = 82.38 − (0.7515 × ADF); dry matter intake (DMI): DMI% = 120/NDF; digestible dry matter (DDM): DDM% = 88.9 − 0.779 × ADF (Rohweder *et al.* 1983).

Statistical analysis: Data were analyzed using the GLM procedure of SPSS 16.0. The following models were used to determine treatment mean differences using a least significant difference method. The model was: $Y_{ij} = \mu + R_i + e_{ij}$
Where μ is the overall mean; R_i is the effect of feeds and e_{ij} is the residual effect. In all the analyses, significant effects were declared at p<0.05.

RESULTS AND DISCUSSION

Chemical composition of the Chanhluong grass: The chemical composition of the three harvest times of grass (pre-flowering, flowering and post-flowering) is shown in Table 1. The DM, CP, EE, ADF, NDF and Ash content significantly differed between the grass's three harvest times. The DM and NDF content of the pre-flowering stage (39.57 and 68.50%) was lower than flowering (40.38 and 70.14%) and post-flowering stage (45.23 and 71.66%) (P<0.001). The CP content of the pre-flowering stage (6.57%) was higher than that of the flowering (6.04%) and higher than the post-flowering stage (5.04%) (P<0.001). The ADF content of pre-flowering (40.34%) and flowering (41.36%) was similar and lower than that of the post-flowering stage (43.78%) (P<0.001). The Ash content was the highest in the pre-flowering stage (2.59%) and lowest in the post-flowering stage (1.95%) (P<0.001). The DM content in Chanhluong grass at three harvest times in this study are higher than those of some grass growth in Vietnam as elephant grass VA06 (15.5%), Ghine hamil grass (21.54%), Decumben grass (21.63%), Ruzi grass (25.6%) (Thang et al., 2019), TD58 grass (23.7%), Mulato II grass (22.3%), Paspalum grass (20.6%) (Mui et al., 2017). Similarly, the CF content in Chanhluong grass in three harvest stages are higher than the results of analysis of CF of grasses (Elephant grass VA06, Ghine Hamil grass) ranges from 20.2 to 30.8% (Thang *et al.* 2019). The NDF content is higher than the NDF of Ruzi grass (65.8%) and sweet Sorgho grass (53.5%), but it is similar to the NDF of Lemongrass grass (69.6)%, Paspalum grass (68.6%) and lower than NDF of Elephant grass (74.1%) (Dung *et al.* 2007).The CP content of Chanhluong grass in this study is lower than the CP content of Elephant grass VA06 (6.8%), Ghine Hamil grass (9.7%), Decumben grass (11%) and Ruzi grass (12.1%) (Thang *et al.* 2019), Lemongrass grass (7.7%), Paspalum grass (8.1%), and sweet Sorgho grass (14.1%)(Dung *et al.*2007).

From the results of chemical composition, it was shown that the Chanhluong grass has a much lower nutritional value than the common grasses grown in Vietnam (higher DM, CF and NDF, lower CP). This is also understandable because the Chanhluong grass is a highly drought tolerant grass, living in arid, water-deficient areas.

***Gas production and nutrient digestibility In vitro**:* Total gas production of Chanhluong grass in three harvesting times incubated to 72h is shown in Fig 2. The gas production ability of Chanhluong grass at different growth stages is different, the highest is in the pre-flowering stage, followed by the flowering stage and finally the lowest at the post-flowering stage. The gas production potential in this study showed that there is a positive relationshipwith CP content, whereas there is a negative correlation with ADF and NDF content. This result is consistent with the conclusion of Kazemi *et al.* (2012) when evaluating the gas production potential of forage species for ruminants.

The results obtained in Table 2 show that the *in vitro* degradability of DM at 24 h after incubation of the grass in pre-flowering stage was significantly different compared to degradability of DM of grass in the flowering and post-flowering stages (P<0.05). Similarly, the OM digestibility of grass at pre-flowering stage is higher than that of grass harvest in flowering or post-flowering period (P<0.001). The OMD of grass at the pre-flowering stage in this study is higher than that of Mui et al. (2017) on TD58 grass (43,3%), VA06 grass (49.4%), Mulato II grass (48.1%), Ruzi grass (47.5%) and Paspalum grass (40.4%). However, the OMD of grass at flowering and post-flowering stage in this study is lower than that of grasses in study of Mui et al. (2017). The estimated metabolizable energy (ME) decreased by stage (pre-flowering > flowering > post-flowering stage) and this difference was statistically significant (P = 0.001). The highest ME at the pre-flowering stage (4.50 MJ/kgDM), the flowering and post-flowering stages had similar results is 3.82 and 3.63 MJ/kgDM, respectively. The ME content of grass in this study is lower than the estimated ME in study of Thang *et al.* (2019) on elephant grass (7.4 MJ), Ghine-Hamol grass (6.2 MJ), Decumben grass (7.34 MJ) and Ruzi grass (7.13 MJ).

The result of DDM, OMD and ME in this study indicated that the DDM, OMD and ME value of Chanhluong grass is lower than that of some other grass varieties, especially at the flowering and post-flowering stage. This shows that it is necessary to harvest grass before flowering for ruminants. Although the quality is lower, it is nevertheless a highly drought tolerant variety that lives in arid regions. This is something to keep in mind when using as feed for ruminants, especially in arid regions.

In situ nutrient degradability: The DM degradation of the grass at the pre-flowering stage was significantly (P<0.05) higher than of the flowering and post-flowering stage at 4, 8, 12, 24, 48 and 72h in rumen (Table 3). No significant difference was found when comparing the degradability of DM between flowering and post flowering stage (P>0.05). Similarly, the OM degradation of the grass in this study is higher at the pre-flowering stage than that at flowering or post-flowering (P<0.05), the OM degradation at flowering and post-flowering stage is similar (P>0.05) (Table 3).

The rumen degradability of nutrient in forage feeds were affected by the cellulose content and the degree of lignifications in the feed, which reflects the difficulty of rumen degradability of the forage (Ma *et al.* 2021; Gutiérrez *et al.* 2012). The DM and OM degradation of the Chanhluong grass is consistent with the trend of ADF and NDF content in grass, the ADF and NDF of the grass at pre-flowering is lower than that at flowering and post-flowering. Shahbazi et al. (2012) reported that the ADF is mainly composed of lignin, cellulose, and silicon dioxide and is thus considered the most indigestible part of roughage. Due to the unique structure of lignin, it might have been difficult to be decomposed by rumen microorganisms. High levels of ADF and NDF content in the grass at flowering and post-flowering may be the main reason for the low of DM and OM degradation in the rumen compared to DM or OM degradation at pre-flowering stage. The degradation time in the rumen prolongs, the rumen degradability of DM and OM gradually increases and eventually stabilizes. The rumen degradability trend of DM of grass in rumen in this study was consistent with the results of Ma *et al.* (2021).

Feeding value evaluation: The TND (52.07%) and DDM (57.48%) of the grass at the pre-flowering stage and flowering stage (TDN: 51.3%, DDM: 56.68%) was higher than of post-flowering stage (TND: 49.48%, DDM: 54.79%). The DMI (1.75%), RFV (78.05%) and RFQ (74.15%) of the grass at the pre-flowering stage was higher than of flowering stage (DMI: 1,71%, RFV:75.18%, RFQ: 71.36%) and higher than of post-flowering stage (DMI: 1.68%, RFV: 71.12%, RFQ: 67.35%). The estimated feeding value of grass at three harvest times (pre-flowering, flowering and post-flowering) has been summarized in Table 5. The RFV (relative feed value) is a comprehensive reflection of ADF and NDF content in feed, and it was used to estimate the roughage quality. Rohweder *at al.* (1983) recommended that the RFV of general high-quality roughage is assumed to be higher than 100. In this study, the RFV of the Chanhluong grass at three harvest times was lower than 100, it is indicated that Chanhluong grass was low-quality roughage. Ma *et al.* (2022) concluded that RFQ was closer to the actual situation than RFV and could accurately classify forage. In the current study, the RFV and RFQ of the grass at the pre-flowering stage were higher than of the grass at the flowering and post-flowering stages. It is well known that TDN reflects the degradation characteristics of the roughage itself. In the current study, the TDN and DDM values of the grass at the pre-flowering and flowering stages were higher than of the grass at the post-flowering stage. The comprehensive analysis of our current study showed that Chanhluong grass at the pre-flowering stage had the highest feeding value. Thus, it is recommended that Chanhluong grass should be harvested and preferred as a roughage resource in ruminant feeding practice.

There were differences in the nutrient contents of the Chanh Luong grass at three harvest times. The grass harvested at the pre-flowering stage had the highest CP and the lowest NDF and ADF contents. At the same time, the grass at the pre-flowering stage had the highest effective degradation rate of DM and OM in the rumen, in general, the feeding values of Chanhluong grass at a pre-flowering stage were higher than those at the flowering and post-flowering. Based on the present study, the grass harvested at pre-flowering had the highest ruminal degradability, therefore, a promising alternative to utilize for ruminant feeding, especially in arid regions.

DECLARATIONS

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Authors' Contribution
Phan Ba Thuy and Doan Thi Phuong Thu, Le Duc Thao, Hoang Dinh Trung, Bui Van Loi collected the plants and took care of animals during the experiment and sample collection. Bui Van Loi and Dinh Van Dung as the main authors conceived the idea, designed, implemented and analysed the study and wrote the first draft. Hoang Huu Tinh, Vo Thi Minh Tam, Le Duc Thao, Hoang Dinh Trung implemented and sample analysed. Bui Van Loi made a final revised and submitted the manuscript.

Conflict of interests
The authors have not declared any conflict of interest.

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Table 1. Chemical composition of the grass (% DM basis)

Kind of feed	DM	CP	EE	CF	ADF	NDF	Ash
Pre-flowering	39.57^a	6.57^a	1.77^a	42.31	40.34^a	68.50^a	2.59^a
Flowering	40.38^b	6.04^b	1.82^b	44.60	41.36^b	70.14^b	2.58^b
Post-flowering	45.23^c	5.04^c	1.52^a	43.84	43.78^b	71.66^c	1.95^c
SEM	0.171	0.031	0.17	0.717	0.243	0.163	0.015
P-value	<0.001	<0.001	<0.001	0.150	<0.001	<0.001	<0.001

DM: dry matter, CP: crude protein, EE: ether extract, CF: crude fibre, ADF: acid detergent fibre, NDF: neutral detergent fibre,^{a,b,c}: means that do not share a letter are significantly different

Table 2. Estimattion of degradability of DM, OM and ME content of the grass

Items	Pre-flowering	Flowering	Post-flowering	SEM	P-value
DDM (%)	48.03^a	37.13^b	35.62^b	1.501	0.002
OMD (%)	51.35^a	36.86^b	31.20^c	1.256	<0.001
ME (MJ/kgDM)	4.50^a	3.82^b	3.63^b	0.078	0.001

DDM: degradability of dry matter, OMD: organic matter digestibility, ME: Metabolic energy,^{a,b,c}: means that do not share a letter are significantly different

Table 3. Rumen degradability of DM and OM of the grass

Items	Pre-flowering	Flowering	Post-flowering	SEM	P-value
Rumen degradability of DM					
4h	21.58^a	12.78^b	9.83^b	1.254	0.001
8h	30.13^a	15.35^b	13.33^b	0.915	<0.001
12h	33.81^a	21.30^b	18.54^b	0.544	<0.001
24h	43.30^a	35.87^b	32.11^b	1.597	0.007
48h	53.93^a	48.79^b	48.02^b	1.023	0.013
72h	64.82^a	62.03^b	60.42^b	0.786	0.020
Rumen degradability of OM					
4h	23.23^a	14.55^b	11.28^b	1.232	0.001
8h	31.15^a	15.80^b	14.09^b	0.050	<0.001
12h	34.83^a	22.98^b	18.98^c	0.537	<0.001
24h	44.21^a	36.33^b	32.21^b	1.587	0.005
48h	54.91^a	49.20^b	48.78^b	1.003	0.009
72h	65.60^a	62.26^b	60.54^b	0.780	0.010

SEM: standard error of the mean,^{a,b,c}: means that do not share a letter are significantly different

Table 4. Estimated feeding value of the grass

Items	Pre-flowering	Flowering	Post-flowering	SEM	P-value
TND (%)	52.07^a	51.30^a	49.48^b	0.257	0.001
DMI (%)	1.75^a	1.71^b	1.68^c	0.004	<0.001
DDM (%)	57.48^a	56.68^a	54.79^b	0.267	0.001
RFV (%)	78.05^a	75.18^b	71.12^c	0.232	<0.001
RFQ (%)	74.15^a	71.36^b	67.35^c	0.241	<0.001

TND: total digestible nutrients, DMI: dry matter intake, DDM: digestible dry matter, RFV: relative feed value, FRQ: relative forage quality,^{a,b,c}: means that do not share a letter are significantly different.

Fig 1. Chanhluong grass in Central Coastal Vietnam

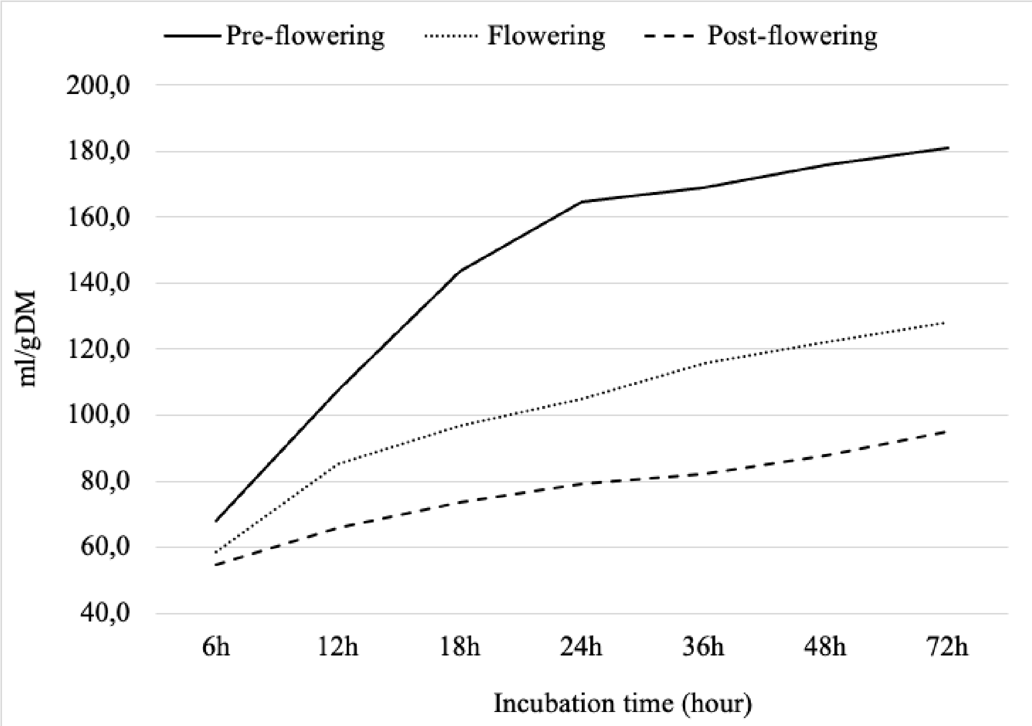

Fig 2. Gas production of Chanh Luong grass in three harvesting times