NUTRIENT COMPOSITION, RUMEN DEGRADATION CHARACTERISTICS AND FEEDING VALUE OF WATERLOGGED GRASS (*BRACHIARIA HUMIDICOLA)* FOR RUMINANT FEED IN VIETNAM

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ABSTRACT:

The objective of this study was to investigate the chemical composition, rumen degradation characteristic and feeding value of *Brachiaria humidicola* grass – a waterlogged grass in Vietnam at three harvest times as ruminant feed, including pre-flowering, flowering and post-flowering stage. 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 fibre (NDF) content of the pre-flowering stage (18.78 and 61.87%) were lower than that of the flowering stage (20.09 and 60.99%) and post-flowering stage (22.49 and 63.46%) ($P<0.001$). The crude protein (CP) content of the pre-flowering stage (8.57%) was higher than that of the flowering stage (8.28%) and higher than the post-flowering stage (6.19%) ($P<0.001$). The rumen DM and OM degradation of the grass at the pre-flowering stage was significantly ($P<0.05$) higher than that of the flowering and post-flowering at four hours in the rumen, and at other times, it was the same ($P>0.05$). The comprehensive analysis of our current study showed that *Brachiaria humidicola* grass at the pre-flowering stage had the highest feeding value. Based on the present study, the grass harvested at pre-flowering had the highest ruminal degradability; therefore, it is a promising alternative for ruminant feeding, especially in flooded regions.

Keywords: Waterlogged grass, nylon bag, feeding values

INTRODUCTION

The grazing land for approximately 60% of global agriculture and forage crops for livestock is essential for ruminant production (FAO, 1997). Continued population growth is predicted to grow to 9.7 billion by 2050, leading to increased demand for animal products coupled with pressure to reduce polluting output. Ruminant production increase has been achieved through continual improvement of forage genetics, with a major focus on traits such as performance and digestibility (Kingston-Smith et al., 2013). However, the lack of forage sources for ruminants still occurs in many countries around the world. Similarly, in Vietnam, Ba at al. (2013) reported that beef cattle production has been a traditional and important component of the smallholder farm system but feeding these livestock has been a major challenge. Especially in the conditions of climate change, high temperature in summer, prolonged rain in winter, very few grass varieties can survive this harsh period. Therefore, the shortage of forage for ruminants occurs throughout the country and is increasingly serious during the flood season.Therefore, it is necessary to find forage that can adapt to inundation conditions (Elizabeth et al., 2022). In Vietnam, *Brachiaria humidicola* grass is a new grass variety grown as forage for ruminants (Loi et al., 2019). The characteristic of *Brachiaria humidicola* grass is that it can live in flooded conditions for a long time. Therefore, the *Brachiaria humidicola* is often planted under rice fields[5]. In the flooded season, the *Brachiaria humidicola* grass is an important source of green fodder for ruminants in 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 a grass is determined by the quality of the animal products (beef, milk,..) and its content (Ma et al., 2021). Gürsoy (2021) reported that, maturity or growth stage is the main factor responsible for 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. Howerver, very limited data is available on the chemical composition and rumen degradability characteristic of low quality forage, especially of *Brachiaria humidicola* grass. Therefore, the aim of this study to investigate the chemical composition, rumen degradation characteristics and feeding value of *Brachiaria humidicola* grass – a waterlogged grass in Vietnam.

MATERIALS AND METHODS

Feed chemical composition analysis

The grass was harvested three times, including the pre-flowering, flowering and post-flowering stages in the rainy 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 using the AOAC method (1990). Acid detergent fibre (ADF) and neutral detergent fibre (NDF) were determined according to the method of Van Soest et al. (1991).

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 two gaps of roughly 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 grass sample was put into the rumen of 2 cattle (each cow had two parallel bags of each grass 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°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.

The degradation data wre fitted to the following exponential equation (Ørskov and Mcdonald 1979).

$P = a + b\,(1 - e^{-ct})$ (1)

P is the nutrient disappearance rate in the rumen at a time “t”; “a” is a rapidly degradable fraction; “b” is the potentially degradable fraction; “c” is the constant rate of degradation of “b”(%/h); and “e” is the base of natural logarithms. “a + b” is the total degradable fraction. The NLIN program in SAS (9.2) was used to calculate the values of a, b, and c.

Then, the effective degradability (ED) of DM and OM of forage samples were calculated by applying the following equation (Ørskov and Mcdonald, 1979):

$ED\ (\%) = a + b \times c/(k + c)$ (2)

a, b and c are the same parameters represented in Equation (1), and “k” is the rumen outflow rate of the nutrient component (%/h). In this study, the value of k was 2%/h. The NLIN program in SAS (version 8.2; SAS Institute, Inc., Cary, NC, USA) was used to calculate the values of a, b, and c.

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 (body weight) 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 and Ripley, 1997); total digestible nutrients (TDN): TDN% = 82.38 − (0.7515 × ADF) (Rohweder et al., 1983), dry matter intake

(DMI): DMI% = 120/NDF (Rohweder et al., 1983); 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 of mean; R_i is the effect of harvest time and e_{ij} is the residual effect. In all the analyses, significant effects were declared at $p<0.05$.

RESULTS

Nutrient content of grass at three harvest times

There were significant differences between the harvest time of the grass in terms of nutrient contents (P<0.001) (Table 1). The DM, CF, ADF and NDF content of grass at pre-flowering stage were lower than that of flowering stage, and these nutrients of grass at flowering stage were lower than that of post-flowering stage (P<0.001). The CP, EE and Ash content of grass at pre-flowering stage was higher than that of the flowering stage. Similarly, the CP, EE and Ash content of grass at flowering stage was higher than that of post-flowering stage (P<0.001).

Rumen degradability and degradation parameters of the DM and OM of the grass

The DM degradation of the grass at pre-flowering stage was significantly (P<0.05) higher than that of the grass at flowering and post-flowering stage at 4h in rumen. The DM degradation of the grass at pre-flowering and flowering stage was higher than that of post-flowering stage (P<0.05). Meanwhile, the DM degradation of grass at the time of 8, 12, 24 and 72h had no significant difference between the time of harvesting. The rapidly degradable fraction (a) of grass at pre-flowering stage was significantly higher (P<0.05) than of post-flowering, while the constant rate (c) of the potentially degradable fraction and the potentially degradable fraction (b) and the effective DM degradation (ED) of DM were no significant difference between the time of harvesting of grass (P>0.05) (Table 2). Similarly, the results of Table 3 showed that OM degradation of grass at pre-flowering stage was significantly (P<0.05) higher than that of the grass at flowering and post-flowering stage at 4h in rumen. Whereas, no significant difference was found when comparing the OM degradability of grass at the time of harvest at 8, 12, 24, 48 and 72h in rumen (P>0.05). The rapidly degradable fraction (a) of grass at pre-flowering stage was significantly higher (P<0.05) than of post-flowering, while the constant rate (c) of the potentially degradable fraction and the potentially degradable fraction (b) and the effective DM degradation (ED) of OM were no significant difference between the time of harvesting of grass (P>0.05).

DISCUSSION

Brachiaria humidicola grass is a new grass variety grown as forage for ruminants in Vietnam (Loi et al., 2019). The characteristic of *Brachiaria humidicola* is that it can live in flooded conditions for a long time. Therefore, the *Brachiaria humidicola* is often planted under rice fields. Compared with the common grass varieties grown in Vietnam such as elephant grass VA06, Ghine hamil grass, Decumben grass, Ruzi grass (Thang et al., 2019), TD58 grass, Mulato II grass, Paspalum grass (Mui et al., 2017), the DM, EE, NDF, ADF and CF content of *Brachiaria humidicola* grass are similar. However, the CP content of *Brachiaria humidicola* is lower than that of common grassses grown in Vietnam. The harvest time of forage grass is one of the important factors affecting nutrients contents (Ma et al., 2021). In present study, the content of CP was higher than and the content of GF, NDF and ADF was lower in *Brachiaria humidicola* grass at pre-flowering stage, these results is consistent with the findings documented in the previous studies (Ma et al., 2021; Zhao et al., 2021). The CP is the most limiting factor of *Brachiaria humidicola* grass in present study, some previous authors reported that the rumen activity of microbial was inhibited and intake of forage was decreased when the CP content in forage was below the lowest threshold level (7.0%) (Soest, 1994; Naves et al., 2013). In present study, the CP content of grass at pre-flowering and flowering stage was higher than 7.0%, which implies that the CP content in these harvest times can suffice the basic requirements of rumen microbial activity. However, the CP content of grass at post-flowering stage was lower than 7.0%, which indicated that the grass should be harvested at pre-flowering or flowering stage instead of post-flowering harvest. The DM and OM degradation of *Brachiaria humidicola* grass is the same. As the degradation time in the rumen prolongs, the DM and OM degradation gradually increases and eventually stabilizes. The DM and OM degradation trend of grass in present study was consistent with the results of previous studies (Ma et al., 2021; Mehrez and Rskov, 1997). The ED value of DM and OM is the same and higher than ED value of DM of some grass was studied in China such as Chinese rye grass or Barley grass (Ma et al., 2021).

Ma et al. (2021) recommended that feed value was used to evaluate an important economic characteristic of forage. 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 *Brachiaria humidicola* grass at three harvest times was lower than 100, it is indicated that *Brachiaria humidicola* grass was low quality roughage. Ma et al. (2021) 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 pre-flowering stage was higher than of the grass at flowering and post-flowering stage. 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 pre-flowering and flowering stage were higher than of the grass at post-flowering stage. The comprehensive analysis of our current study showed that *Brachiaria humidicola* grass at pre-flowering stage had the highest feeding value. Thus, it is recommended that *Brachiaria humidicola* grass should be harvested at pre-flowering and preferred as a roughage resource in ruminants feeding practice

CONCLUSION

There were differences in the nutrient contents of the *Brachiaria humidicola* grass at three harvest times. The grass harvested at pre-flowering stage had the highest CP and the lowest NDF and ADF contents. In general, at the same time, the grass at pre-flowering stage had the highest effective degradation rate of DM and OM in the rumen, in general the feeding values of *Brachiaria humidicola* grass at 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 flooded areas

DECLARATIONS

All of the authors agreed to publish, respond to all of the steps, and submit our manuscript. All of the data are available when the journal asks for a commitment that experiments have been done under consideration of the university scientific committee.

Authors' Contribution

Bui Van Loi, the main author, conceived the idea, designed, implemented, and analysed the study and wrote the first draft. Contribution by Dinh Van Dung and Hoang Huu Tinh for some steps in the faculty of Animal Sciences and Veterinary Medicine.

Conflict of interests

We have no conflict of interest for this article.

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Table 1- *Chemical composition of the grass at three harvest times (% DM basis)*

Items	DM	CP	EE	CF	ADF	NDF	Ash
Pre-flowering	18.78[a]	8.57[a]	2.01[a]	31.30[a]	37.14[a]	61.87[a]	9.20[a]
Flowering	20.09[b]	8.28[b]	1.82[b]	32.00[b]	37.69[b]	62.99[b]	8.54[b]
Post-flowering	22.49[c]	6.19[c]	1.52[c]	34.19[c]	40.21[c]	63.46[c]	7.84[c]
SEM	0.006	0.061	0.17	0.096	0.208	0.147	0.046
P-value	<0.001	<0.001	<0.001	<0.001	<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- *Rumen degradability and degradation parameters of DM of the grass*

Items	Pre-flower ng	Flowering	Post-fl wering	SEM	P-value
Rumen degradability (%)					
4h	26.06[a]	23.25[b]	23.14[b]	0.593	0.015
8h	30.39	32.35	28.40	2.397	0.578
12h	35.98	33.72	34.92	0.861	0.238
24h	47.95	44.42	44.06	2.336	0.483
48h	59.79[ab]	60.28[a]	56.37[b]	0.832	0.033
72h	66.65	63.23	65.11	2.475	0.674
Degradation parameters					
a (%)	24.73[a]	20.48[ab]	18.72[b]	1.422	0.040
b (%)	46.14	50.57	50.14	2.685	0.480
c (%/h)	3.31	3.52	3.54	0.640	0.960
a+b (%)	60.95	71.05	68.86	2.858	0.832
ED (%)	51.0	49.7	50.0	1.210	0.231

SEM: Standard error of the mean ED: Effective degradabiliy, a,b: Means that do not share a letter are significantly different

Table 3- Ru en degradability and degradation param ters of OM of the grass

Items	Pre-flower ng	Flowering	Post-flowering	SEM	P-value
Rumen degradability (%)					
4h	26.70^a	24.20^b	24.40^b	0.557	0.010
8h	30.56	32.82	28.77	2.502	0.578
12h	37.73	33.96	35.13	1.579	0.243
24h	48.28	44.87	44.84	2.121	0.612
48h	60.17	61.28	57.33	0.895	0.054
72h	65.41	63.97	62.55	2.534	0.642
Degradation parameters					
a (%)	21.72^a	19.03^{ab}	17.25^b	0.986	0.031
b (%)	47.99	52.82	54.55	3.272	0.380
c (%/h)	3.46	3.33	3.25	0.651	0.973
a+b (%)	69.71	73.58	70.07	3.420	0.688
ED (%)	51.5	49.9	50.0	1.102	0.122

SEM: Standard error of the mean, ED: Effective degradability, a,b: 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 (%)	54.47^a	54.06^a	52.16^b	0.156	<0.001
DMI (%)	1.94^a	1.91^b	1.81^c	0.004	<0.001
DDM (%)	59.97^a	59.54^a	57.57^b	0.162	<0.001
RFV (%)	90.16^a	87.93^b	80.58^c	0.365	<0.001

RFQ (%)	85.89[a]	83.73[b]	76.57[c]	0.360	<0.001

SEM: Standard error of the mean, 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