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Characteristics of Ini Vitro Fermentative Digestibility of Odot Grass (*Pennisetum Purpureum Cv. Mott*) At Different Planting Spacing and Defoliation Age

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ABSTRACT

This study aimed to evaluate the characteristics of in vitro fermentative digestibility of odot grass planted at different spacing and defoliation ages-days after planting (DAP). The study was conducted in the Field Laboratory of the Faculty of Animal Science of the Halu Oleo University and the Laboratory of Nutrition for Dairy Sciences of the Faculty of Animal Husbandry, IPB University. The study was designed by using factorial randomized block design (3x3). The first factor was planting spacing (60 cm x 90 cm, 75 cm x 90 cm and 90 cm x 90 cm), and the second factor was defoliation age (60 DAP, 90 DAP, and 120 DAP), consisted of 4 groups and there were 36 treatment combinations. The grouping was based on the slope of the land. The study data were analyzed by using analysis of variance using SPSS 21 and if the treatment had a significant effect, a different test between treatments was tested by using the Duncan's Multiple Range Test (DMRT) test. The experimental results showed that the interaction between planting spacing and defoliation age did not affect the characteristics of fermentation and nutrient digestibility in vitro. However, the in vitro fermentation characteristics were affected significantly (p<0.05) by defoliation age, but were not affected by planting spacing whereas the pH level was not affected by spacing and defoliation age. Further tests of the differences between treatments showed that the planting spacing treatment was not significantly different (p>0.05), whereas among the defoliation age treatments were significantly different (p<0.05). The conclusion of this study was the treatment of planting spacing and defoliation age did not affect digestibility in vitro fermentative of grass odot. The treatment of defoliation age independently influenced the in vitro digestibility of odot grass.

Keywords : Digestibility, fermentative, *in vitro,* nutrient, *pennisetum purpureum* cv. Mott

I. INTRODUCTION

Odot grass is one of the superior grasses because it can produce a high quality of forage, palatable, easily cultivated, resistant to disease, and able to adapt to varied environmental conditions (Kozloski et al 2005). However, the management factor of defoliation is crucial to get enough production and good quality forage continuously (Djajanegara et al 1998).

The quantity and quality of odot grass are influenced by several factors including planting spacing,

defoliation age and the combination of planting spacing and defoliation age. According to Sirait et al (2015), the spacing of planting in the cultivation of fodder forage allows for more optimal forage productivity so that the availability of feed both in terms of quality and quantity can be fulfilled and available throughout the year. Planting spacing affects the level of forage production in one area (Sulistiawati and Maryono 2013). Odot grass production varies greatly and is influenced by various factors, including agro-climate, planting spacing and cultivation management. Odot grass production in several different locations (Halim et al 2013)

The defoliation age at a more mature age of forage is greatly affecting the content of crude protein. Budiman et al (2012) showed a very drastic decrease in crude protein from 12.94% at eight weeks of harvesting time to 8.77% at 12 weeks of harvesting. Bilal (2009) obtained crude protein content of odot grass at 45 days of harvesting by 13.90% and decreased to 11.75% at 60 days of harvesting.

The quality of odot grass could be known through proximate analysis and fermentative digestibility and digestibility analysis in vitro. In vitro technique or often called artificial rumen technique is an anaerobic fermentation experiment in a fermenter tube and uses a buffer solution (artificial saliva) to evaluate the digestibility of feed nutrients including dry matter digestibility (DMD), digestibility of organic matter (OMD), production of VFA and NH_3 (ammonia).

II. MATERIAL AND METHOD

Location, Time and desain research

The research was conducted at the Field Laboratory of the Faculty of Animal Science of Haluoleo University and the Laboratory of Dairy Nutrition Sciences Faculty of Animal Science, IPB University in December 2019. The experimental design used was a factorial randomized block design (RBD) and consisted of 4 groups (Steel and Torrie 1995). The first factor is the planting spacing (60 cm x 90 cm, 75 cm x 90 cm, and 90 cm x 90 cm) and the second factor is the defoliation age (60 DAP, 90 DAP, and 120 DAP). The grouping is based on the slope of the land.

Research Method

In vitro fermentation digestibility was conducted according to the method of Tilley and Terry (1963). The stages of the procedure for measuring the characteristics of digestibility in vitro were as follows: McDougall solution preparation, 0.2% pepsin solution preparation, formulating of an indicator of borate solution, preparation of materials and equipment, taking rumen fluid, using a fermentor tube. The parameters measured in this experiment are pH level, N-NH_3 concentration, total VFA production, and total gas production.

Measured Parameters

a. The pH value of fermentation was measured by using a digital pH meter merk Hanna 70. The concentration of N-NH_3 was analyzed by using *the micro diffusion Conway* (Conway,1962) method. N-NH_3 content was measured based on the formula:

$$\text{Content of N-NH}_3\text{ (mM)} = \frac{\text{mlH2SO4 x NH2SO4 x 1000}}{\text{sample(g)}x\ DMsample(g)}$$

b. The total production of VFA was analyzed by using the steam distillation method according to *General Laboratory Procedure* (1966). Total production of VFA was measured by formula:

Total Production of VFA (mM/ml) = (volume of blanko – volume of sample) x solution normality-HCl x 1000/5 mM

c. The total gas production was measured by the gas test method from Menke et al (2009). Total gas production (GB) was measured by using the formula:

Gb (ml/230 mg DM 24 hours) = {(Gb24 – Gb0)*200*((gas standar+gas standar/actual production)/2)/DM of feedstuff

Analized data

The data obtained in this experiment were analyzed by using analysis of variance. If the treatment had a significant effect, a different test between treatments was continued by using the Duncan Multiple Range Test (DMRT).

III. RESULT AND DISCUSSION

a. Ph Value

The average pH value of the odot grass by planting spacing and defoliation age treatments is presented in Figure 1.

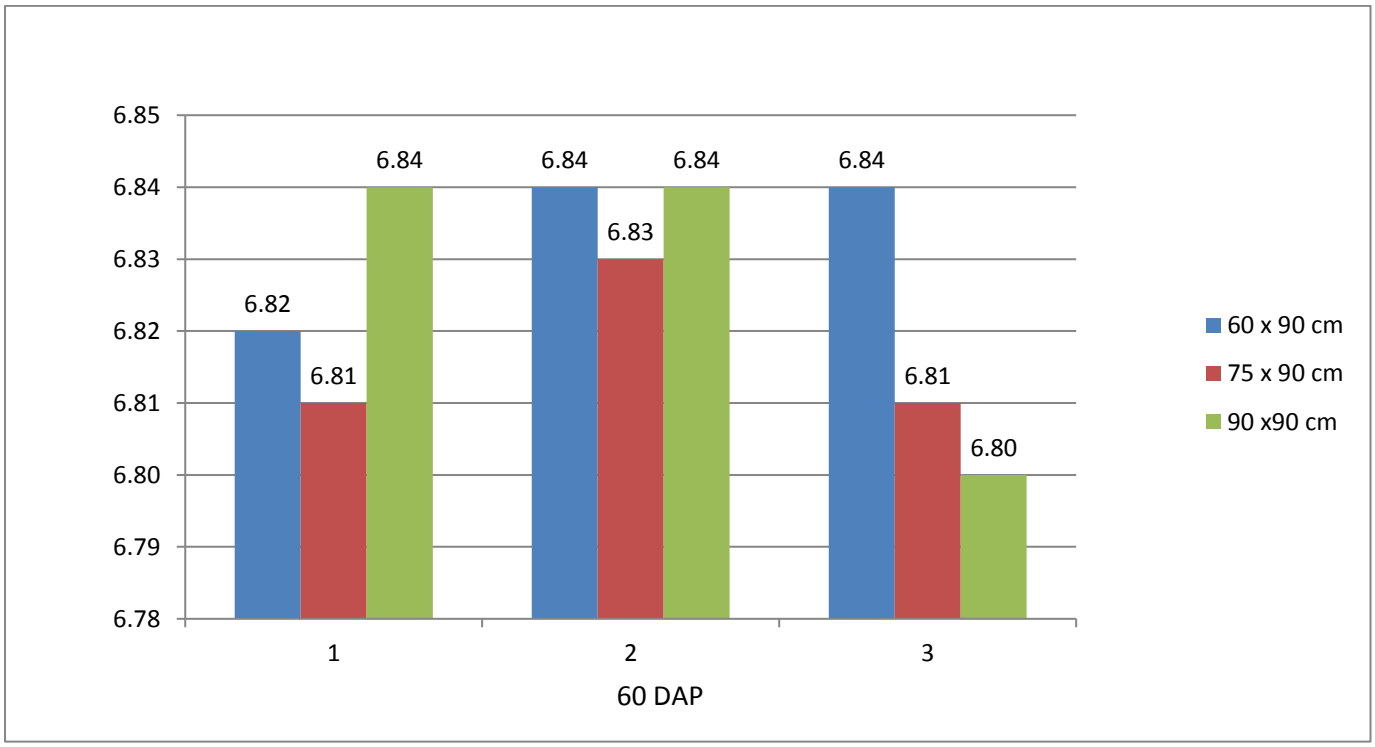

Fig. 1. Diagram of average ph value of odot grass with the different planting spacing and defoliation age treatments

The results of the analysis of variance showed that the interaction of planting spacing and defoliation age had no significant effect (p>0.05) on the average pH level of odot grass. The highest pH level of odot grass was at the defoliation age of 60 DAP which was 6.84. While the lowest average pH level was at the defoliation age of 90 DAP of 6.82. Diagram 1 showed that the interaction of planting spacing and defoliation age did not affect the pH value of odot grass. The average pH of in vitro fermentation of odot grass in all treatment combinations was 6.82. The pH level achieved indicated that in vitro fermentation of grass produced sufficient and optimal fermentation pH value to support the continuation of the fermentation process by microbes during the digestion process in vitro. This is in line with Johnson's report (1996) that rumen microbial activity continues normally when the rumen pH ranges from 6.7 to 7.0. The optimal rumen pH range for the process of cellulolysis, proteolysis, and deamination ranges from 6-7. The pH level achieved in this study was relatively similar as the pH achieved by Bain et al (2017) found pH 6.75 in experiments related to the effect of soybean calcium soap supplementation on fermentation characteristics of rations based on local feed ingredients and natural grass. Degradation of fiber feed takes place optimally at pH 6.5 to pH 6.8. If the pH falls below 6.2 the activity of cellulolytic bacteria starts to be disrupted. While Owens and Goetsch (1988) reported that this pH range is still in accordance with good rumen pH for the fermentation process, which is 5.5-7.22. The accuracy of the result

of in vitro digestibility is influenced by rumen fluid pH, amount of rumen fluid, amount and particle size, and incubation temperature and fermentation duration (Rahmadi et al 2004). Proteolytic enzymes will hydrolyze feed protein into oligopeptides, and then be converted to peptides and amino acids which can be used by some rumen microbes for growth. Ammonia from degradation will be used by bacteria as a source of N for the formation of body protein (McDonald et al 2010). Hindratiningrum et al (2011) added that easily degraded protein sources can increase ammonia production in the rumen.

b. Concentration of N-NH3

The average concentration of N-NH3 (ammonia) from the fermentation process in vitro of odot grass planted with a combination of planting spacing and defoliation age is presented in Figure 2.

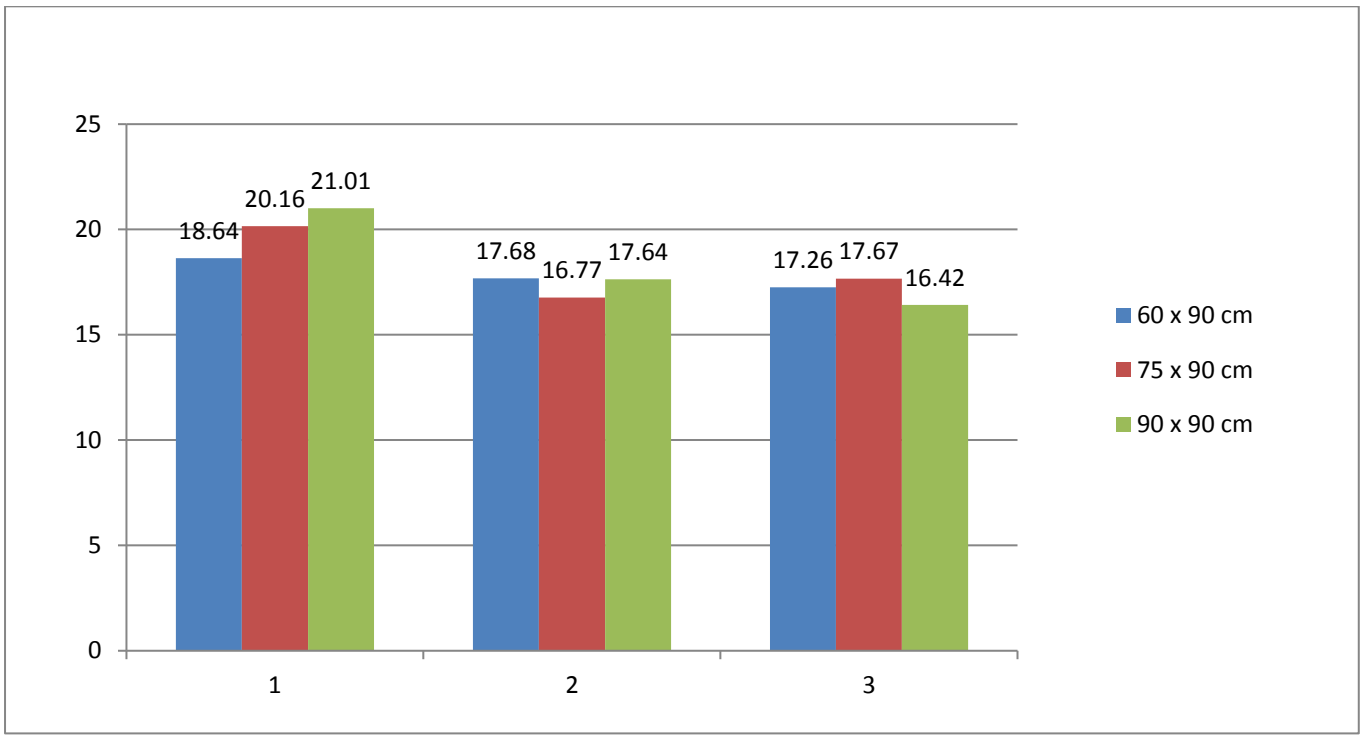

Fig. 2. Diagram of average N-NH3 (µM/ml) in vitro fermentation of odot grass with the different planting spacing and defoliation age treatments

The results of the analysis of variance showed that the interaction of planting spacing and defoliation age had no significant effect (p>0.05) on the average concentration of N-NH3 fermentation in vitro. Defoliation age gave a significant effect (p<0.05) independently on N-NH3 concentration, whereas planting spacing had no significant effect (p>0.05) on the average of N-NH3. The average N-NH3 concentration of odot grass decreased with increasing of planting age. The highest concentration of N-NH3 odot grass was at the defoliation age of 60 DAP which was 19.94 mM/ml while the lowest average of N-NH3 concentration was at the defoliation age of 120 DAP which was 17.12mMol / ml. The average value of N-NH3 concentration in all treatments produced in this study was 18.14 mMol/ml. The highest N-NH3 concentration level was obtained at the defoliation age treatment of 60 DAP. This was probably related to the dynamics of the protein content of odot grass where at the age of young plants tend to have high protein content. According to Haaland et al. (1982) that the high protein content of feed provided, the ammonia production will increase due to increased proteolytic activity. N-NH3 levels obtained in this study ranging from 16.42-21.01 mM/ml were very good levels of N-NH3 when compared to in vitro research reported by Bain et al (2018), which used rumen fluid from Bali cattle, which was only around

9.43 mM/ml to 10.55 mM/ml. This indicated that the use of odot grass as animal feed material is very supportive in creating the condition of the fermentative digestive ecosystem in vitro.

Feed protein that enters the rumen will initially undergo proteolysis by enzymes that become peptides, then hydrolyzed into amino acids which are then quickly deaminated into ammonia. Amino will be used by rumen microbial in the formation of microbial proteins. Measurement of in vitro N-NH_3 can be used to estimate protein degradation and its utilization by microbes (Wahyuni 2008). Excess of N-NH3 will be absorbed through the walls of the rumen and brought to the liver for urea synthesis, conversely, deficiency of N can reduce the production of digestible carbohydrate-digesting microbes. Ammonia concentration is influenced by the crude protein content (Getachew et al 2004; Firsoni et al 2011; Firsoni and Yunita, 2014).

c. Total Production of VFA

The total VFA production of in vitro fermentation using odot grass substrate that is cultivated by different planting spacing and defoliation age treatments is presented in Fig. 3.

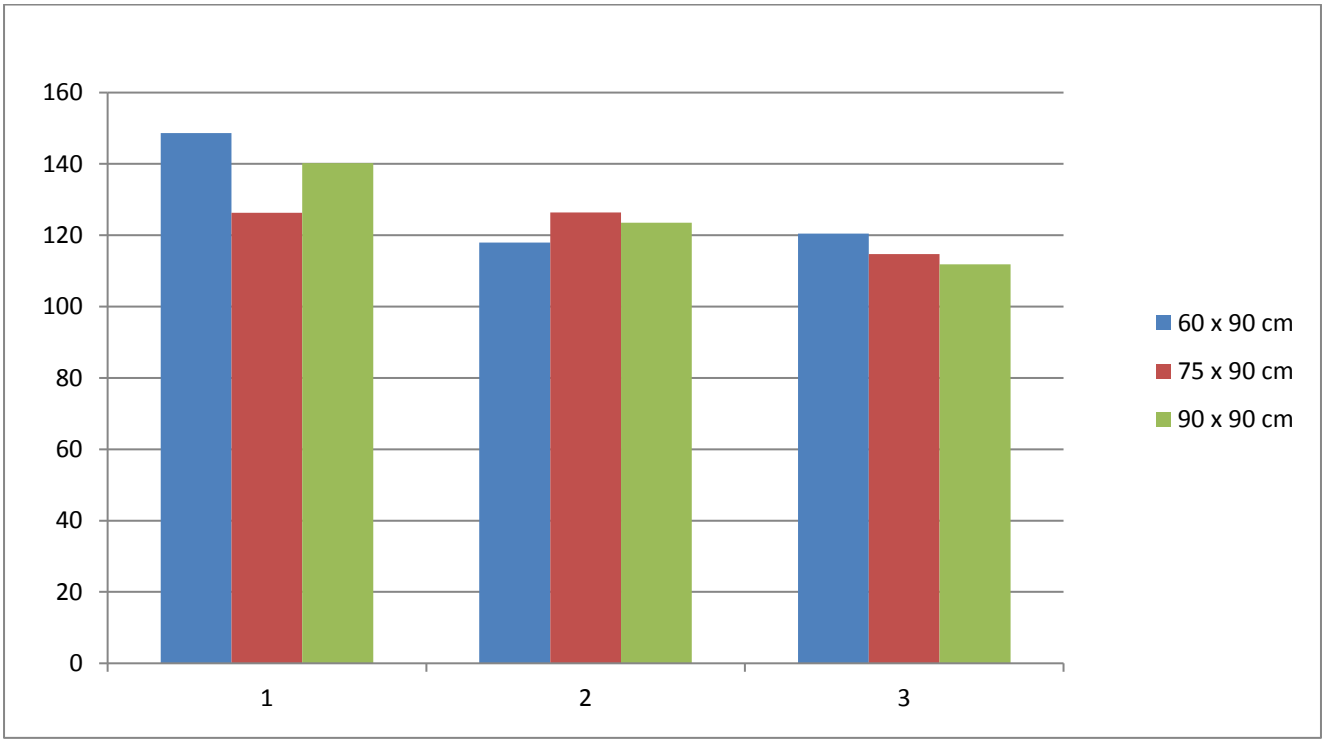

Fig. 3. Diagram of total VFA production of in vitro fermentation of odot grass at the different planting spacing and defoliation age treatments

The results of the analysis of variance showed that the interaction of planting spacing and defoliation age had no significant effect (p>0.05) on the average of VFA total production of in vitro fermentation of odot grass. However, independently, treatment of defoliation age had a significant effect (p<0.05) on total VFA production, while treatment of planting spacing gave no significant effect (p>0.05) on the average of total VFA production. The highest total production of VFA in vitro fermentation of odot grass was obtained at the defoliation age of 60 DAP of 138.39 mM/ml while the lowest average of VFA production was at the defoliation age of 120 DAP which is 115.72mMol/ml. Diagram 3 showed that the total of VFA production of odot grass at different planting spacing and defoliation age was 125.58 mMol/ml. Total VFA production resulted in this study was in line with the average value of VFA production found by McDonal et al (2010) ranging from 129.87 mMol/ml to 146.69 mMol/ml. The results

of the study were relatively similar to the report of Bain et al (2018), which ranged from 125.29 to 167.89 mMol/ml. Meanwhile, McDonald et al (2010) reported that the total production of VFA that can support the process of rumen microbial synthesis in the normal range is around 70 mMol/ml to 150 mMol/ml. VFA is the result of carbohydrate fermentation in the rumen which can be directly used by an animal as an energy source (Parakkasi 1999). Hindratiningrum et al (2011) added that feed with different carbohydrate sources produced different total VFA concentration. This is due to the different levels of fermentability of the feed. VFA can be utilized as an indication that easy or not feed is degraded in the rumen. The high VFA production indicated the capability of rumen microbial to ferment odot grass and became a measure of feed fermentability. According to Sutardi et al (1993), VFA has a dual role as the main energy source for ruminants and a carbon skeleton source for microbial protein formation. VFA production that supports optimal microbial growth is between 80 - 160 mMol/ml.

d. Total of Gas Production

The total gas production of odot grass at different planting spacing and defoliation age treatments is presented in Figure 4.

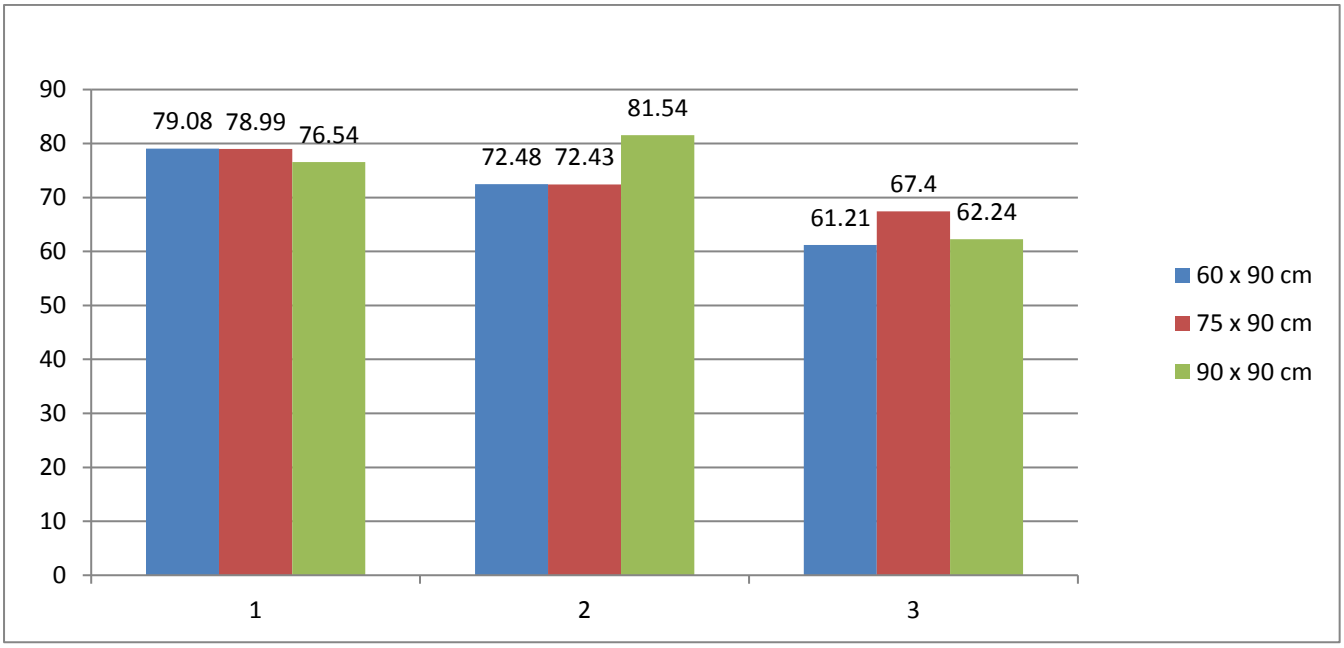

Fig. 4. Diagram of the total of gas production in vitro fermentation of odot grass with the different planting spacing and defoliation age treatments

The results of the analysis of variance showed that the interaction of plant spacing and defoliation age treatments had no significant effect (p>0.05) on the average total of gas production in vitro fermentation. Treatment interactions resulted in an average total gas production of 72.44 mL/200 mg DM). The treatment of planting spacing did not have a significant effect (p>0.05) on the total of gas production, while the defoliation age treatment had a significant effect (p<0.05) on the total of gas production. The highest average of the total of gas production was obtained at the defoliation age of 60 DAP that is 138.39 mL/200 mg of DM while the lowest total of gas production was at the defoliation age 120 DAP that is 115.72 mL/200 mg of DM. Gas production is reduced with increasing crude fiber. Bain et al (2017) reported that gas production in the fermentation process was associated with high total sugar content in the ration. Crude ingredients and carbohydrate content in rations contribute 40% of total gas production (McDonald et al 2010). The defoliation age of 60 DAP was higher in gas production because, at that age, the

levels of digestible carbohydrates and high protein are still quite high. Akinfemi et al (2009) reported that the speed and high production of gas are influenced by the fraction of dissolved carbohydrates available in food that can be used by microbes. Gas production is an indication of a well-fermented of organic material in the rumen (Pellikaana et al 2011). Protein, as concentrate ingredients, can enrich the rumen fermentation results (Wanapat et al 2013). Some things that cause low gas production are the role of antinutrients contained in plants (Jayanegara 2008; Hariadi and Santoso 2010; Firsoni 2014).

IV.CONCLUSION

Characteristic of in vitro fermentative digestibility of odot grass feed was not affected by the interaction of the plant spacing and defoliation age treatments. The attribute of in vitro fermentation characteristics obtained in this study (pH, N-ammonia, a total of VFA production and total of gas production) are quantitatively very supportive for the continuity of the optimal fermentation process.

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