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In Vitro Gas Production and Rumen Degradability of Lactating Dairy Cow's Rations Containing Three Different Grasses

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ABSTRACT

This research aimed to ascertain whether dwarf elephant grass (DEG) is compatible to substitute the common forages, viz. elephant grass (EG) and maize stover (MS) in dairy cow rations (denote as R) using *in vitro* gas production (IVGP) technique. A completely randomized block design was applied to 4 feeding regimes and 3 replicates, viz. R1 consisted of 40% concentrate (C) + 30% MS + 30% EG; R2: 40% C + 30% MS + 30% DEG; R3: 40%C + 60% EG; and R4: 40% C + 60% DEG. The results showed that rations containing DEG improved both DM and OM degradabilities concomitant with an increase in rumen efficiency of microbial protein synthesis (EMPS). Nevertheless, total and potential of gas production (GP) were higher in rations containing MS than other counterparts suggesting that there was a diverting usage of ROMD available for GP and EMPS. In conclusion, the use of DEG in dairy rations is recommended.

Keywords dwarf elephant grass, maize stover, *in vitro* gas production, microbial protein synthesis

INTRODUCTION

Dairy cattle farming in Indonesia is predominantly occupied by smallholder farmers. This means only a small number of cattle-ownership that generally have less than 5 heads/farm. There are many obstacles that cause smallholder farmers in Indonesia to not develop, including lower technological inputs (Ibeawuchi et al., 2015), land scarcity for quality forage cultivation (Duguma and Janssens, 2021), and traditional management systems (Anggraini et al., 2021). Apart from being influenced by these environmental factors, internal factors that arise from the body of the livestock are very influential. Differences in environmental conditions from sub-tropical to tropical areas cause livestock productivity to adapt to new environmental conditions, therefore milk yield seldom reaches the genetic potential of dairy cows according to their breed of origin. They rely heavily on ration composting of fodder and medium-quality of commercial concentrate (usually produced by the local dairy cooperative). The fodder commonly used by farmers in Indonesia are elephant grass (EG) and maize stover (MS).

Elephant grass (EG) is widely grown in Indonesia because it is resistant to hot weather and can grow on less productive land. Meanwhile, MS is widely used as animal feed in Indonesia because of the availability of waste from the maize plant, where the fruit of maize is used to meet human needs. The high fiber content in EG and MS is needed by the rumen to produce energy for ruminants. Discovering a new variety of elephant grass in the last 5 years, called dwarf elephant grass (DEG) has been used by farmers as fodder for livestock. Dwarf elephant grass (DEG) has high productivity and nutritional content and also has high palatability for livestock (Sirait, 2017). Thus, this research aims to ascertain whether DEG is compatible to substitute the common forages, viz. EG and MS in the dairy cow's rations using *in vitro* gas production (IVGP) technique.

MATERIAL AND METHOD

This experiment was a demo plot of smallholder practices in Ngantang Sub-district, Malang Regency, and conducted at the Laboratory of Animal Nutrition and Feed, the Department of Animal Science, Universitas Brawijaya from August

August until October 2017. Materials used were fodder consisting of EG, MS, DEG, and concentrate (C) which was obtained from the local dairy cooperative of Sumber Makmur, Ngantang Sub-district of Malang Regency. Rumen fluid was obtained from a female fistulated Friesian-Holstein crossbred cow. Methods used for this experiment were randomized block design with 4 feeding regimes and 3 replicates as follows.

R1 : 40% C + 30% MS + 30% EG
R2 : 40% C + 30% MS + 30% DEG
R3 : 40% C + 60% EG
R4 : 40% C + 60% DEG

Feed Preparation

Fodder was dried in a 60 degree-oven to a constant weight, and then milled using a grinding machine with a 0,1 mm sieve. Each material is weighed according to the proportion of each feeding regime. Each feeding regime was analyzed proximate to determine the chemical composition of each treatment. The chemical composition was shown in Table 1. as follows.

Table 1. Chemical composition of each feeding regime

Feeding Regime	Dry Matter (%)	Organic Matter (%)	Crude Protein (%)	Crude Fat (%)
R1	88.46	89.41	10.94	1.20
R2	88.19	88.89	11.59	1.41
R3	88.48	88.71	9.67	1.35
R4	87.80	91.59	13.32	1.74

Source: Proximate analysis results in the Laboratory of Animal Nutrition and Feed, The Department of Animal Science, Universitas Brawijaya (2017)

Rumen Fluid Preparation

Before filling with rumen fluid, the vacuum flask is filled with warm water (39°C). The water in the flask is discarded, then the rumen fluid is taken using a plastic syringe and put into the flask. The first three times taking of rumen fluid is used to rinse the inside of the flask, which aims to equalize the temperature of the flask with the temperature of the rumen fluid. The rumen fluid is accommodated at the fourth intake until the flask is full. The flask containing rumen fluid must be immediately taken to the laboratory for further analysis.

In Vitro Analysis

The feed sample weighed 500 mg and was entered into a 100 ml glass syringe, and incubated overnight before *in vitro* analysis was conducted. Rumen fluid is filtered by 3 layers of filter cloth. Filter rumen fluid added into Erlenmeyer fill of McDoughal fluid consisting of 1095 ml distilled water, 730 rumen buffer solution, 365 ml macromineral solution, 0.23 ml micromineral solution, 1 ml resazurine, 60 ml freshly prepared reduction solution. The mixture was kept stirred under CO_2 at 39°C using a magnetic stirrer fitted with a hot plate, 50 ml of mixture solution was transferred into each syringe and incubated in a water bath at 39°C. This procedure was according to Makkar et al. (1995) with modification as described by Soetanto et al. (2021). Parameter observed was gas production with interval time incubation of 0.5; 1; 1.5; 2; 2.5; 3; 4; 5; 6; 9; 12; 18; 24; 30; 36; 48; 60; 72; 96 hours, potential (b) and rate (c) of gas production is measured using SPSS v.16 program.

At 24-hour-incubation, several syringes were transferred to a fermentor tube for the centrifugation process. Several samples were centrifuged at 3000 rpm for degradability purposes and the other samples were centrifuged at 8000 rpm for microbial protein synthesis purposes according to Blümmel et al. (1997).

Data Analysis

The data were analyzed using one-way of Analysis of Variance (ANOVA) according to the completely randomized block design. If there were differences between treatments, it would continue with Duncan's Multiple Range Test.

RESULT AND DISCUSSION

The result from Figure 1. showed gas production which measured in time incubation interval of 0.5; 1; 1.5; 2; 2.5; 3; 4; 5; 6; 9; 12; 18; 24; 30; 36; 48; 60; 72; 96 hours. Figure 1. shows that there is an increase in the value of GP in accordance with the activity and development of microbes in the rumen. Gas production increases slowly at the beginning of the incubation time (0-6 hours) because microbial growth is in a lag or adaptation phase, where microbes begin to adapt to their new environment. At 6 to 24 hours there was a high increase in GP, this indicates that microbial growth was in the log phase. After 24 hours, GP has increased but the amount is less than the incubation period before 24 hours. This shows that microbial growth is in a stationary phase. The peak of GP will be reached in the first 24 hours of incubation, then the rate of GP will decrease until 96 hours of incubation and finally, it reaches zero. The decrease in the amount of fermentable substrate will result in a decrease in GP which is the result of fermentation (Jayanegara et al., 2008; Mukmin et al., 2014).

The results of this experiment showed in Table 2. Statistically, feeding regimes showed a significant difference ($p<0.05$) in the GP total and EMPS, it also showed a significant difference ($p<0.01$) in the rumen dry matter and organic matter degradability (RDMD and ROMD, respectively), but it showed no significant difference ($P>0.05$) on the potential and rate of GP.

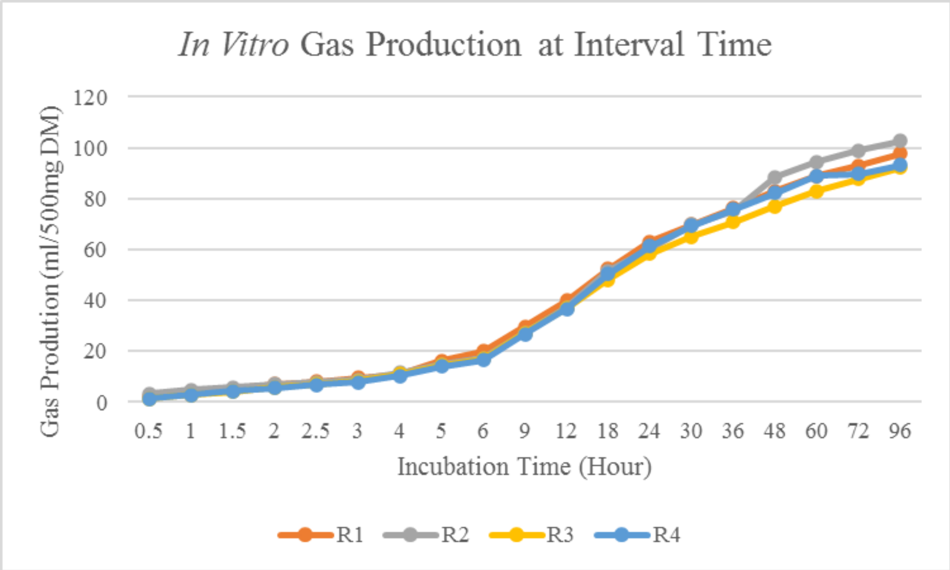

Figure 1. Graphic of gas production for 96 hours of incubation

Table 2. Effect of varying proportion of forages on IVGP main parameters

Parameter	Feeding Regime				SEM	p-value
	R1	R2	R3	R4		
Total GP (ml/500mg DM)	97.547^{ab}	102.684^{b}	92.224^{a}	93.215^{a}	2.435	0.042
Potential of GP (ml/500mg DM)	98.902	108.644	93.772	97.894	2.667	0.099
Rate of GP (fraction/hour)	0.040	0.034	0.038	0.039	0.001	0.405
RDMD (%)	61.249^{a}	65.914^{b}	59.840^{a}	64.808^{b}	0.916	< 0.01
ROMD (%)	58.199^{a}	62.240^{b}	56.166^{a}	63.162^{b}	1.065	< 0.01
EMPS (g N/kg FOM)	40.117^{b}	37.726^{a}	36.116^{a}	42.238^{c}	1.150	0.017

Note: GP = Gas Production; RDMD = Rumen Dry Matter Degradability; ROMD = Rumen Organic Matter Degradability; EMPS = Efficiency of Microbial Protein Synthesis; DM: Dry Matter; FOM: Fermented Organic Matter; R1-4: Ration 1-4; SEM: Standard Error of the Mean. Different superscripts in the same row show significant ($p<0.05$) or very significant ($p< 0.01$) difference

Ration 2 (R2) showed the highest value of GP (102.7ml/500 mg DM) dan the lowest value is showed in R3 (92.2 ml/500 mg DM) followed by the potential of GP, even though the highest value of organic matter (OM) and crude protein (CP) showed in R4. It was indicated that the rations had highly insoluble material but were potentially fermented in the rumen to produce gas (Anggraeni et al., 2020). However, the high total GP was not followed by high EMPS which was the highest value of EMPS showed in R4 (42.238 g N/kg ROMD). This is probably due to the fact that some of the fermented feed products cannot be used for EMPS. Microbial activity in R4 in fermenting of OM to VFA (Volatile Fatty Acid) is low and is not used as an energy source for livestock, but OM fermented is more widely used for EMPS (Dagaew et al., 2021). Feed degradation that is not followed by an increase in GP indicates that the degradation products are widely used for EMPS (Makkar et al., 1995). The process of fermentation and degradation in the rumen produces gas which is an illustration of the amount of OM that can be degraded in the rumen. Blümmel et al. (1997) explained that knowing the substrate that is not degraded shows the amount of substrate available for fermentation and GP shows the part of the substrate. The volume of GP can be used as an indicator of apparent degradability in the rumen while residual dry matter (DM) is lost to form true digestibility.

The highest value of RDMD is shown in R2 and the highest value of ROMD is shown in R4. However, R2 and R4 are not different statistically. The highest value of R2 in RDMD was caused by ratio composition which contains MS. This result showed higher than that Gómez-Vázquez et al. (2011) that only used EG (without added MS or C in the ratio) which is equal to 56.1%. The value of DEG application in this treatment showed higher than Sarwanto et al. (2019), but the substitution of DEG up to 50% of indigenous forage showed an increased degradation value of ±2%. The higher value in this study was due to the addition of C and MS in the feed. The material of MS is a maize plant with a cutting age of 60 days, where there are young maize kernels used in the ration. According to Nasriya et al. (2016), MS contains energy sources contained in maize grains and the contents of forage maize cells which are a group of readily available carbohydrates (RAC). Energy sources are needed as a carbon framework for optimal microbial growth because the more RAC is available in the feed, the microbial growth rate will increase so that RDMD increases. Both EG and DEG are the fodder that has high palatability for ruminants, but as plants grow, the complex carbohydrate content in plants such as cellulose, hemicellulose, and lignin will increase, so degradability will decrease Novianti et al. (2014). The lowest value showed in R3 was caused by material content in the rations, which contain EG with a cutting age of 90 days. The older the EG cutting, the higher the lignin content, so that degradability decreases (Rambau et al., 2016). Degradability value was an indicator of the ability of a feed to provide nutrient needs for livestock.

CONCLUSION

The rations containing DEG improved both DM and OM degradability with concomitant increases in rumen EMPS. Nevertheless, the total and potential of GP were higher in rations containing MS than other counterparts suggesting that there was a diverting usage of ROMD available for GP and EMPS. In conclusion, the use of DEG in dairy rations is recommended.

CONFLICT OF INTEREST

There is no potential conflict of interest was reported by the authors.

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