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Supplementation of Herb Plants Containing Bioactive Components Against Rumen Fermentation (Study *In Vitro*).

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Abstract – Protozoa are related to methane-producing microbes. Approximately 9% - 25% of methanogens living in symbiosis by sticking on the surface of protozoa. The purpose of this research was to reduce the population of protozoa by utilizing various herbal plants in vitro. The study consisted of two phases. Phase I was screening of seven plants, i.e. pasak bumi leaves, galinggang leaves, cottonwood leaf, cola leaves, kendal leaves, kunir leaves and meniran leaves. Selection was based on high total gas production and relatively low protozoa. Three selected plants based on such parameters were continued to the second phase. The experimental design used was a randomized complete block design, consisted of 7 treatments and 3 replicates. Variables observed were the total gas production, gas production kinetics and total protozoa population. Results showed that the total gas production between each plant showed differences ($P<0.05$). The total gas production was high in cola (80.33 ml), meniran (38.50 ml) and kunir (37.83 ml), while the lowest protozoa population consecutively contained in pasak bumi (4.11 log cells/ml), kendal (4.43 log cells/ml) and galinggang (4.64 log cells/ml). High total gas production indicates more intensive nutrient utilization by rumen microbes so that the feed is used more optimal. It can be concluded that, based on protozoa population and total gas production, the most optimal materials are cola, pasak bumi, and meniran.

1. INTRODUCTION

Methane gas is a product of fermentation of feedstuffs by rumen microbes in ruminants and is the second biggest contributor to the greenhouse gas CO2 in the atmosphere layer and has the ability to retain heat 25 times greater than CO2 [9]. Ruminant livestock sector in particular is one of the contributors to the accumulation of anthropogenic methane gas (about 28%) [2]. Besides an impact on global warming, methane emissions from ruminant livestock also impact the loss of feed energy that would otherwise be used to support productivity. The amount of energy lost from ruminant about 8-14% of the total energy ingested [2]. Methane is formed in the rumen by methanogenic microbes, which is 70% of the population makes the body protozoa as habitat. Many nutrition experts seek to reduce methane production of cattle, because they feel responsible for the main ruminant livestock sector contribute to atmospheric pollution by methane. Efforts are usually done to overcome the disadvantages and negative effects of agricultural wastes as animal feed is to improve the nutritional quality of agricultural waste prior to administration to the rumen of cattle and manipulation performance. Defaunasi technology implementation can be done to control the protozoa population obtained so that a balanced ecological conditions for the growth of rumen microbes [1]. Partial Defaunation can use oil or natural ingredients that many containt saponin and the results can reduce protozoa, accompanied by the increase in rumen bacterial populations and the growth of livestock ([3].. Plants considerable potential as a source of rumen bacteria and nutrients defaunator are herbal plants. Some herbs are also believed to contain nutrients and compounds that contribute to improving the production and quality of spermatozoa. Thus, the identification and exploration of herbal plants that contribute to improving the performance of rumen needs to be done. Alternative plants used in this study include pasak bumi leaves, galinggang leaves, cottonwood leaf, cola leaves, kendal leaves, kunir leaves and meniran leaves. The purpose of this study was to evaluate the effect of supplementation of herbal plants on rumen fermentation and methane emissions.

2. MHETODS

2.1 Chemicals

Herbs (pasak bumi leaves, galinggang leaves, cottonwood leaf, cola leaves, kendal leaves, kunir leaves and meniran leaves), liquid cow's rumen, digital scales, water bath, magnetic stirrer, syringe, centrifuge, flask-scale 2-liter, cloth filter, hot plate, tube capacity 120 ml glass vial, eppendorf tubes, spectrophotometers, reflux, HPLC and Gas Chromatography, H2S04 0005 M, 6 N NaOH, pepsin-HCl 0.2%, TBFS.

2.2 Procedures

Making of Buffer liquid

Making the buffer used by [7] method. Ingredients constituent include NaHCO3 (9.8 g), Na2HPO4 (3.71 g), KCl (0.57 g), NaCl (0.47 g), MgSO4 (0.12 g) and CaCl2 (0.04 g) all of these materials are mixed with the addition of 1000 ml of distilled water (needs 1 liter) and 25 ml rumen fluid.

Implementation of *In Vitro* Test

In vitro fermentation techniques performed by methods [8]. A total of 75 mg (0.75 g) treatment sample vial inserted into the bottle injection size of 100 ml, and 75 ml bottle is input buffer rumen fluid as the media has been saturated incubation using CO2 gas. Rumen fluid used was taken from the Holstein Frisian cows (FH) fistula home Livestock Research Institute (Balitnak), Ciawi, Bogor on the morning before the cattle fed. Rumen fluid is then filtered using a filter cloth and put into a thermos to take to the lab. This mixture of sample treatment and rumen buffer liquid in the bottle is then closed with a rubber cover and pressed aluminum cover using a pressure tool that further incubated in a water bath at a temperature of 39-410C. During the incubation period, is done manually shaking the bottle every hour on the first 4 hours, and every subsequent gas measurement.

Total Gas Production and Gas Production Kinetics

Total gas production was measured at hours 2, 4, 8, 12, and 24. While the gas production kinetics were measured in hours to 2, 4, 8, 12, 24, 36, 48, 60 and 72. Total and gas kinetics were measured using the plastic syringe with a volume of 60 ml (Terumo). [4] Plugged end portion of the syringe through the rubber cover in the bottle go to the chamber of the bottle without the liquid inside, automatically total gas produced in pushing the syringe upwards. Once the total gas is already pushing the syringe completely, then the tip of the syringe removed from the bottle and read. The total volume of gas (ml) can be known by reading the manual on a scale that is contained in the syringe.

Total Protozoa

Calculations are based on the total protozoa [5]. 1 ml samples of rumen fluid in vitro results of a 24-hour incubation mixed into a solution of MFS (Methylgreen Formal-Copy) composed of: 100 ml of 35% formaldehyde, 900 ml of distilled water, 0.6 grams methilgreen, and 8 grams of NaCl. Two drops of the mixture was placed on a hemocytometer by the number of readings box of 25 pieces with the total volume of 10-4. Protozoa population calculations performed using a microscope (Olympus) at a magnification of 10 times. Total protozoa / ml rumen fluid is calculated by multiplying the number counted by a factor penganceran protozoa. Total protozoa are then ditranformasi into log units.

3. RESULTS AND DISCUSSION

Total accumulated value of gas production and gas production kinetics for 24 hours and 72 hours is presented in Table 1 and Table 2. Results of analysis of variance showed that there were differences between treatments in total gas production of herbaceous plants ($P < 0.05$). The total gas production of the highest seen in the treatment cola. The total gas production of kunir and meniran treatment ($P > 0.05$) lower than cola treatment but higher than other treatments ($P < 0.05$). These results indicate that treatment cola, kunir, and meniran has a total gas production was higher than the other treatments herbal plants. Lowest protozoa population indicated by pasak bumi treatment ($P < 0.05$). Kendal and galinggang ($P > 0.05$) has a population of protozoa higher than pasak bumi treatment, but lower than the other treatments ($P < 0.05$). The highest population seen in Kunir treatment ($P < 0.05$). This may imply that the low protozoa population shown by pasak bumi, kendal, and galinggang. High gas production with low protozoa population on treatment indicates that herbs treatments can increase the performance and reduce the population of bacteria rumen protozoa. Mean protozoa population is presented in Table 3. The decline has been suggested that treatment of active substances in the form of saponin. Saponin performance is through the reduction of the population of rumen protozoa [4]. We know that frequent protozoa feed on bacteria in the rumen under normal conditions. The results of this study can be concluded that the herbs treatment is good in maintaining the balance of rumen microbes are pasak bumi, cola, and galinggang. It can be seen from the total gas production is high. This is because of the high performance indicates total gas production in the rumen bacterial fermentation process is not compromised.

Table 1. Mean of Total Gas Production at 24 h

total accumulation of gas 24 h	
Herbs	**means of gas total**
Pasak bumi	14.00 ± 1.00**d**
Galinggang	27.67 ± 3.55**c**
Kapuk	24.83 ± 1.26**c**
Cola	80.33 ± 4.54**a**
Kendal	4.17 ± 0.58**e**
Kunir	37.83 ± 2.02**b**
Meniran	38.50 ± 0.50**b**

Description: Symbol of different letters in the table shows the difference (P <0.05).

Table 2. Mean of Total Gas Production at 72 h

total accumulation of gas 72 h	
Herbs	**means of gas total**
Pasak bumi	36.50 ± 3.28**e**
Galinggang	53.67 ± 6.17**cd**
Kapuk	48.50 ± 2.29**d**
Cola	123.33 ± 9.02**a**
Kendal	11.67 ± 3.06**f**
Kunir	76.33 ± 8.62**b**
Meniran	61.33 ± 2.02**c**

Table 3. Mean of Protozoa Population

Herbs	mean protozoa (Log CFU/ml)
Pasak bumi	4.11 ± 2.30**d**
Galinggang	4.64 ± 2.63**b**
Kapuk	4.85 ± 2.79**ab**
Cola	4.76 ± 2.71**b**
Kendal	4.43 ± 2.54**c**
Kunir	5.00 ± 2.88**a**
Meniran	4.85 ± 2.77**ab**

Description: Symbol of different letters in the table shows the difference (P <0.05).

4. CONCLUSIONS

The results of this study can be concluded that the herb is good in maintaining the balance of rumen microbes is pasak bumi, cola, and galinggang. Judging from the total gas production is high. This is because of the high performance indicates total gas production in the rumen bacterial fermentation process is not compromised.

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