In vitro gas profile of dairy goat basal diet added with Coleus amboinicus lour extracted with different solvents

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Abstract. The aim of this study was to reveal the in vitro gas profile of basal diet added with Coleus amboinicus lour (CAL). The basal diet was added with CAL extracted with different solvent of each water and ethanol. There were four treatments of this experiment namely P0: basal diet (control), P1: basal diet added with 2% of CAL powder, P2: basal diet added with 2% of CAL extracted with water, P3: basal diet added with 2% of CAL extracted with ethanol. Each sample was incubated for 48 h following the Reading Gas technique methods with slight modification. Gas was collected at 2, 4, 6, 8, 12, 18, 36 and 48 h. The parameters measured were the total gas production (P), potential gas production (b) and rate of gas production (c). The results of this experiment showed that P and b were significant (P<0.05) different. The total gas production was 122.16, 94.78, 122.34 and 62.34 ml/g for P0, P1, P2 and P3 respectively and b was 124.88, 100.13, 117.05 and 80.76 ml/g for P0, P1, P2 and P3 respectively. The value of c was not significant (P>0.05) among four treatments. The value of c was 0.0625, 0.0525, 0.0525 and 0.0425 for P0, P1, P2 and P3 respectively. It could be concluded that the use of water to extract CAL was the best among four treatments.

1. Introduction

Coleus amboinicus lour (CAL) is a tropical plant with a typical aroma. This plant is grouped as shrubs, having thick and woody stem with branch and a height of almost one meter. Santosa and Hertiani [1] reported that CAL was an ethno-botany from North Sumatra and was used as every day menu. Siburian et al [2] reported that this plant had been processed in making green and black tea. In Kutai, Kalimantan, CAL has been used for traditional medicine for fever, thrush and rheumatic [3]. This plant could be found in most area of Indonesia. In addition, Sing et al [4] declared that CAL was a native plant from East India and usually recognized as “Indian Borage”.

Syarief et al [5] stated that CAL leaf constitutes as a food plant functioning as lactagogum. It could increase the secretion and production of breast milk. In fact, this plant leaf has been consumed by mothers for increasing breast milk in North Sumatra [6]. Some experiments using rats have been done to investigate the effect of CAL in diet on the milk production [1,7]. The supplementation of CAL into goat basal ration could be possible. Adriani, et al [8] reported that CAL could potentially increase the in vitro digestibility of ration consume by animal. In addition, the effect of solvent in extracting of CAL might also influence on the digestibility of completed ration as it might also influence of the rumen environment and the digestibility.
In vitro gas is one of method to figure out the digestibility within rumen. This method could evaluate the profile gas within rumen such as total gas production, potential gas production and rate of gas production. Base on the above information, it would be done the effect of different solvent in extracting CAL within ration on the gas profile. The aim of this study was to evaluate the total gas production, potential gas production and the rate of gas production of the ration supplemented with different extracted CAL.

2. Methodology

2.1. Sample preparation
CAL was collected from Fapet Farm, Faculty of Animal Husbandry, Universitas Jambi and transported to the Laboratory of Ruminant Nutrition, Universitas Jambi. Sample of CAL was sundried for 1 day, oven-dried over night at 60°C and then ground to pass through a 1 mm sieve into a grinder machine to be powder. The powder sample was extracted using hot water and some other sample was extract using ethanol following Method performed by Afdal [9]. The sample of field grass and rice brand was also oven-dried and ground to pass through a 1 mm sieve into a grinder machine to be powder. The basic ration consisted of 70% field grass and 30% rice brand on the dry matter basic. The chemical composition of ration was 9.09% of crude protein (CP), 2.12% of ether extract (EE) and 30.26% of crude fiber (CF).

2.2. Experimental procedure
Inoculum was collected from rumen fistulated cow before morning feeding at 08.00 from. In vitro gas trial following Reading Gas Technique Method with slight modification was applied [10]. The ration was treated according to treatment design of P₀ (control), P₁ (add with 2% of unextracted CAL), P₂ (add with 2% of water extracted CAL) and P₃ (add with 2% ethanol extract). Gas was collected at 2, 4, 6, 8, 12, 18, 36 and 48 h. The profile gas produced was fixed following model p=a + b (1-e⁻⁰⁵) [11].

2.3. Parameter and experimental design
Parameter measured was Total gas Production, potential gas production (b) and rate of gas production (c). The design of experiment was Completely Randomized Design with four replications. Anova followed with Duncan Test was applied for statistical analysis [12].

3. Results and discussion
3.1. Total gas production
The total gas production was significantly different (P<0.05) among four treatments (table 1). Treatment P₁ shows the highest total gas production of 122.34 ml/g among other treatments. Gas production normally figures out the fermentation activity within rumen. It seems the ration added with hot water extracted CAL shows the highest fermentation activity. The fermentation activity within the rumen is normally due to the existence of bacteria. It seems the addition of ration with hot water extracted CAL would create the bacteria population to increase the fermentation activity within rumen. In fact treatment P₂ provided tannin into ration that inhibits protozoa growth as bacterial predator. Makkar [13] mentioned that tannin could be as de-fauna agent that could reduce the protozoa population in rumen. The reduction of protozoa, as predator of bacteria, could increase the bacterial population that increases the fermentation activity in rumen.

The treatment P₃ with the total gas production of 62.34 ml/g, show slower activity of fermentation than treatment P₂. Low activity of fermentation in P₃ could be due to so high tannin in this treatment. Ethanol could extract much more tannin in comparison with using hot water [14]. Wahyuni et al [15] stated that tannin function as defaunation agent, its phenolic group also functions as antibacterial [16]. Apart from this, Gram positif bacteria was sensitive on the certain polyphenol [17] whereas some fibrolytic bacterium is categorized as Gram positive bacteria. Therefore it might reduce the fermentation and degradation of ration.
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Table 1. Total gas production, potential gas production and rate of gas production among four treatments.

| Treatment                          | Total Gas (ml/g) | b (ml/g) | c (ml/h) |
|------------------------------------|------------------|----------|----------|
| Control (P0)                       | 122.16 ± 5.10a   | 124.88 ± 1.71ab | 0.0625 ± 0.0047 |
| 2 % of CAL (P1)                    | 94.78 ± 3.36b    | 100.13 ± 3.41b | 0.0525 ± 0.0025 |
| 2 % of hot water extracted CAL (P2)| 122.34 ± 5.66a   | 117.05 ± 4.38a | 0.0525 ± 0.0025 |
| 2 % of ethanol extracted CAL (P3)  | 62.34 ± 14.53c   | 80.76 ± 17.11c | 0.0425 ± 0.0025 |

The value are mean of 4 replications and standard error of the mean. Means with different Superscript within the same column is significantly different (P<0.05).

3.2. Potential gas production

The potential gas production was significantly different (P<0.05) among four treatments. The effect of treatment was proportionally in concordance with effect on the total gas production. The effect of CAL and ethanol extracted CAL within ration was significantly low on ‘b’ value of 100.13 and 80.76 ml/g for P1 and P3 respectively, in comparison with control and hot water extracted CAL on the potential gas production. It looks the use of CAL and ethanol extracted CAL might influence not only inhibits the protozoa growth [15] but also disturb the bacteria activity in rumen as an antibacterial and antioxidant agent [16]. Therefore, the bacteria population would lessen that it could reduce the fermentation activity and also decrease the potential gas production from ration.

3.3. Rate of gas production

The rate of gas production was not significantly different (P>0.05) among four treatments. It is contrary to the experimental hypotheses in which the addition of extracted CAL in ration would influence on the rate of gas production during incubation. It seems that the addition of extracted CAL in ration demonstrates no effect on the rate of gas production. It is different from Patra and saxena [18] stated that the addition of phytoherbs as additive feed on rumen fermentation and rumen microbe was undoubtedly subject to on the interaction among chemical composition of ration and phytochemical used in ration. This might possibly be due to the chemical composition and physical property of each ration was similar. In fact, all rations were prepared with 70% of field grass and 30% of rice brand and their chemical composition of ration was 9.09%, 2.12% and 30.26% for CP, EE and CF respectively. Consequently the rate of gas production would be the same. Ørskov [19] mentioned that physical property of feed source and rumen environment were the main factor in determining characteristic of degradation including solubility of feed, the rate of ration degradation in rumen, the availability of fermented substrate, microbial population, particle size, physical appearance and rumen pH.

Apart from this, the ration was only incubated for 48 h so it only represents the rate of potential gas production for 48 h. This degradation might initially happen mainly on only rice brand and or few field grasses that contain the nonstructural substrate. Therefore the rate of degradation above could not figure out the rate of degradation of the whole ration yet. While we need more incubation time since the ration mainly consisted of high fiber feed of field grass. Normally the degradation of nutrient in rumen is initiated with nonstructural component and followed with structural insoluble feed component. The structural component of ration is a complex matrix insoluble component in which there is no primarily standard method to evaluate it [20].

Furthermore, the amount of treat of both hot water extract CAL and ethanol extracted CAL was relatively very little in ration (2%). Thus the data collected for 48 h did not influence on the rate of degradation of whole ration component yet. This looks contrary to the Patra and saxena [18] studied on phytochemical. They stated that the interaction amongst the chemical structures and levels of phytochemicals used, nutrient composition of diets and microbial components in the rumen possibly was influenced by phytoadditive in ration [19].
4. Conclusion
It could be concluded that the use of water to extract CAL was the best treatment among four treatments that show good fermentation profile of total gas production, potential gas production and rate gas production.

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