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Feed additive of betel leaves meal (*Piper betle L.*) use on ruminants as one of methane mitigation efforts

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Abstract

Methane (CH_4) is one class of greenhouse gases that could lead to global warming when the concentration of greenhouse gases in excess. Ruminant is one of the biggest methane contributors in agriculture sector. The aim was to evaluate supplementation of Betel leaves meal to decrease methane production. This research was conducted using *in vitro* technique for 48 hours of incubation time. Methane produced in the rumen was estimated by using data of volatile fatty acids (VFAs) partial concentration, particularly three main acids, i.e. acetic acid, propionic acid and butyric acid. The experiment used was randomized block design with three replicates and four treatments. The treatments were the addition 0%, 1%, 2%, and 3% of betel leaves meal in feed. The results showed that addition of betel leaves meal into the ration decreased methane production. Betel leaves meal also decreased protozoa population and C2/C3 ratio ($p < 0.05$), but increased propionic acid (C_3) and butiric acid (C_4) proportion ($p < 0.05$). The best dose of betel leaves meal to reduce methane and to maintain optimum rumen condition was 2%.

Keywords: betel leaves meal, gas production, methane (CH_4), VFA

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Introduction

Methane (CH4) is one class of greenhouse gases that could lead to global warming when the concentration of greenhouse gases in excess. Approximately 50% of methane emission is the result of human activity that comes from the agriculture where the 27% comes from the livestock sector. Ruminant is the biggest methane contributor agriculture sector. Methane in ruminant livestock comes from two sources is derived from the fermentation of the digestive tract (enteric fermentation) and feces (manure).

The production of methane gas reduces the utilization efficiency of feed as it can reduce of gross energy that would otherwise be converted in the form of fermented products. Various efforts have been made in reducing methane emissions of ruminants either by modifying the microbial ecosystem (Morgavi et al., 2010) such as the use of plants that contain saponin and tannin as killer agent for protozoa in the rumen. One of the plants containing tannin and saponin is a betel leaves (*Piper betle* L.). The use of betel leaves meal as a feed additive to reduce the amount of methane naturally in the gastrointestinal tract has not been studied before.

The purpose of this study was to evaluate the use of betel leaves meal on the methane production in the digestion of ruminants to obtain the proper dosage for use.

Materials and Methods

Rumen fluid from fistulated Holstein Friesian cows was taken in the morning before feeding time. Feed samples were incubated in vitro by the method of Tilley and Terry (1963) and total VFA concentration was measured by using steam distillation technique (General Laboratory Procedure, 1966). VFAs analysis was conducted using a gas partial Cromatograph Bruker® Scion 436-GC, and SHO-40 with auto inject system. Protozoa population was calculated using counting chamber and microscope at 100 times magnification. Methane gas production was calculated by the approach of stoichiometric proportion of VFAs partial (Moss et al., 2000). Samples were incubated by the method of Menke et al. (1979) and production of gas was estimated by an exponential equation described by Ørskov and McDonald (1979).

The experimental design used in this study was a randomized block design (RBD), which consists of four treatments each with three replicates. The treatments were:

P0 = basal ration (concentrate: forage = 1: 1) as a control
P1 = basal diet with the addition of betel leaves flour 1%
P2 = basal diet with the addition of betel leaves flour 2%
P3 = basal diet with the addition of betel leaves flour 3%

Data obtained from the study were analyzed using analysis of variance (ANOVA) if there was a significant difference would be tested further using orthogonal polynomial test (Steel and Torrie, 1995). The chemical composition of experimental diets were shown in Table 1.

Table 1. Chemical composition of experimental diets (%DM)

Nutrient*	The addition level of betel leaves meal			
	0%	1%	2%	3%
Dry matter (DM) (%)	90.860	90.830	90.800	90.770
Crude protein (CP) (%)	11.947	11.969	11.990	12.012
Crude fibrear (CF) (%)	32.644	32.628	32.613	32.598
Ether extract (EE) (%)	3.676	3.652	3.623	3.603
Ash (%)	9.118	9.104	9.088	9.073
Nitrogen free extract (NFE) (%)	42.615	42.648	42.681	42.713

Results and Discussion

Production of VFAs partial

Results of VFAs partial measurement in this study are presented in Table 2. Giving betel leaves meal showed significant effect ($p < 0.05$) on the proportion of propionic, butyric and the ratio of C2: C3, but did not show any significant effect on the proportions of acetate, isobutyrate, valerate, and isovalerat. The proportion of acetate produced in this study ranged from 53.910% - 66.044%. Decreased production of acetate with the addition of betel leaves meal was expected to reduce the production of methane gas. The highest proportion generated in this study were acetate. Propionic acid generated in this study significantly affected by treatment ($p < 0.05$). Propionate increased with the addition of betel leaves meal with the average 19.016% - 24.202%.

Protozoa population

The existence of protozoa affect the amount of methanogens in the rumen, because methanogens living in symbiosis with the protozoa. A total of 37% methanogens living in symbiosis with the protozoa, and the rest move freely in the rumen (Newbold et al., 1995). The results of protozoa population count in this study are presented in Table 3.

The addition of betel leaves meal have a significant effect ($p < 0.05$) on the decrease of rumen protozoa number. The average number of the rumen protozoa were 3.784 – 3.966 log cells/ml rumen fluid. The more the addition of betel leaves meal the more the decreases of rumen protozoa number. This is presumably because betel leaves meal contain tannins and saponins. When additional dose of betel leaves meal increasing, its tannin and saponin content also are also increase. Betel leaves contain tannins and saponins of 2.61% and 3.55%, respectively. Saponins can interfere with the development of the protozoa population as saponins is able to create a complex bond with the surface of the cell membrane sterols protozoa, causing protozoa cell membrane rupture and undergo cell lysis and ultimately death (Wallace et al., 2002). Tannins have the potential to decrease the number of protozoa without affecting the normal conditions of the rumen.

Table 2. Production of VFAs parsial

Proportion of VFAs parsial (%)	The addition level of betel leaves meal				P-value
	0%	1%	2%	3%	
Asetate (C2)	66.044±3.483	58.026±0.141	53.910±9.062	58.625±5.548	0.238
Propionate (C3)	19.016±1.126a	24.202±0.741b	22.332±1.559b	22.281±0.539b	0.011
Butirate (C4)	9.076±0.645a	13.133±0.585b	13.749±2.198b	11.129±1.135ab	0.029
Isobutirate (IC4)	1.379±0.517	1.244±0.015	2.237±1.404	2.103±1.547	0.671
Valerate (C5)	0.822±0.137	1.634±0.118	4.488±3.547	3.572±1.980	0.247
Isovalerate (IC5)	1.020±0.217	1.761±0.021	3.283±2.279	2.290±0.799	0.276
C2/C3 ratio	3.475±0.098b	2.399±0.079b	2.419±0.428a	2.636±0.308a	0.014

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Tabel 3. Population of rumen protozoa

The addition level of betel leaves meal	Protozoa population (log cell/ml)
0 %	3.966±0.088b
1 %	3.904±0.085ab
2 %	3.828±0.113ab
3 %	3.784±0.190a

Methane Production

The formation of methane in the rumen occurs through CO_2 reduction by H_2 catalyzed by enzymes produced by methanogenic archaea through the reaction as follows:

$$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O;\ \Delta G^0 = -32{,}75\ kJ\ mol^{-1}\ H_2$$ (Vlaming, 2008).

The result of the calculation of methane production in this study is shown in Figure 1. The addition of betel leaves meal up to the level of 3% in the ration has not been able to reduce methane production significantly.

The addition of 2% betel leaves meal produced the lowest methane production, i.e., 23.618 mmol/100 mmol. This is likely due to the significant ($p < 0.05$) increase of ruminal propionate. Simultaneously the proportion of acetate in ration with the addition of 2% betel leaves meal was also lower. When the acetic acid produced in the rumen, H_2 and CO_2 is also produced which is then used by methanogens for methanogenesis process. If the amount of acetate produced declining, H_2 and CO_2 produced is also decreased. This will disrupt the process of methanogenesis, so that methane production will decline.

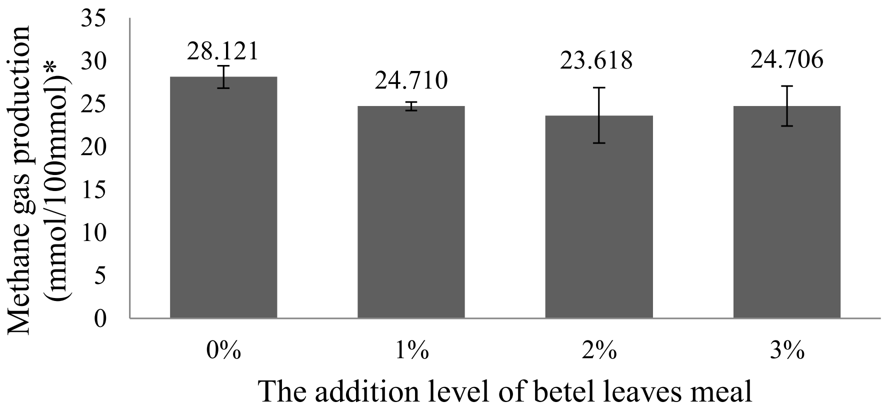

Figure 1. Production of methane (CH_4).
*(p = 0206) Calculations using the method of Moss et al. (2000).

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