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Product fermentation and gas production in vitro of feed content from Moringa oleifera, Lamm and Paraserianthes falcataria leaves

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Abstract: *This study aims to determine the best composition Moringa oleifera, Lam (MOL) and Paraserianthes falcataria (PFL) leaves supplementation in the feed to produce fermentation process and low CH_4 gas production. Concentrate feed is composed of MOL, PFL, plus coconut cake (CM), and solid cassava waste (O). The study was conducted with a factorial experiment, arranged in a randomized complete block design (RBD). The first factor is 4 types of concentrate: A, B, C and D. The second factor is the proportion of concentrates A, B, C, D and maize stover (Zea mays) (M). as follows: P1, P2 and P3. The usage of concentrate feed (MOL 30% : PFL 10% : coconut cake 45% : solid cassava waste 15%) with the proportion of concentrate 50% : forage 50% was be able to produce normal fermentation process with indicated DM degradation 66.4%, OM degradation 70.41%, gas production 94.8 ml, microbial biomass 46 mg, pH 6.67, NH_3 concentration 7.48 - 8.29 mg/100 ml rumen fluid and proportion VFA (C_2:C_3:C_4 = 65:26:9) and the lowest CH_4 gas production (< 1% 24-hour incubation). In the future studies are in-vivo testing of the usage of MOL 30% : PFL 10% in the concentrate feed.*

Keywords: *CH_4, in vitro, leaf of moringa, supplementation*

I. Introduction

Manipulation of rumen fermentation processes aimed at improving the productivity of ruminants. The result of the fermentation process in the rumen is volatile fatty acid (VFA), ammonia (NH_3), microbial protein and methane (CH_4) and CO_2. CH_4 production in the rumen of ruminant livestock in the tropics is relatively high due to the low quality feed. Feed low-quality low impact on livestock production and increased CH4 emissions. Total CH_4 emission fermented feed on cattle, buffalo, sheep and goat estimated 72 million tons in 2004 and contributed to the pollution of CH_4 in the atmosphere by 17-30% [1]. Efforts to control the production of CH_4 with antibiotics, growth hormones and chemicals cause residue in the product as well as toxic effects on livestock, so it is not recommended for use [2].

Alternatives to suppress CH_4 production has been carried out with the use of natural additives of some legume plants containing secondary compounds tannins and saponins. [3],[4],[5] and [6] reported on the presence of condensed tannins (CT) on several of forage crops was be able to protect protein from excessive degradation processes in the rumen, so that to increase the amount of protein that is readily absorbed in the small intestine. Other reports suggested that saponin compounds are toxic to bacteria and protozoa in the rumen [7],[8],[9],[10],[11]. Some methanogens symbiosis with protozoa. Which is it may act as a donor H_2. Although the effect on methanogens do not always correlate with the effects on protozoa [12]. Inhibited the growth of protozoa, the availability of H_2 from reduced protozoa, methanogens number attached to the protozoa will be reduced, thus decreasing the production of CH_4.

Leaves of *Moringa oleifera*, Lam (MOL) has 26 kinds of antioxidants and amino acids essential to the ideal composition according to nutritional standards of FAO [13]. Benefits of adding MOL in lactating dairy cattle feed showed that the administration of as much as 25% in the molasses block (approximately 100 g / head / day) was able to increase the milk production of 4% FCM of 9.80 kg / head / day to 10.6 kg / head / day. The results of the study [14] proved that supplementation of urea molasses block-based MOL significant effect ($P < 0.05$) to increase feed intake of DM by 18% and average daily gain of goats were very real to 100 g / head / day ($P < 0.01$). Approach through in-vitro studies, the addition of MOL was able to increase microbial protein synthesis significantly [15] that is thought to be a factor supporting the increase in milk production of dairy cows. Report [16] in goats also showed that the MOL replace cotton oilcake and produce weight gain 20% higher when compared with the control feed. However, the publication of MOL and PFL utilization for animal feed in Indonesia is still very limited. MOL and PFL selected because of the high protein content and low CH_4 yield. This study aims to determine the best composition MOL and PFL supplementation in the feed concentrate to produce a fermentation process with low CH_4 gas production.

II. Methodology

The experiment was conducted at the Laboratory of Animal Nutrition and Feed, Faculty of Animal Husbandry, Brawijaya University. Leaves of tree foliage (MOL and PFL) collected from Malang areas were air forced dried in the oven at 60^0C until the weight was constant. Milled material with a size of 1 mm for proximate [17] and fibre analysis [18]

Concentrate feed is composed of MOL, PFL, plus coconut cake (CM), and solid cassava waste / *onggok* (O). The study was conducted with a factorial experiment, consisting of 4 types of concentrate, 3 proportions of forage provision, arranged in a randomized complete block design (RBD). The first factor is 4 types of concentrate: **A (**MOL 0%: PFL0%; CM 85% : O 15%), **B (**MOL 10%: PFL 10% : CM 65% :O 15%), **C (**MOL 20%: PFL 10% : CM 55% :O 15%) and **D** (MOL 30%: PFL 10%: CM 45% : O 15%). The second factor is the proportion of concentrates A, B, C, D and maize stover (Zea mays) (M) as follows: P1 (concentrates 30% : M 70%), P2 (concentrates 40%: M60%) and P3 (concentrates 50%: M50%). Each treatment combination was repeated 3 times. The variables measured were (1) nutrient compositions (2) gas production, (3) degradation of dry matter (dDM), degradation of organic matter (dOM), (4) microbial biomass, and (5) methane gas production.

The process of fermentation in vitro using techniques [19]. Rumen fluid taken in the morning before the fistulated dairy cows was fed. The fistulated dairy cows with body weight approximately 350 kg were fed maize stover (7.93 % CP) 20-25 kg $head^{-1}day^{-1}$ and commercial concentrate (16 % CP) 5 kg $head^{-1}day^{-1.}$ Drinking water was always available *ad libitum*. After collecting, the rumen fluid filtered with a nylon sieve size of 100 m was added to the buffer solution. Measurement of levels of $N\text{-}NH_3$ rumen fluid was performed according to the instructions [20]. Determination of rumen fluid pH is done by inserting a portable pH meter on a sample of 50 ml rumen fluid that has been filtered and placed in a glass beaker. Calibration is done by using a buffer of pH 6.82 and pH 4.

Methane gas content was measured by gas chromatography (Shimadzu Brands 2005, Type GC 2010 Detector FID (Flame Ionization Detector) which was calibrated with standard methane yield of 0.1%. The CH_4 gas measurement was performed following procedures developed by [11]. The substrate was incubated in rumen fluid in vitro tube at vacuum conditions. After observing the total gas volume, the gas produced was transferred into 10 ml volume vacutainer vacuum at observation time of 12 hours and 24 hours after incubation. Then the gas was injected as much as 1 ml into the inlet of Detector FID, the similar conditions to the standard conditions of 0.1% methane. The data obtained were in the form of methane content percentage in the total gas content. The implementation of CH_4 measurement was performed in Integrated Research and Testing Laboratory (IRTL) at Gadjah Mada University.

$$\text{Methane (\%)} = \frac{\text{(width of sample area)}}{\text{(width of standard area)}} \text{ X standard concentration of 0.1\% methane}$$

The data were statistically analyzed by PASW STATISTICA 18 according to a randomized block design followed with a honesty significant difference test [21]. Standard errors were calculated from the residual mean square in the analysis of varians.

III. Results And Discussion

Nutrient content

Table 1. high CP content (> 18%) at MOL and PFL potentially be used as a feed supplement in improving the quality of rations in ruminants. Use of MOL (as much as 10%, 20% and 30%) and PFL (by 10%) in the feed concentrate treatment resulted in increased protein levels ranging from 9.40% to 12.0%. The increased use of concentrates of 30%, 40% to 50% in the feed (P1 = concentrates 30% : M 70%; P2 =concentrates 40%: M60% ; and P3 = concentrates 50%: M50%) resulted in CP content feed increased. Feedstuffs protein sources are expensive, because its has a high digestibility due to the high content of CP and CF are low.

Results of preliminary in-vitro studies (Table 2.) on MOL and PFL potential as a protein supplements. Degradation value, the total gas production, microbial biomass, number of protozoa (10^4 sel ml^{-1} rumen fluid), NH_3 concentration at 4, 12 and 24 hours incubation at MOL significantly values ($P < 0.01$) more high than PFL. The higher CT content of the PFL produces lower gas production.

Concentrate feed composed of MOL and PFL. The result of in-vitro, MOL more rapidly degraded in the rumen, therefore more available nitrogen as a source of amino acids for rumen microbial synthesis. PFL contains high tannin. The high tannin content of PFL was able to protect protein from excessive degradation processes in the rumen [11].

Table 1. The content of dry matter, organic matter, crude protein and crude fiber of feedstuffs and feed treatment

Item	DM (%)	OM* (%)	CP* (%)	CF* (%)
Moringa oleifera leaves (MOL)	18.4	87.1	36.6	10.8
Paraserianthes falcataria leaves (PFL)	41.3	96.2	23.3	37.9
Coconut cake (CM)	86.7	92.1	21.8	13.3
Solid cassava waste (O)	46.7	98.5	3.34	11.6
Maize stover (M)	18.4	92.5	7.93	26.4
concentrate : maize stover (M)				
AP1 A30% : M70%	91.1	92.2	9.41	20.0
AP2 A40% : M60%	90.4	92.3	10.2	18.6
AP3 A50% : M50%	89.9	92.9	10.7	17.2
BP1 B30% : M70%	88.0	92.3	9.91	20.5
BP2 B40% : M60%	87.8	93.1	10.8	19.3
BP3 B50% : M50%	89.7	92.7	11.4	17.9
CP1 C30% : M70%	90.4	92.0	9.91	20.8
CP2 C40% : M60%	89.1	92.7	10.9	19.6
CP3 C50% : M50%	89.9	92.4	11.6	18.4
DP1 D30% : M70%	91.1	92.0	10.2	21.0
DP2 D40% : M60%	90.8	92.1	11.0	19.9
DP3 D50% : M50%	90.3	92.1	12.1	18.8

*) Based 100% DM . DM = dry matter, OM = organic matter, CP = crude protein, CF= crude fiber, **A**= MOL 0%: PFL 0%; CM 85% : O 15%; **B**= MOL 10%: PFL 10% : CM 65% :O 15%; **C**= MOL 20%: PFL 10% : CM 55% :O 15%; **D**= MOL 30%: PFL 10%: CM 45% : O 15%. Analysis of Animal Nutrition and Feed Laboratory, Faculty of Animal Husbandry, University of Brawijaya

Table 2. Chemical composition and fermentation products in vitro from *Moringa oleifera* leaves and *Paraserianthes falcataria* leaves

Variables	*Moringa oleifera* leaves	*Paraserianthes falcataria* leaves
Dry matter (%)[1]	18.4	21,3
Organic matter (%) [1]	87.1	96.3
Crude protein (%) [1]	36.6	23.3
Crude fiber (%) [1]	10.8	37.9
Extract etter (%) [1]	5.79	5.41
Neutral Detergent Fiber (%) [1]	16.1	52.3
Acid Detergent Fiber (%) [1]	12.7	43.1
Total phenol (TP)[*2]	8.37	26.7
Total tannin (TT)[*2]	3.39	20.4
Condenzed tannin (CT)[*2]	0.19	5.89
Total saponin (TS)[*2]	5.89	3.98
Degradation of DM (%)[3]	68.3±1.81	27.7 ±4.55
Degradation of OM (%)[3]	76.8 ±2.30	28.3±4.29
Microbial biomass (mg/0.5gDM) [3]	114±8.75	62.1±15.9
Apparent digestibility (mg/0.5gDM) [3]	275±4.99	119±6.27
True digestibility (mg/0.5gDM) [3]	389±6.12	180±9.30
Number of protozoa (10^4 cells/ml rumen fluid)[3]		
Incubate 4 hours	3.33±1.31	2.93±0.87
Incubate 12 hours	2.93±1.06	2.53±0.94
Incubate 24 hours	3.07±0.87	2.93±0.63
NH_3 (mg/100 ml rumen fluid) [3)]		
Incubate 4 hours	13.3±0.42	8.23±0.19
Incubate 12 hours	13.4±0.37	6.09±0.30
Incubate 24 hours	13.6±0.58	8.32±0.31
CH_4 (%/ml) [4)]		
Incubate 12 hours	0.29	0.16
Incubate 24 hours	0. 57	0.35

[1]) Based 100% DM, Analysis of Animal Nutrition and Feed Laboratory, Faculty of Animal Husbandry, University of Brawijaya

[2]) Analyzed at the Laboratory Animal Research Center Ciawi.

[3]) Fermentation products in vitro incubation of 48 hours

[4]) Analyzed at Integrated Research and Testing Laboratory (IRTL), Gadjah Mada University.

Fermentation products in vitro

Feed treatment resulted in very significantly differences (P<0.01) to the value of DM and OM degradation (Table 3). DM degradation value and the value of feed OM degradation ranged from 64% -70%.

Table 3. DM degradation value, OM degradation value, microbial biomass, apparent degradability and true degradability of feed treatment in vitro incubation of 48 hours

Feed	DM degradation value	OM degradation value	microbial biomass	Apparent degradability	True degradability
concentrate : maize stover(M)	(%)	(%)	(mg/0.5gDM)	(mg/0.5gDM)	(mg/0.5gDM)
AP1 = A30% : M 70%	75.1 ^{d} ±0.42	78.4 ^{g} ±1.34	68.8 ^{d} ±4.65	284±4.59	356 ^{f} ±0.53
AP2 = A40% : M 60%	72.9 ^{d} ±0.79	76.3 ^{efg} ±0.53	59.2 ^{bcd} ±3.11	290±0.80	348 ^{e} ±1.65
AP3 = A50% : M 50%	72.9 ^{d} ±1.61	76.7 ^{fg} ±1.12	60.7 ^{bcd} ±7.15	304±13.67	353 ^{f} ±2.21
BP1 = B30% : M 70%	66.6 ^{ab} ±1.39	70.5 ^{abc} ±1.81	57.9 ^{abcd}±2.87	277±10.56	330 ^{a} ±1.81
BP2 = B40% : M 60%	67.3 ^{ab} ±0.75	72.4 ^{bcd} ±1.32	67.4 ^{cd} ±0.47	277±2.94	343 ^{de} ±0.90
BP3 = B50% : M 50%	69.8 ^{c} ±2.08	73.7 ^{bcd} ±2.39	68.3 ^{cd} ±3.29	292±9.10	355 ^{f} ±1.18
CP1 = C30% : M70%	66.2 ^{a} ±1.11	71.4 ^{abcd} ±1.59	61.7 ^{bcd} ±3.55	277±2.51	339 ^{cd} ±0.89
CP2 = C40% : M 60%	65.4 ^{a} ±1.06	68.4 ^{a} ±1.33	50.2 ^{ab} ±3.71	284±3.50	334 ^{ab} ±0.90
CP3 = C50% : M 50%	69.4 ^{a} ±1.08	73.3 ^{cde} ±1.17	57.9 ^{abcd}±5.20	288±4.34	344 ^{de} ±4.57
DP1 = D30% : M 70%	64.7 ^{a} ±1.52	68.5 ^{a} ±1.32	55.5 ^{abc}±10.1	288±2.15	338 ^{bc} ±1.95
DP2 = D40% : M 60%	64.3 ^{a} ±0.76	69.5 ^{ab} ±1.65	52.3 ^{ab} ±6.51	289±0.92	346 ^{e} ±0.47
DP3 = D50% : M 50%	66.4 ^{a} ±0.45	70.4 ^{abc} ±1.03	46.0 ^{a} ±1.68	298±1.57	343^{e}±1.85d
A	73.7 ^{c} ±3.74	77.2 ^{b} ±3.28	62.9 ^{bc} ±15.5	293 ^{b} ±30.7	353 ^{c} ±12.4
B	67.7 ^{b} ±5.08	72.2 ^{a} ±4.98	64.6 ^{c} ±17.3	282 ^{a} ±25.9	343 ^{b} ±37.1
C	66.9 ^{b} ±6.37	71.0 ^{a} ±7.36	56.6 ^{ab} ±17.6	283 ^{a} ±16.6	339 ^{a} ±15.3
D	65.1 ^{a} ±3.25	69.5 ^{a} ±2.83	51.3 ^{a} ±14.6	292 ^{b} ±15.6	342 ^{b} ±12.4
P1	68.2 ^{ab} ±14.1	72.2 ^{ab} ±12.9	61.0 ^{a} ±17.4	282 ^{a} ±16.5	341 ^{a} ±32.7
P2	67.4 ^{a} ±11.5	71.7 ^{a} ±10.6	57.3 ^{a} ±23.4	285 ^{a} ±18.1	343 ^{a} ±19.1
P3	69.6 ^{b} ±8.14	73.5 ^{b} ±7.77	58.2 ^{a} ±27.7	296 ^{b} ±20.9	349 ^{b} ±18.9

Based 100% DM, Analysis of Animal Nutrition and Feed Laboratory, Faculty of Animal Husbandry, University of Brawijaya $^{a-e}$: Different superscripts in the same column showed very significantly differences (P<0.01). ^{a} The same superscript in the same column indicates no significant difference (P>0.05).

The feed with MOL and PFL produce DM and OM degradation value is lower than the feed without MOL and PFL (AP1, AP2 and AP3). Feed without MOL and PFL is more easily degraded by rumen microbes. DM and OM degradation is used as an indicator to determine feed quality due to degradation of the value indicates the amount of nutrients in feedstuffs that can be utilized by rumen microbes and animals. Feed DP3 (D50%: M50%) resulted in a lower degradation rate, because the feed DP3 difficult to degrade due to the presence of tannins in the leaves. Feed quality protein sources can be protected by the tannins of rumen microorganisms degradation in neutral pH conditions, which is expected to be available at post-ruminal digestive tract. Low pH conditions in the abomasum causing bond tannin-protein complex can be separated and the protein can be digested by the enzyme pepsin that amino acids available to the animal.

Production of microbial biomass indicates the number of rumen microbes (bacteria, protozoa, fungi) that play a role in degrading the feed in the rumen. DP3 feed produces the lowest microbial biomass production. CT compounds and saponins from MOL and PFL in feed resulted DP3 protease and cellulase enzyme activity inhibited rumen microbes, which marked the production of microbial biomass and low feed degradation. Production of microbial biomass and high digestibility true value on feed AP1, BP2 and BP3 show potential feed readily soluble and easily degraded in the rumen.

NH_3 concentration ranged from 6.99 to 11.7mg/100ml rumen fluid allows for optimal microbial growth. NH_3 levels for optimal microbial growth that ranged from a minimum of 5-8 mg / 100 ml rumen fluid. NH_3 is one result of an overhaul of protein by rumen microbes. 24-hour in vitro incubation accumulation reshuffle results so that the rumen microbial protein by NH_3 concentration is higher than the 12-hour incubation. Feed DP3 produce high NH_3 concentration at 24 hours incubation. This case shows the proportion of 50% forage: 50% concentrate on feed D produces optimal microbial growth. The secondary compounds in MOL and PFL on Feed DP3, causing feed more protected from microbial degradation processes in the rumen, so that the value of DM degradation and degradation OM are low (Table 3).

Table 4. NH_3 concentration, ratio $(C_2+C_4)/ C_3$ of feed at 4, 12 and 24 hours incubation

Feed	NH_3 (mg/100ml rumen fluid)			Ratio $(C_2+C_4)/ C_3$		
concentrate : maize stover(M)	4 hours	12 hours	24 hours	4 hours	12 hours	24 hours
AP1 = A30% : M70%	9.26±2.98	11.7 [b] ±0.70	9.89±0.60	4.58	3.28	2.99
AP2 = A40% : M60%	8.75±3.72	8.90 [a] ±3.10	10.6±1.11	5.13	3.03	2.99
AP3 = A50% : M50%	8.83±3.27	8.59 [a] ±2.62	8.53±0.79	4.73	2.91	2.65
BP1 = B30% : M70%	7.78±2.00	8.22 [a] ±1.96	9.46±0.20	5.01	3.23	3.13
BP2 = B40% : M60%	8.12±1.40	9.22 [a] ±2.14	9.44±0.53	5.04	3.28	3.11
BP3 = B50% : M50%	7.48±1.71	8.14 [a] ±3.19	10.3±2.19	4.97	3.58	3.03
CP1 = C30% : M70%	8.76±3.60	8.92 [a] ±3.19	8.75±2.73	4.97	3.46	3.29
CP2 = C40% : M60%	8.16±1.63	8.29 [a] ±2.61	8.71±2.68	4.64	3.59	2.95
CP3 = C50% : M50%	7.90±1.05	9.20 [a] ±1.99	8.61±1.75	4.63	3.56	3.56
DP1 = D30% : M70%	7.17±2.21	6.99 [a] ±0.80	8.48±1.85	4.58	3.52	3.08
DP2 = D40% : M60%	8.04±2.10	8.65 [a] ±2.34	8.08±1.54	4.81	3.58	3.24
DP3 = D50% : M50%	7.48±1.71	8.29 [a] ±2.61	8.10±1.09	4.66	3.54	2.88
A	8.94±0.82	9.72 [b] ±5.07	9.67±3.15	4.80	3.07	2.88
B	7.79±0.96	8.53 [ab] ±1.80	9.72±1.40	5.01	3.35	3.09
C	8.27±1.34	8.80 [ab] ±1.39	8.69±0.20	4.73	3.54	3.27
D	7.56±1.32	7.98 [a] ±2.62	8.22±0.67	4.68	3.54	3.06
P1	8.24±2.83	8.95±5.94	9.14±1.94	4.78	3.37	3.12
P2	8.27±0.97	8.76±1.17	9.21±3.24	4.89	3.36	3.06
P3	7.92±1.91	8.56±1.40	8.88±2.84	4.74	3.37	3.02

[a-b] : Different superscripts in the same column showed very significantly differences ($P<0.01$). [a] The same superscript in the same column indicates no significant difference ($P>0.05$).

Fermentation of feed with higher results of acetic acid (C_2) and butyric acid (C_4), produce more greater ratio $(C_2 + C_4) / C_3$. Data Feed DP3 in Table 4, shows the proportion of C_3 increased compared the other feed, so that the value of the ratio $(C_2 + C_4) / C_3$ is lower. Variation of VFA production (C_2, C_3, C_4) causes differences in CH_4 production in the rumen [2]. C_2 and C_4 play a role in the formation of CH_4, when there is competition with the formation of H ions using propionate (C_3) in the rumen. Increased ratio $(C_2 + C_4) / C_3$ will produce high availability C_2 and C_4 so that the higher the production of CH_4. C_2 and C_4 are the source of energy for oxidation. C_2 is a non glukogenik compounds, and almost all tissues of the body are able to oxidize. Oxidation process produces high heat increament so that the lower feed efficiency. C_3 is a sugar compound precursors or primary glukogenik. C_3 of the reticulo-rumen is absorbed into the blood, through the portal vein to the liver to be converted into glucose through gluconeogenesis.

Methane production in vitro

Feed AP3 produce the highest gas production at 48 hours incubation in the amount of 90.8 ml / 0.5gDM. Feed AP3 without MOL and PFL easily degraded so that the resulting high gas production. Feed DP3 produces gas production, degradation of DM and OM were lower (Table 2.). Gas production depends on the amount of substrate degraded [22]. Feed DP3 able to produce CH_4 gas production 34% lower than the feed treatment without MOL and PFL. Feed without MOL and PFL produce CH4 gas production averaging 1.25%, while feed DP3 produce CH4 gas production of 0.76%. Low CH_4 production on feed DP_3, predictable result to supplementation MOL 30% and PFL10%. PFL leaves fermentation at 24 hours of incubation proved to produce CH_4 gas production amounted to 0.35%, while MOL fermentation, CH_4 production yield of 0.57% (Table 5.).

Supplementation of feed use MOL 30% and PFL 10%, causing a low CH_4 production. Ruminant feed is expected to increase production because it produces less energy in the form of CH4 is lost from the body. MOL-based feed supplements provide a positive response because it has a balanced content of essential amino acids [13]. This research results are consistent with previous research on *Moringa oleifera* (30%) and *Samanea saman* (10%) leaves supplementation. Supplementation of *Moringa oleifera* (30%) and *Samanea saman* (10%) leaves in the concentrate feed (18% crude protein) is given by 1.0% BW on basal diet of maize stover produce average daily gain of 87.7±18.3 g/head/day and normal range blood profile of growing rams. [23]

Table 5. Total gas production (ml/0.5 gDM), gas CH_4 (ml/0.5 gDM) of feed treatment in vitro at 12 and 24 h incubation

Feed concentrate : maize stover(M)	Total volume of gas[1] (ml/ 0.5g DM)		concentration CH_4* (%/ml)		Volume CH_4** (ml/ 0.5g DM)	
	12 hours	24 hours	12 hours	24 hours	12 hours	24 hours
AP1 = A30% : M70%	52.9	86.5	0.60	1.27	0.32	1.10
AP2 = A40% : M60%	57.8	90.8	0.55	1.24	0.32	1.13
AP3 = A50% : M50%	57.1	90.7	0.58	1.24	0.33	1.12
BP1 = B30% : M70%	51.8	84.2	0.41	1.02	0.21	0.86
BP2 = B40% : M60%	53.9	86.3	0.51	0.90	0.27	0.78
BP3 = B50% : M50%	54.7	85.4	0.38	0.95	0.21	0.81
CP1 = C30% : M70%	45.8	76.5	0.49	0.87	0.22	0.67
CP2 = C40% : M60%	45.8	74.0	0.48	0.82	0.22	0.61
CP3 = C50% : M50%	48.1	78.0	0.47	0.79	0.23	0.62
DP1 = D30% : M70%	35.1	61.8	0.57	0.91	0.20	0.56
DP2 = D40% : M60%	44.0	73.8	0.52	0.86	0.23	0.63
DP3 = D50% : M50%	41.9	71.8	0.47	0.76	0.20	0.55
A	55.9	89.3	0.58	1.25	0.32	1.12
B	53.4	85.3	0.43	0.96	0.23	0.82
C	46.6	76.2	0.48	0.83	0.22	0.63
D	40.3	69.1	0.52	0.84	0.21	0.58
P1	46.4	77.3	0.52	1.02	0.24	0.80
P2	50.4	81.2	0.52	0.96	0.26	0.79
P3	50.4	81.5	0.48	0.94	0.24	0.77

Specification: [1] Gas production through syiring, * Analysis of CH_4 at Integrated Research and Testing Laboratory (IRTL), Gadjah Mada University., ** volume CH_4 = total volume of gas[1] x concentration CH_4*

IV. Conclusion

It can be concluded that the usage of concentrate feed (MOL 30% : PFL 10% : coconut cake 45% : solid cassava waste 15%) with the proportion of concentrate 50% : forage 50% was be able to produce normal fermentation process and low CH_4 production. Indicator of normal fermentation at 48 hours of incubation *in-vitro* indicated DM degradation value 66.36%, OM degradation value 70.41%, gas production 94.83 ml, microbial biomass 46 mg, pH 6.67, NH_3 concentration 7.48 until 8.29 mg/100 ml rumen fluid and proportion VFA (C_2:C_3:C_4 = 65:26:9)) and the lowest CH_4 gas production (< 1% 24-hour incubation). It is suggested that in the future studies are in-vivo testing of the usage of MOL 30% : PFL 10% in the concentrate feed.

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