In vitro gas production and cinetical fermentation of a few local feed in Kupang East Nusa Tenggara

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Abstract. Research objectives were to evaluate and to analyze in vitro gas production and cinetical fermentation of Kupang’s local feed. The feed materials were dried and grinded for nutrient and gas produced analysis. Collected data were chemical composition, in vitro gas production, cinetical fermentation, short chain fatty acids, energy metabolic, and organic matter digestion. The result showed that the materials have different gas production. Commonly, the higher the carbohydrate content the higher the gas production; the higher the protein and the fat content the lower the gas production. Total gas productions of each material after 96 hours incubation were 53.88 ml/0.5 g DM for the high fat content feed, 92.26 and 159.25 ml/0.5 DM for the high protein and high carbohydrate content. Regarding total production, the fermented fraction gas productions were 44.662; 82.54 and 153.462 ml/0.5 g DM. The short chain fatty acids produced were 0.31; 0.51 and 1.28 mM. The feed organic matter digestations produced were 42.2; 55.91 and 68.8 % for each feed with a high content of fat, protein, and carbohydrate. Conclusively comparing with the protein and carbohydrate feed sources, the higher the fat content the lower the gas production, short chain fatty acids, and organic matter digestion.

Keywords: cinetical fermentation, energy metabolic, gas production, in vitro, short chain fatty acids

1. Introduction
There are many technologies can be used to evaluate the opportunity of using feed for livestock. The technologies are approachments to evaluate feed utilising. Feed evaluation plays important roles to inform individual feed capacity to fulfill the livestock needed and to evaluate more accurately the livestock performance [1].

Feed nutrition value for the livestock depends on consumption and nutrient absorption content during in digestive tract. Application of feed evaluation method can be done based on chemical method, digestion method which covers in vivo method and in situ method as well as in vitro method comprises the in vitro method of dry matter and the in vitro method of gas production [2].
In vitro method of gas production is a relatively cheaper, easier, and faster method [3] and it can be applied to stimulate rumen fermentation of feed materials [4]. The in vitro gas production also a method used for evaluating nutrition content of ruminant feed [5].

Based on the method, it can be known surely the fermentation kinetics that describes the amount of feed consumption, digestion of dry matter and organic matter, preparation of microbes nitrogen, the quantity of short chain fatty acids, CO2 and feed metabolic energy for the ruminants or as a technique to evaluate feed nutrition value and ration in anaerob fermentation [6].

Fermentation kinetics of a kind of feed material can be studied based on the gas production. The gas produced during anaerob fermentation shows the livestock feed utilising and it probably utilised as feed source for the livestock. Inoculum source in fermentation process and the fermentation kinetics evaluation can be taken from the ruminant rumen liquid.

The research objectives were to evaluate and to analyze the gas production and fermentation kinetics of local feed in East Nusa Tenggara.

2. Materials and method

2.1. Preparation of feed material

The research materials were local feed available in Kupang and its sorrounds. The materials with a high fiber content such as grass, and the materials with a high protein content namely the leaves of *Leucaena leucocephala* and *Glyricidia sepium* include cereals such as mayze and mung bean, as well as rice bran and coconut meal. Those materials, particularly the fresh leaves, were dried under shade during 2-3 days in order to release their water content. Then, the dry leaves were sunny dried in 3-4 hours until crysp. Further, the crysp leaves were milled to be flour. In addition, each kind of feed material was taken as sub sample and used for data collecting.

2.2. Analysis of chemical composition

The chemical composition analysis of the feed materials were done in Chemical Feed Laboratory of Brawijaya University based on an [7] mainly the analysis of DM, CP, EE, CF, NFE, and ash contents.

2.3. Evaluation of in vitro gas production

In vitro gas production was evaluated based on the method of [8] as follows. First, prepare 340 ml filtered rumen liquid. Second, prepare mixed buffer solution comprises: aquades 564 ml; buffer 376 ml; macro mineral 188 ml; micro mineral 0,12 ml; rezasurin 0,52 ml; reductor 30,9 ml. Then, fill the mixed buffer solution into erlenmeyer tube, mixed it and hotted at 39°C temperature. CO2 gas was flown while the reductor was added. The bluish solution will change into redness and colorless. The rumen liquid was fill into the tube and the CO2 was constantly flown and kept the temperature at 39°C. The gas production will be read at the hours of 2,4,8,12,16,24,36,48,72,96.

Metabolizable energy (ME, MJ/kg DM) and the organic matter digestion (%) according to [9]; ME (MJ/kg DM) = 2.2 + (0.136*Gv + (0.0057*PK) + (0.00029*EE). The organic matter digestion = 14.88 + (0.899*Gv) + (0.45*PK) + (0.651*ash).

The short chain fatty acids refers to [10]. The short chain fatty acids (mM) = 0,0239*Gv - 0,0601 where: Gv = gas volume at 24 hours; CP = Crude Protein; EE = Extract Ether; contain in incubated sample.

2.4. Data analysis

Data parameter of gas production was estimated based om exponential function model by (11) using computer program pocket Neway (12) as follows: Y = a + b (1-e^ct) where Y = amount of gas production at t time; a = gas production quantity of dissolved fraction (ml/500 mg DM); b = gas production of unresolved fraction (ml/500 mg DM); c = gas production constant of unresolved fraction (ml/hour); and
\[ a + b = \text{total gas production of fermented fraction (ml/500 mg DM)}; \ t = \text{incubation time (hour)} \text{ and } e = 2.7182 \ (\text{natural algorithm}). \]

3. Result and discussion

3.1. Nutrient content
Chemical composition data of the feed materials used in this study were shown on Table 1.

| Components                        | DM (%) | Nutrient content (\% DM) |
|-----------------------------------|--------|--------------------------|
| Native grass                      | 88.99  | 7.04 34.18 2.83 48.36 7.60 |
| Flour of Glycemia sepium leaves   | 87.18  | 24.28 11.41 5.08 47.42 11.29 |
| Flour of Leucaena leucocephala    | 87.99  | 29.26 14.29 5.66 40.50 10.28 |
| Flour of Corypha gebanga Stem     | 86.82  | 2.79 8.30 1.14 83.63 4.14 |
| Flour of Moringa oleifera leaves  | 87.97  | 31.10 7.95 5.49 46.49 8.97 |
| Mung bean                         | 86.64  | 22.51 7.07 0.83 65.90 3.69 |
| Coconut meal                      | 90.22  | 22.65 2.93 4.17 65.28 4.97 |
| Milled Mayze                      | 87.24  | 9.75 2.66 4.45 81.49 1.64 |
| Rice bran                         | 90.28  | 8.98 26.24 3.11 45.95 15.72 |

Table 1 data showed that each feed material has a different nutrient content. Besides the difference of genetic characteristics, the difference also influences rumen fermentation due to the quantity of gas production.

Commonly, the feed material protein content was a determination factor of feed quality. The higher protein content feed will influence the growth and activities of the rumen microbes. The higher the number of the rumen microbes, the faster the fermentation speed and the higher the gas production.

Besides the protein content, the crude fiber content of the feed materials also plays as feed quality indicator. In common, the crude fiber content has a negative correlation to digest value. The higher the crude fiber content, the lower the digest value produced [13].

3.2. In vitro gas production
Observation result on the in vitro gas production showed that there was a difference quantity of gas produced at each observation time as figured on Table 2.

Data of Table 2 described that there were a difference of gas quantity produced after 96 hours incubated. The difference showed that every feed material has a different fermentation speed of rumen microbes. Comparing with the high protein content, it was found that the higher carbohydrate content of the feed materials, the higher the gas production. It was occurred because common fermentation by rumen microbes in carbohydrate feed produced a higher gas quantity than that of the other nutrient feed sources.

The higher the protein content of the feed materials, the lower the gas production, because the protein content has a negative correlation to the gas produced [14] However, the higher the protein content has a correlation to N-NH3 production and synthesize microbes protein.

A high carbohydrate content in Corypha gebanga flour has a different effect on microbes growth and activities. Availability of easier fermentation carbohydrate will influence bactery growth, so it will produce more gas during fermentation. The bacterial speed growth very depends on the carbohydrate availability [15] and principally the gas production was a fermentation towards carbohydrate compound which produces acetate acid, propionate and butirate [16].

The lower the gas production, the lower the feed degradation in the rumen, and the higher the gas production the higher the easier fermented component. The higher the protein content, the lower the gas production during fermentation although it has a high degradation [17]; [18]. Protein fermentation will
produce ammonia influences buffer carbonate equilibrium by ion H neutralization of VFA without release CO₂ [19].

Besides the technical evaluation process, gas production quantity during fermentation was influenced by some factors such as incubation period, time of rumen liquid collecting, livestock donor species used, available or unavailable of nitrogen in buffer solution added into the rumen liquid include NDF content of utilised feed [5].

### Table 2. In vitro gas production of feed at any point of observation.

| Components                  | Gas Production (ml/0.5 g DM) at any point of observation (at hour of.....) |
|-----------------------------|--------------------------------------------------------------------------|
|                             | 0  | 2  | 4  | 8  | 12 | 16 | 24 | 48 | 72 | 96 |
| Native grass                | 0  | 3.33| 7.59| 15.38| 24.35| 41.30| 53.32| 85.61| 100.74| 103.30|
| Flour of Glyricidia sepium  | 0  | 5.82| 14.07| 27.51| 37.03| 49.99| 64.54| 87.81| 95.48| 95.75 |
| Flour of Leucaena leucocephala | 0  | 4.56| 10.51| 24.68| 32.73| 48.56| 55.27| 76.73| 83.44| 84.24 |
| Flour of putak              | 0  | 4.01| 8.31 | 34.05| 105.16| 118.68| 140.50| 145.97| 153.98| 159.24|
| Flour of Moringa oleifera   | 0  | 10.63| 23.12| 32.14| 49.78| 65.57| 59.61| 78.26| 86.04| 92.26 |
| Mung bean                   | 0  | 8.14| 19.64| 40.71| 75.83| 89.57| 108.70| 116.03| 124.68| 129.77|
| Coconut meal                | 0  | 10.11| 14.96| 22.98| 29.62| 34.98| 39.07| 42.38| 47.75| 53.88 |
| Milled mayze                | 0  | 8.86| 15.95| 42.22| 77.15| 83.93| 95.66| 100.09| 105.57| 111.30|
| Rice bran                   | 0  | 3.64| 8.36 | 19.74| 27.53| 38.70| 44.15| 55.84| 61.29| 66.75 |

### 3.3. Fermentation kinetics

Result of analysis on gas production produced obtained fermentation kinetics value as shown by the values of a, b, c and a+b, each value figured the quantity of gas production of dissolved fraction, quantity of gas production of unresolved fraction, gas production constant of unresolved fraction, and total gas production of unresolved fraction and total gas production of fermented fraction. The fermentation kinetics’ values were shown on Table 3.

### Table 3. Fermentation kinetics’ values of feed.

| Kind of Feed Materials          | Values of Fermentation Kinetics (ml/0.5 g DM) |
|---------------------------------|---------------------------------------------|
|                                 | a   | b   | c   | a+b           |
| Native grass                    | 4.019| 82.582| 0.031| 86.602        |
| Flour of Glyricidia sepium      | 6.319| 81.708| 0.037| 88.028        |
| Flour of Leucaena leucocephala  | 5.094| 71.452| 0.039| 76.546        |
| Flour of Corypha gebanga Stem   | 0   | 153.462| 0.060| 153.462       |
| Flour of Moringa oleifera leaves| 8.449| 74.095| 0.057| 82.544        |
| Mung bean                       | 1.688| 120.487| 0.059| 122.175       |
| Coconut meal                    | 5.508| 43.154| 0.057| 48.662        |
| Milled mayze                    | 0   | 104.662| 0.077| 104.662       |
| Rice bran                       | 3.821| 55.420| 0.042| 59.241        |

Data of Table 3 showed that different kinds of feed have a different gas production produced by fermented fraction. Quantity of gas production very depend on the fiber content mainly NDF and ADF. The lower the content of NDF and ADF fraction, the higher the gas production. This condition occured because of enzyme penetration produced by microbes during fermentation time.

### 3.4. Metabolisable Energy (ME), Digestible of Organic Matter (DOM) and Short Chain Fatty Acids (SCFA)

Regarding the in vitro gas production process, the quantity of gas produced during the process, it can be calculated the value of organic matter, metabolic energy, and short chain of fatty acid as shown on Table 4.
Table 4. Metabolisable Energy (ME), Digestible of Organic Matter (DOM) and Short Chain Fatty Acid (SCFA) of Feed Materials.

| Kinds of Feed                  | ME (MJ/kg DM) | DOM (%) | SCFA (mM) |
|--------------------------------|---------------|---------|-----------|
| Native grass                   | 5.52          | 41.95   | 0.45      |
| Flour of Glyricidia sepium leaves | 7.14        | 56.10   | 0.56      |
| Flour of Leucaena leucocephala leaves | 6.92       | 54.39   | 0.47      |
| Flour of Corypha gebanga Stem  | 10.01         | 68.80   | 1.28      |
| Flour of Moringa oleifera leaves | 7.26        | 55.91   | 0.51      |
| Mung bean                      | 9.46          | 66.07   | 0.98      |
| Coconut meal                   | 5.96          | 42.20   | 0.31      |
| Milled mayze                   | 8.00          | 54.35   | 0.85      |
| Rice bran                      | 5.14          | 44.86   | 0.36      |

Data of Table 4 described that each feed material has a different metabolic energy. The difference of metabolic energy indicated that during fermentation the microbes in the digestive tract also produced a different metabolic energy. The difference metabolic energy of each feed material has a significant correlation to the fermentation speed in digestive tract. The faster the speed of the feed material fermentation, the faster the available of metabolic energy for livestock.

Besides, the content of crude protein was different of each kind of feed. The breaking protein in rumen will produce amino acid and it followed by ammonia. The ammonia was a source of nitrogen to increase rumen microbes activities and it has a function as a source of microbes amino acid formulation, and besides, it needs easier degradation carbohydrate [20]; [21].

4. Conclusion

It can be concluded that:

1. Production of gas, fermentation kinetics, metabolic energy, organic matter digestion, and short chain fatty acids among the feed materials were different each other because the differences of the nutrient content.
2. The feed contains a high easier fermented carbohydrate tends to produce higher short chain fatty acids and organic matter digestion

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