***In vitro* gas production and its prediction on metabolize energy of complete feed using rumen fluid of three *Indigenous* cattle as inoculum taken from abattoir**

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ABSTRACT: *In vitro* gas production (IVGP) technique has been routinely used to evaluate the nutritional value of ruminant feed. The pre-requisite of using rumen fluid from fistulae animals is now facing a raising pressure from the animal welfare organization to ban this practice. Alternatively, rumen fluid (RF) from abattoir has been suggested to be used as source of inoculum by a number of scientists. The objective of this study was to evaluate the potential of rumen fluid taken from cattle that were slaughtered at Surabaya abattoir as inoculum for IVGP measurement and its prediction on metabolic energy (ME) of a complete feed. Fresh rumen fluid of three indigenous cattle, namely Ongole Cross Breed (OCB), Madura (MC) and Bali Cattle (BC) was transferred into a vacuum thermo flask and immediately transported to the animal nutrition laboratory of Brawijaya University for further processes to measure IVGP of complete feed in a completely randomized block design with two replicates each. Gas production was monitored at time intervals and terminated after 48 hours incubation in the oxygen-free glass syringes at 38°C. Feed degradability was calculated by gravimetric method at 48 hours, while prediction of ME content was based on 24 h GP and estimated using the equation described by Menke and Steingass (1979). The results demonstrated that inoculum from OCB resulted in the superiority in all parameters, namely total GP (ml/500 mg DM), potential of GP (ml/500 mg DM), rate constant of gas production for insoluble fraction (ml/hour), and estimated ME content (MJ/DM) compared with MC and BC. Based on these findings it can be concluded that the fermentation process of complete feed in the rumen of OCB is more efficient than MC and BC which reflects to the more efficient interaction among rumen microbes to ferment the feed ingredients.

Keywords: *In vitro* gas production; Metabolize energy; Ongole Cross Breed Cattle; Bali Cattle; Madura Cattle

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INTRODUCTION

The main factor affecting the increase in livestock production in tropical countries is the uncertain quality and quantity of feed. Providing good quality feed can increase and maintain livestock productivity. Smallholder farmers in Indonesia generally provide feed based on by-products from agriculture and plantations which contain high fiber and low protein, minerals and vitamins. Assessment of feed quality can be seen from the digestibility value of the feed. Parameters in determining the quality of feed can be done by using the *in vitro* method. There are three types of *in vitro* digestibility test techniques, namely 1) rumen microorganisms (Tilley and Terry, 1963), 2) gas method (Menke *et al.*, 1979) and 3) in situ with nylon bags (Mehrez and Orskov, 1977)) citation (Menke *et al.*, 1979). The method that is mostly used is the modification gas method.

The advantage of the gas method (Getachew *et al.*, 2004) is that it does not require sophisticated equipment and can be used with many feed samples at the same time. The displayed results provide an overview of the product, the fermentation rate, which is indicated by the increase in gas that can be observed over time. This method also results in an evaluation of the degradation of organic matter and estimation of metabolize energy. Gas production is basically the result of fermentation of carbohydrates into acetate, propionate and butyrate. Weaknesses in this study using rumen fluid obtained from

fistulae cattle. Fistulas are cattle that are intentionally injured in their stomach to extract rumen fluids. This experiment was carried out on many livestock as part of the study (Prasad *et al.*, 2014). This has a serious clinical impact resulting in trauma, injuries to internal organs, injuries and complications (Sangwan *et al.*, 2011; Shah *et al.*, 2019).

This fistula experiment contradicts the principles of animal welfare. Etim *et al* (2014) state that the principles of animal welfare state that people who raise animals must be able to meet their 5 needs such as eating, drinking, protection, care, reducing pain and suffering and other needs. Steps that can be used to carry out digestibility analysis *in vitro* with respect to animal welfare are taking rumen fluids in slaughterhouses. This technique can be a solution to keep research going without hurting the livestock. Lutakome *et al* (2017) state that the results obtained are supported by previous journals, showing gas production data for several feed ingredients incubated with rumen fluid from abattoir is also a viable option for feed evaluation.

MATERIALS AND METHODS

The material used in this research is complete feed obtained from the beef cattle research workshop in Grati, Pasuruan, and East Java. Information obtained from the raw materials used consisted of 50% straw and 50% concentrate. The nutritional content of the complete feed is presented in Table 1.

Table1. The Nutrition value (%DM) of Complete feed

Parameters (%)					
DM	Ash	OM	CP	CF	EE
90,09	9.93	90,07	13,36	24,96	5,4

Note: Results of analysis at the Laboratory of Animal Nutrition Faculty of Animal Science, Brawijaya University (2020).

The method used in this study was the *in vitro* digestibility test of feed with inoculums from the slaughterhouse obtained in Pegirian, Surabaya, East Java. Rumen

fluid is collected in the morning, from 01.00 AM to 03.00 AM. Taking rumen fluid begins by filling a 1-2 liter flask with warm water 50-70°C, removing 1/3 of the water in

the flask and adding enough cold water. Next, check the temperature in the thermos with a thermometer until it reaches 40°C. Furthermore, taking rumen fluid from the digestive tract of livestock in the abattoir begins with selecting the cattle by paying attention to the physical characteristics of Madura Cattle (MC), Bali Cattle (BC) and Ongole Cross Breed Cattle (OCB), then taking rumen fluids from the livestock rumen by injuring the rumen (torn) with a size of ± 10 cm which is then check the temperature and pH and the rumen fluid and digested are put into the thermos (the water in the thermos has been wasted) the time needed is ± 5 minutes.

Rumen fluid intake is carried out in 3 parts, namely the top, middle and bottom. The liquid that has been taken is then taken to the laboratory to be used as an inoculum in gas production with a time of ± 3.5 hours. Arriving at the Animal Nutrition Laboratory of the Faculty of Animal science UB, Malang, the thermos containing the rumen fluid is checked for temperature and pH then coated with CO₂ and ensures the condition is still anaerobic (time ± 5 minutes) then the

rumen and digested fluids are mixed using a mixer at low speed (time $\pm 1-2$ minutes) and then filtered using a filter cloth as much as 4 layers and put into a solution in the Erlenmeyer.

The solution used in the *in vitro* digestibility test consisted of aquadest (376 ml), macro mineral solution (125 ml), micro mineral solution (0.08 ml), buffer solution (251 ml) and resazurin solution (0.34 ml) as well as reducing agent (20.6 ml) and rumen fluid (227 ml). The solutions are mixed sequentially and stirred with a magnetic stirrer and flowed with CO₂ gas. A bluish solution will turn pink and then become colorless. Before adding rumen fluid, the pH and temperature are first measured according to optimal conditions (pH 6.9-7, temperature 39-40°C). The homogeneous liquid is then put into 50 ml syringe using a dispenser, then record the initial volume and put it in water bath temperature 39°C. Incubated for 48 hours with checks for 0, 2, 4, 8, 16, 24, 48 hours. The calculation of the Metabolize Energy value uses the formula from the Menke and Steingass (1988) cited by Getachew *et al* (2002):

Forage (MJ/Kg DM)
($2.20 + 0.36 \text{ GP} + 0.057 \text{ CP} + 0.0029 \text{ CF}^2$)

Concentrate (MJ/Kg DM)
($1.06 + 0.1567 \text{ GP} + 0.084 \text{ CP} + 0.22 \text{ CF} - 0.081 \text{ CA}$)

Information:

The gas production used is a 200 mg feed dry matter after 24 h of incubation. Where, GP: Gas production, CP: Crude Protein, CF: Crude Fat and CA: Ash.

Statistical Analysis

Data for *In vitro* gas production (IVGP) were analyzed using a completely randomized block design with two replicates.

The treatment group is based on the source of 3 indigenous, namely Cattle Ongole Cross Breed, Madura and Bali cattle. Data were analysis of Varian (ANOVA) from one way if there were significant effect between treatments then continued with Least Significance Different (LSD).

RESULT AND DISCUSSION

In-vitro gas production and fermentation parameter values for complete feed

This *in vitro* gas production method can be used to measure and predict the digestibility value of feed ingredients and the effect of feed ingredients on fermentation in the rumen (Kurniawati, 2007). The gas produced during the feed fermentation process is 40% CO₂, 30-40% CH₄ and around 20% hydrogen and nitrogen gas (McDonald, 2010). Measurement of gas

production was observed for 48 hours (every hour 2, 4, 8, 12, 16, 24 and 48 the increase was calculated). The results of measurement of gas production for 48 hours are presented in Figure 1.

The results can be seen in Figure 1. It shows that OCB have the highest gas increase rate compared to other cows, it can be seen from the observation that the 4th to 48th hour OCB have the highest increase in gas production. This shows that the fermentation yield produced by OCB rumen fluid is more than that of MC and BC. The

gas yield obtained can be used as an indicator of acid fermentation production and the amount of gas produced as a prediction and rate of feed digestion (Getachew *et al.*, 2004). If so, this indicates that OCB have a more optimal fermentation result than other cows. This is indicated by the results of research from Umar *et al* (2011) with the highest total acetic, butyric and propionic acid in OCB with the highest value, namely 115.71 mmol and followed by MC with a total of 102.4 mmol and the lowest is BC with a value of 30.54 mmol.

Figure 1. Graph of total gas Production with Incubation time of 48 hours. OCB: Ongole cross-bred, MC: Madura cattle and BC: Bali cattle.

Table 2. Effect of complete feed on IVGP main parameters

Inoculum	Gas Production (ml/500mg DM)	b value (ml/500mg DM)	c value (ml/H)
OCB	116.79 ± 7.8	115.84 ± 6.5	0.099 ± 0.0098 ^b
MC	99.35 ± 2.5	105.97 ± 4.8	0.051 ± 0.0007 ^a
BC	100.73 ± 0.9	110.96 ± 0.1	0.048 ± 0.0007 ^a

Note : ^{a-b}different superscripts indicate significant differences (P<0.005)

b value: Potential of GP; c value: rate constant of GP for insoluble fraction; OCB : Ongole cross-bred; MC : Madura Cattle and BC: Bali Cattle

The potential rate of gas production per ml in feed can be determined by knowing the gas production value calculated in the SPSS program with the formula $Y = b(1 - e^{-ct})$. The results given have a value of b and a value of c. The value of b is the average value of the potential for gas production which is used as a parameter of the part of the organic material that has the potential to ferment in the rumen, while the

value of c is the rate of fermentation of feed in the rumen per hour (Olfaz *et al.*, 201). The results of the parameter values in the complete feed sample are presented in Table 2. Table 2. Shows that the source of inoculum from the cattle of the three breeds of cattle has not significant effect (P> 0.05) on the total gas and b value, but has a significant effect (P <0.05) on the c value. Total gas production and the highest b value came

from the inoculums OCB, BC and MC. A high b value has an impact on the feed fermentation rate so that the c value at the inoculum source has a significant effect ($P < 0.05$) on complete feed with the highest value on the OCB inoculum. This indicates that the OCB inoculum has microbes that are able to degrade high fiber feed compared to BC and MC. The function of microbes in the rumen has a role as feed degradation capable of producing fermentation products in the form of VFA (*volatile fatty acid*) as a source of energy, protein and vitamins that can be utilized by host livestock (Creevey *et al.*, 2011). Thus, it shows that PO cattle have characteristics that are more able to survive

in tropical countries compared to other cattle with the ability to degrade high fiber feed which is better (Sutarno and Setiawan, 2015).

Rumen Degradability

Feed digestibility is meant here is the degradability which is obtained from the residue after the incubation process of gas production for 48 hours in the rumen. The high and low digestibility values indicate the quality of the feed because the results of feed degradation can meet livestock needs (Mohamed and Chaudhry, 2008). The complete feed digestibility values with different inoculums in vitro are presented in Table 3.

Tabel 3. Rumen Degradability and ME in vitro with an incubation period of 48 hours

Inoculum	Parameters		
	RDMD (%)	ROMD (%)	ME (MJ/Kg DM)
OCB	50.79 ± 3.3	55.58 ± 2.3 ^b	11.33 ± 0.532 ^b
MC	33.46 ± 5.4	40.31 ± 4.3 ^a	9.35 ± 0.319 ^a
BC	42.85 ± 0.8	41.45 ± 0.9 ^{ab}	9.67 ± 0.008 ^a

Note : ^{a-b}different superscripts indicate significant differences ($P < 0.005$)

RDMP: Rumen Dry Matter Degradability; ROMD: Rumen Organic Matter Degradability; ME: Metabolize Energy; OCP : Ongole cross-bred; MC : Madura Cattle and BC: Bali Cattle

The results of statistical analysis showed that the treatment of inoculum sources in complete feed showed a significant difference ($P < 0.05$) in the (ROMD) and ME values. The results obtained showed that the highest RDMD and ROMD came from the OCB inoculum with a value of 50.79% and 55.58%, and followed by BC inoculum with a value of 42.85% and 41.45% and the lowest was in MC with a value of 33.46% and 40.31%. Digestibility results are strongly influenced by feed and microbes in the rumen. The complete feed given was of low quality because of its very high fiber content, namely 24.96% and CP 13.36%. According to Schneider (1975) the value of feed digestibility ranges from 50-70%. The good digestibility results were shown from the OCB inoculum which had a dry matter digestibility value of 50.79% and organic

matter 55.58%. This result is in line with the high value of gas produced by OCB because the increase in gas production rates is high, the better the feed degradation process carried out by rumen microbes (Rodriguez *et al.*, 2019).

Energy is one of the main indicators in determining the need for feed for ruminants. This energy can be obtained from various sources of organic feed ingredients, such as fiber, carbohydrates, fats and proteins. The potential that comes from each of these organic material sources as a provider of energy varies according to the level of degradability and ferment ability (Haryanto, 2012). The results are presented in Table 3. It shows that the source of inoculum of the three breeds of cattle has a significant effect ($P < 0.05$) on the ME value. The highest ME value is owned by the inoculum OCB, followed by BC and the lowest from MC.

The overall data generated shows that OCB has a very high ability to digest feed compared to BC and MC cows at low feed quality. This is consistent with the statement of Ngadiyono *et al* (2008) which states that OCB has a very high adaptability in tropical areas with low feed quality.

CONCLUSIONS

Based on the overall data generated from gas production parameters, degradability and ME in complete feed with low feed quality, it shows that OCB have the highest value which can be interpreted as OCB as a breed of cattle that is more efficient or able to degradability feed better than Bali.

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