Comparison of three different rumen fluid as a source of inoculum to evaluate in vitro gas production and digestibility of elephant grass-concentrate mixture

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Abstract. This study aimed at elucidating the use of three different rumen fluid (RF) of indigenous cattle breeds i.e. Bali, Madura and Crossbred Ongole immediately after slaughtered at abattoir to evaluate the nutritive value of elephant grass (EG) - concentrate mixture using a standard in vitro gas production (IVGP) technique. Approximately 500 mg feed dry matter/syringe was added with 50 ml RF-buffer solution and incubated in a 39 °C water bath for 48 hours where gas production was observed at time intervals. Following termination of incubation the content was transferred into tare glass crucible to measure rumen dry matter (RDMD) and organic matter (ROMD) digestibility. The results showed that there was no significant different (P>0.05) in gas production parameters. In contrast, RDMD and ROMD differed significantly (P<0.01) among cattle breeds. RF from OCB resulted in the highest IVGP, RDMD and ROMD as compared with other RF sources. In conclusion, the use of RF from abattoir for IVGP measurement can be warranted using the same source of RF. The highest values resulted from OCB suggests that the abundance and variation in rumen microbiota may exist among cattle breeds.

Keywords: Bali, Madura and Crossbred Ongole Cattle, Elephant grass, In Vitro gas production.

1. Introduction

Feeding ruminant animals require a routine laboratory analysis for feed evaluation that meet some criteria such as simple, valid, highly reproducible, robust and conform to animal welfare consideration. In vitro gas production (IVGP) technique has been accepted worldwide to be employed routinely in feed evaluation for ruminant animals. The historical review on in vitro techniques for measuring feed digestibility has been discussed thoroughly by [1]. The intensive use of Tilley and Terry method [2] to study in vitro forage digestibility since 1963 was challenged by in vitro gas production technique introduced by [3] and in the later days this technique underwent some development when a test feed contains secondary compounds such as tannin [4,5,6] and by the type of incubator used [7].

Most in vitro digestion techniques require rumen-microbial inoculum either taken directly from cannulated animals or aspirated using a stomach tube inserted from the mouth. These common practices are currently subjected to criticism not only from the cost-demanding and tediousness of keeping such rumen-fistulated animals but the pressure from animal lovers for animal welfare reasons have forced ruminant scientists to seek other alternatives to obtain rumen fluid.

For this reason some ruminant scientists [8,9,10] have done intensive in vitro digestion studies using RF taken from slaughtered cattle as microbial inoculum. The results seem promising and they all concurred that RF from slaughtered cattle can replace rumen fluid from cannulated animals for IVGP
although precautions such as similarity in pre-slaughter conditions of feeding and cattle breed differences should be considered as sources of variation.

However, under the practical conditions of Indonesia cattle that are slaughtered at the abattoir consume various quality of feed as the majority of them come from a smallholder farmer. It therefore is a challenging problem to resolve the notion of such variation as sources of microbial inoculum. In addition to that, the most frequent breeds of cattle that are slaughtered at the abattoir throughout the country are generally indigenous breeds such as Bali, Madura cattle and Crossbred Ongole cattle [11] which lack of information in the literature upon appropriateness of their RF post-slaughtered to be used for microbial inoculum for IVGP measurement.

The study reported herein aimed at investigating the effect of using three different indigenous cattle as sources of RF on IVGP and feed digestibility

2. Material and methods

2.1. Material

The material used in this study was RF as the source of microbial inoculum from three breeds of cattle, namely Crossbred Ongole (OCB), Madura Cattle (MC), and Bali Cattle (BC), collected from the abattoir at Pegirian, Surabaya, East Java. A diet consisted of EG and feed concentrate obtained from Beef cattle Research Station, Grati, East Java at a ratio of 60:40 was used throughout the study. Table 1 below describes the chemical composition of the diet.

| Ingredients       | Chemical composition (%) |          |          |          |
|-------------------|--------------------------|----------|----------|----------|
|                   | DM          | OM       | CP       | CF       | EE       | (% DM) |
| EG                | 15.25       | 84.57    | 6.90     | 33.34    | 2.83     |        |
| Concentrate       | 90.39       | 88.14    | 15.35    | 19.16    | 5.85     |        |
| *Diet             | 93.41       | 86.00    | 10.28    | 27.67    | 4.04     |        |

2.2. IVGP Methods

The methods of measuring IVGP and feed digestibility are essentially according to those described by [4 and 5]. In brief, 500 mg air dry weight of sample was inserted into calibrated glass syringes of 100 ml and kept warm at 39–40°C in the incubator until being added with 50 ml RF buffer mixture solution. Incubation lasted for 48 hours and observations on gas production were made at 0, 2, 4, 8, 16, 24, and 48 hours.

The potential gas production rate per ml in feed can be determined by knowing the gas production value calculated by the formula as follows:

\[ Y = b(1 - e^{ct}) \]

of which the values of b and c denote the potential for gas production and the rate of feed fermentation (ml/hour). The ME (MJ/Kg DM) content of feed was estimated using the formula described by [12 and 13] as follows:

\[ \text{Forage} = 2.20 + 0.36 \text{GP} + 0.057 \text{CP} + 0.0029 \text{EE}^2 \]

\[ \text{Concentrate} = 1.06 + 0.1567 \text{GP} + 0.084 \text{CP} + 0.22 \text{EE} - 0.081 \text{CA} \]

Note: gas production (GP) obtained from this study was calibrated to 200 mg of feed samples in dry matter with an incubation time of 24 hours.

2.3. Data Analysis

A randomized block design was used to analyse the data of IVGP where three different sources of microbial inoculum taken from Bali (BC), cross bred Ongole (CBO) and Madura cattle (MC) acted as blocks. After calculating a one way analysis of variance (ANOVA), the significant differences of the data were subjected to a Least significant difference test (LSD).
3. Results and discussion

As shown in Table 1, the chemical composition of the diet used in this experiment represents a low to medium quality feed commonly given to beef cattle by smallholder farmers in Indonesia [14] and somewhat higher than generally found in the smallholder farmers in other tropical countries such as Viet Nam [15,16]. At the present level of CP density beef cattle receiving this diet may only gain less than 0.48 kg/d [14]. Similarly in Viet Nam [15] reported that with the CP content of the diet <10 % beef cattle daily gain varied from 0.43 to 0.69 kg/d. The recommended quality of beef cattle ration in the tropical climate should contain at least >12 % CP with >11 MJ ME/kg DM and 20-75% of NDF [14].

3.1. In vitro gas production

Figure 1 depicts the effect of microbial inoculum sources from three different cattle breed of which OCB demonstrated the highest production of gas as compared with other sources of inoculum although not statistically significant (P>0.05). It is generally agreed that gas production reflects the microbial activity in feed fermentation process plus the indirect contribution of buffering short chain fatty acids [17]. From this study the highest value of total gas production (Table 2) from OCB microbial inoculum may reflects the more abundance and activity of rumen microbes than its counterparts, that is BC and MC which may be linked to the higher values of OMD and RMOD presented in Table 3.

![Figure 1](image-url)  
Figure 1. Cumulative gas Production (ml/ 0.5g DM) from in vitro fermentation of feed using three indigenous slaughtered cattle as sources of microbial inoculum.

| RF  | Total gas Production (ml/500mg DM) | b (ml/500mg DM) | c (ml/h) |
|-----|-----------------------------------|-----------------|---------|
| OCB | 124.38 ± 7.512                    | 128.60 ± 8.799  | 0.072 ± 0.005 |
| MC  | 105.90 ± 9.533                    | 117.42 ± 14.092 | 0.051 ± 0.008 |
| BC  | 116.94 ± 0.624                    | 137.89 ± 5.315  | 0.041 ± 0.004 |

Note: b: gas production potential and c: gas production rate.

As shown in Table 2 there was no significant differences attributed to inoculum sources (P> 0.05) on the values of b and c. Cattle age has been reported to influence the abundance of rumen microbes as generally demonstrated by feed, race, and genetics of each individual differences[18]. [19] reported that the microbial population in the rumen of CBO showed a bacterial population of $2.3 \times 10^8$ (cfu / g); Protozoa $76.33 / \mu l$ of RF, while the characteristics of RF of Bali cattle were reported by [20] having a total number of bacteria ranged from $1.10 \times 10^9$ (cfu / ml) and other researchers [21] reported the number of rumen protozoa in Bali cattle was $4.69 \pm 0.64$ (log cells/ml), respectively. The difference in microbial populations in the rumen will affect the fermentation process as has been reported by a number of researchers [18, 22].
3.2. Rumen degradability

The results of feed degradation in the rumen and the estimated ME values are presented in Table 3.

| RF  | ME (MJ/KgDM) | RDMD(%) | ROMD(%) |
|-----|--------------|---------|---------|
| OCB | 9.31 ± 0.373 | 52.93 ± 0.388⁹ | 52.94 ± 1.552⁹ |
| MC  | 7.90 ± 0.027 | 39.73 ± 2.093⁸ | 43.84 ± 0.589⁸ |
| BC  | 8.21 ± 0.113 | 48.28 ± 1.090⁹ | 46.94 ± 1.118⁹ |

Note: a-b Different superscript denotes highly significant difference (P<0.01).

As shown in Table 3 the predicted ME values of the feed ranged from 7.90 - 9.31 (MJ / kg DM) and it fell within the typical feedstuffs given to beef cattle reared by smallholder farmer. ME values of feed is generally associated with the amount of fermentable carbohydrate in the feed [22] and [23] found that ME values varied significantly among the feedstuffs generally fed to ruminants and it therefore is likely that ME value of feed derived from IVGP may not be considered as an absolute value [24]. In line with this notion, the ME values derived from this study may represent the variation in rumen microbiota responsible to ferment the nutrients rather than the real energy content of the diet. It thus is evidence that the higher RDMD, ROMD and hence the estimated ME value determined using OCB RF reflected the more abundance and variety of rumen microbiota than other two counterparts [25]. Recently [26] reported a higher average daily gain of Sumba Ongole, that is the ancestor of OCB, utilizing the same diet as compared with Bali and Madura cattle and they concluded that Sumba Ongole could gain more weight was attributable to the higher ratio between energy retention to digested energy intake when expressed in g/kg BW⁰⁷⁵.

4. Conclusions

Evaluation of gas production in forage and concentrate (60:40) using three different RFs showed a significant effect (P<0.01) on RDMD and ROMD with the best inoculum of OCB. These results indicate that the microbes in the OCB rumen have better degradation ability than the other two counterparts.

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