

Determination of *in Vitro* Digestibility of Tropical Feeds Using Cattle Faeces as Rumen Fluid Alternative

R. Utomo^{a,*}, M. Soejono^a, B. P. Widyobroto^a, & Sudirman^b

^aFaculty of Animal Science, Gadjah Mada University
Jln. Fauna No. 3, Kampus UGM Bulaksumur, Yogyakarta 55281, Indonesia

^bFaculty of Animal Science, Mataram University
Kampus Gomong, Jln. Majapahit 62, Mataram – Nusa Tenggara Barat, Indonesia

(Received 29-10-2010; accepted 04-10-2011)

ABSTRAK

Penelitian ini dilakukan untuk membandingkan penggunaan feses yang dilarutkan dalam akuades (P1), NaCl-fisologis (P2), saliva buatan (P3), dan cairan rumen (rumen fluid/RF) sebagai sumber inokulan pada penetapan pencernaan *in vitro* bahan organik (KcIVBO) jerami padi, jerami jagung, rumput gajah, dan rumput pangola. Cairan rumen diambil dari dua ekor sapi peranakan ongole berfistula rumen dengan bobot badan 306 dan 333 kg. Sapi diberi pakan 3% dari berat badan terdiri atas 70% rumput gajah dan 30% konsentrat komersial selama 30 hari. Koleksi RF dan feses (grab sampling) dari ternak yang sama dilakukan berurutan pada minggu terakhir. Feses segar 350 g dilarutkan dalam 1 l pelarut, dihomogenkan menggunakan *blender* selama 30 detik, RF diambil langsung melalui fistula. Setelah disaring menggunakan kain kasa 4 lapis, dicampur dengan saliva buatan, rasio 1 : 4 (v/v). Setiap inokulan diambil 50 ml dimasukkan ke dalam tabung reaksi (100 ml) yang telah berisi 500 mg sampel pakan, kemudian diinkubasi pada suhu 39 °C selama 48 jam. Hasil penelitian menunjukkan bahwa KcIVBO rumput gajah, jerami padi, jerami jagung, maupun rumput pangola, tidak berbeda nyata antar pelarut, tetapi secara nyata ($P < 0,05$) lebih rendah daripada RF. Hasil penetapan KcIVBO menggunakan larutan feses mempunyai tingkat keeratan tinggi dengan cairan rumen yang ditunjukkan oleh koefisien regresi yang tinggi. Disimpulkan larutan feses menggunakan pelarut akuades (P1) dapat digunakan sebagai inokulan pengganti cairan rumen pada pendugaan penetapan KcIVBO pakan berserat. Namun demikian penggunaan larutan feses sebagai inokulan menghasilkan pencernaan *in vitro* yang lebih rendah daripada cairan rumen.

Kata kunci: in vitro, pencernaan bahan organik, larutan feses, cairan rumen, koefisien regresi

ABSTRACT

The research was conducted to compare the ability of faeces dissolved in distilled water (P1), saline solution (P2), artificial saliva (P3), and rumen fluid (RF) as sources of inoculant in *in vitro* organic matter digestibilities (IVOMD) of rice straw, corn stover, napier grass, and pangola grass. The rumen fluid was collected from two rumen fistulated Ongole Crossbred Cows of 306 and 333 kg body weight (BW). The cows were fed 3% of BW consisted of 70% napier grass and 30% concentrate. At the end of 30 days feeding, faecal solution was made out of 350 g fresh faeces dissolved in 1 l of each solvent, homogenized using blender for 30 second, while rumen liquor were collected directly from fistula. After straining with four layers of cheesecloth both faecal solution and rumen liquid were mixed with artificial saliva (1:4 v/v). Fifty ml of each inoculants was pipetted into each incubator tube (100 ml) containing 500 mg sample. The tubes were then incubated at 39 °C for 48 h. Value of IVOMD of napier grass, rice straw, corn stover, and pangola grass did not differ among the faecal solvents, but significantly lower ($P < 0.05$) compared to the RF. The value of IVOMD determined using faecal solvent inoculant highly correlated to the RF. The highest regression coefficient was shown by the relation between IVOMD of P1 solvent with RF. It is concluded that faecal dissolved in distilled water could replace rumen fluid as inoculants source in *in vitro* organic matter to predict digestibility of fibrous feed determination. However, the faecal solvent as inoculant produced lower *in vitro* digestibility than that of rumen fluid.

Key words: in vitro, organic matter digestibility, faecal solution, rumen fluid, regression equation

* Corresponding author:
E-mail: rastianuto_utomoto@yahoo.com

INTRODUCTION

Stability of ruminant productivity in Indonesia is determined by forage availability throughout the year (Abdullah & Suharlina, 2010). Information of digestibility values of each feedstuff is essential as asset feed information. The value could be obtained by *in vivo*, *in sacco*, or *in vitro* procedures. Even though the *in vivo* procedure produce more accurate information, but it is inefficient, labor and time consuming, high costs, and labor intensiveness (Adesogan, 2004). Many scholars (Williams, 2000; Mahadevamma *et al.*, 2004; Damiran *et al.*, 2007; Palić & Leeuw, 2009) have developed faster, less cost, and need small quantity of sample to get more numbers of feed used *in vitro* method. The method needs shorter time to predict digestibility (Utomo, 2010).

One of the most widely used *in vitro* digestibility method was developed by Tilley & Terry (Harris, 1970). The method depended on the availability of rumen fluid as inoculant source for fermentative process of digestibility. For some areas, collecting rumen fluid was difficult work because the need for rumen fistulated animal, which is not only high operational cost, easy to contaminate by diseases especially in tropical warm climate, hurting animal to made fistula or using stomach tube for rumen liquor collection, but also against animal welfare (Mauricio *et al.*, 2001; Dhanoa *et al.*, 2004).

The results of some experiments in sub tropic region showed that rumen fluid inoculants (RF) for determining *in vitro* digestibility could be replaced with faecal solution (FS) from some species of animal (Omed *et al.*, 2000; 1988; Akhter *et al.*, 1999). It has been reported that microbial species found in the faeces as inoculant were influenced by the kind of feed consumed by animal donor (Bauer *et al.*, 2004). Since, tropical feed are largely different with subtropics ones, the microbial species in ruminant are assumed to be different. Therefore the *in vitro* procedure using faecal solution as inoculant which has been developed in the subtropics might not be used

to predict Indonesia feedstuff value correctly. Artificial saliva (buffer) is commonly used as solvent for faeces by previous investigators; the solvent is expensive and less available in some areas.

The aim of this research was to compare the effectiveness of faeces and rumen fluid as inoculant source for *in vitro* organic matter digestibilities (IVOMD) determination and to find more affordable faecal solvent as an alternative to artificial saliva.

MATERIALS AND METHODS

The research was done in the Laboratory of Feed Technology, Department of Animal Nutrition and Feed Science, Faculty of Animal Science, Gadjah Mada University, Yogyakarta. Two fistulated rumen Ongole crossbred cows, about 5 years old, 333 and 306 kg body weight were kept in concrete floor individual stalls. In order to have an optimal and stable rumen ecological condition, the cows were fed 3% of body weight, consisted of 70% napier grass and 30% concentrate for 30 days including adaptation period. The concentrate was fed at 07.00 a.m. and 16.00 pm, while napier grass was given after the concentrate was consumed. The cows were offered water *ad libitum*. The chemical compositions of feedstuff used in the experiment were shown in Table 1, and the chemical compositions of the diet were shown in Table 2.

The rumen fluid and faeces were taken from both cows after three weeks of the ration feeding. Rumen fluid was taken using aspirator via rumen fistula and poured fully into the thermos bottle which has already been warmed by keeping hot water prior to collection to keep the temperature around 39 °C. While faeces was taken directly from the caecum (grab sampling), and kept at 39 °C in a thermostatic container.

Rumen fluid from both donor cows were mixed, strained with 4 layers of cheesecloth and then mixed with artificial saliva (McDougall's Artificial Saliva), in an

Table 1. Chemical composition of feedstuffs (%DM)

Feedstuffs	DM	CP	CF	EE	NFE	Ash	NDF	ADF
Rice straw	85.48	5.36	29.25	1.59	42.21	21.79	76.88	49.12
Corn stover	68.65	8.57	27.92	2.44	38.93	16.24	72.01	36.92
Napier grass	14.05	11.19	33.77	1.69	42.86	10.49	63.98	36.79
Concentrate	88.46	13.36	21.23	1.87	54.62	8.92	50.69	29.84

Note: Feed analysis of Lab. of Feed Technology, Faculty of Animal Science University of Gadjah Mada. CP= Crude protein, CF= Crude fiber, EE= Ether extract, NFE= nitrogen free extract, NDF= neutral detergent fiber, ADF= acid detergent fiber.

Table 2. Chemical composition of cows diet (%DM)

Feedstuffs	Level (%)	CP	CF	EE	NFE	Ash	NDF	ADF
Napier grass	70	7.83	23.64	1.18	26.66	10.69	44.79	25.75
Concentrate	30	4.01	6.37	0.56	16.39	2.68	15.21	8.95
Total	100	11.84	30.01	1.74	43.04	13.36	59.99	34.70

Note: Feed analysis of Lab. of Feed Technology, Faculty of Animal Science University of Gadjah Mada. CP= Crude protein, CF= Crude fiber, EE= Ether extract, NFE= nitrogen free extract, NDF= neutral detergent fiber, ADF= acid detergent fiber.

Erlenmeyer flask at 1 : 4 (v/v) ratio (Harris, 1970). Before pouring into the incubation tubes which were already filled with feed sample, the inoculant were pre warmed in incubator at 39 °C and flushed with CO₂.

The inoculants of fresh faeces from both donor cows were mixed homogeneously, and dissolved in three different solvent (distilled water, saline, and artificial saliva) at concentration 35% (w/v) (Akhter *et al.*, 1999; Sudirman, 2007). The mixture was homogenized using a blender for 30 second. The mixtures were strained with 4 layers of cheesecloth and then processed like rumen fluid.

Four kind of fibrous feed namely rice straw (*Oryza sativa*), corn stover (*Zea mays*), napier grass (*Pennisetum purpureum*), and pangola grass (*Digitaria decumbens*) as control sample were used to examine the effectivity of faecal in comparison to rumen fluid for determining *in vitro* digestibility. All feeds were ground using a Wiley mill with 2 mm screen in order to have same particle size. Feeds samples were stored in scaled container in the laboratory at room temperature.

Fifty ml of each inoculant were poured into 100 ml incubation tubes which contained 500 mg of feed sample. Each tube was incubated for 48 h (one step

method) anaerobically at 39 °C waterbath, every 8 h the tubes were shaken by hand. After 48 h incubation, the mixture tubes were strained using predetermined weight porous crucible (30 ml) with glass wool on it. The crucibles were dried at 105 °C until its weight constant to obtain indigestible dry matter residue. In order to get the indigestible organic matter, the crucibles were ignited at 550 °C oven temperature for 5 h. *In vitro* digestibility was calculated using formula of Tilley and Terry method (Harris, 1970).

The experiment was conducted in completely randomized design. Four kinds of inoculants types were used as treatments with 5 replications. The means were tested by Duncan multiple range test. While the ability of faecal solvent to replace rumen fluid was examined using simple linear regression analyses (Gomez & Gomez, 2007). The linear regression equation was $Y = a + bX$, the kind of faecal solution were X and rumen fluid inoculant was Y.

RESULTS AND DISCUSSION

The IVOMD values of all feeds digested with faeces dissolved in different solvent (P1-P3) were not

Table 3. Average *in vitro* organic matter digestibility (%) of fibrous feed using different inoculant sources

Feedstuffs	Treatments			
	P1	P2	P3	RF
Rice straw	22.23±0.46 ^b	21.40±0.58 ^b	20.45±1.36 ^b	28.87±0.92 ^a
Corn stover	33.99±1.88 ^b	32.69±3.19 ^b	32.69±2.34 ^b	35.53±0.99 ^a
Napier grass	31.32±0.75 ^b	28.48±1.36 ^b	29.03±1.31 ^b	50.81±1.15 ^a
Pangola grass	38.63±2.97 ^b	31.73±4.96 ^b	30.82±3.19 ^b	61.37±2.34 ^a

Note: P1= faecal inoculant with solvent of distilled water, P2= saline, P3= artificial saliva; RF= rumen fluid. Means in the same row with different super-script differ significantly ($P < 0.05$).

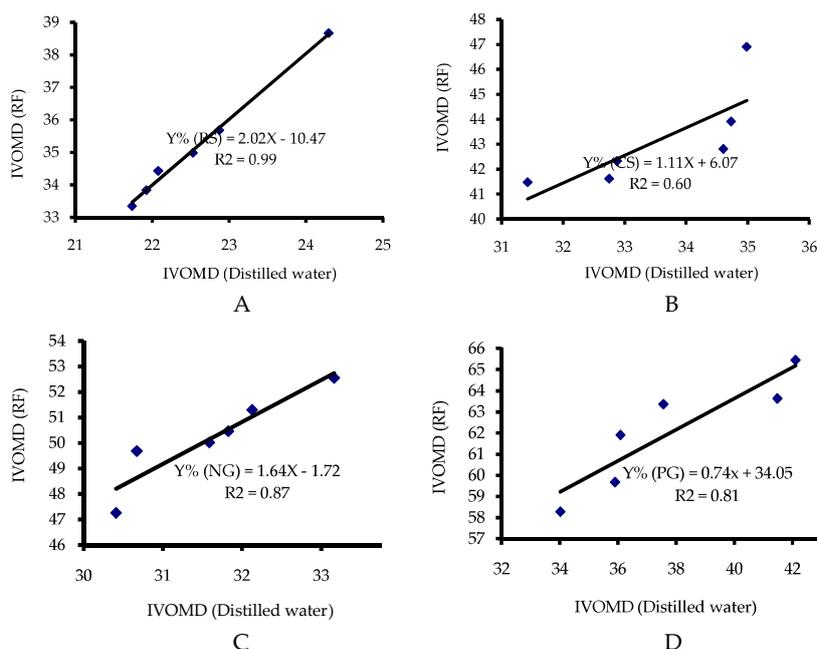


Figure 1. Correlation of feed *in vitro* organic matter digestibilities (%) between inoculant of rumen fluid (Y) and distilled water faecal solution (X). A= rice straw (RS), B= corn stover (CS), C= napier grass (NG), D= pangola grass (PG).

significantly different (Table 3). Therefore, since distilled water was more affordable, it could be used to replace the more expensive solvent such as saline and artificial saliva. The IVOMD obtained using faecal inoculant were significantly ($P < 0.05$) lower compared to that of rumen fluid inoculant, due to the lower total number of microbes in faecal solution than in the rumen fluid. Table 3 also showed that fibrous feeds (rice straw) which contained the highest neutral detergent fiber (Table 1) resulted in the lowest value of *in vitro* organic matter digestibility, followed by napier grass, corn stover, and pangola grass.

Based on simple linear regression analysis, the regression coefficient (R^2), there was close correlation between inoculant of faeces and that of rumen fluid. The equation of feed *in vitro* between inoculant of RF (Y) with faeces (X) using solvent P1 (distilled water) for rice straw was: $Y (\%) = -10.47 + 2.02 X$ ($R^2 = 0.99$), corn stover was: $Y = 6.07 + 1.11X$ ($R^2 = 0.60$), napier grass was $Y = -1.72 + 1.64X$ ($R^2 = 0.87$), and pangola grass was: $Y = 0.74X + 34.05$ ($R^2 = 0.81$), respectively (Figure 1). The equation using solvent P2 (saline) and P3 (artificial saliva) were shown in Figure 2 and Figure 3, respectively. The equation and regression coefficient value (R^2) as shown in

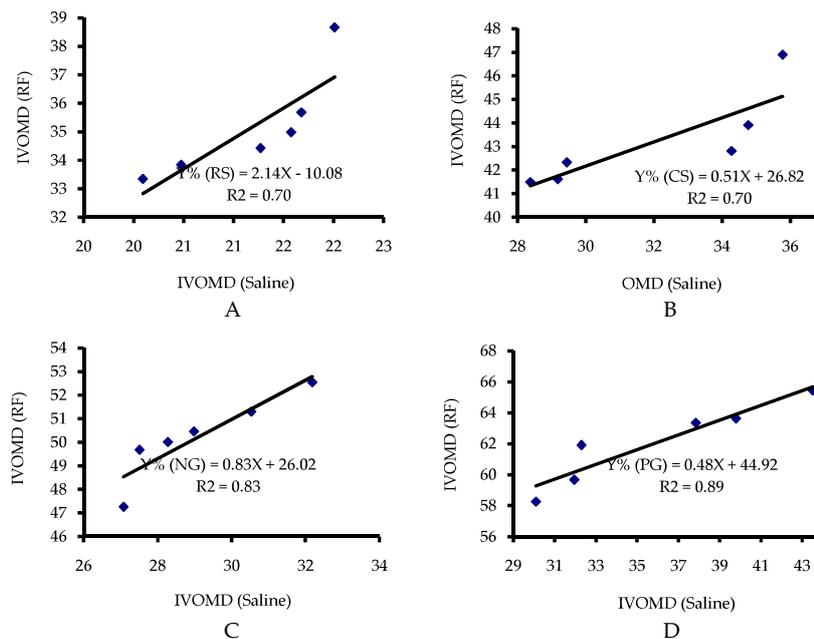


Figure 2. Correlation of feed *in vitro* organic matter digestibilities (%) between inoculant of rumen fluid (Y) and saline faecal solution (X). A= rice straw(RS), B= corn stover (CS), C= napier grass (NG), D= pangola grass (PG).

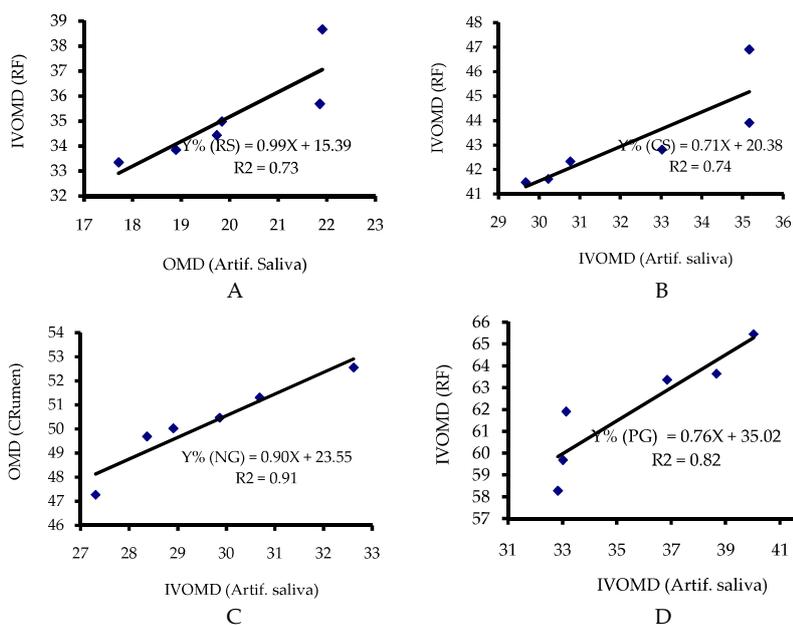


Figure 3. Correlation of feed *in vitro* organic matter digestibilities (%) between inoculant of rumen fluid (Y) and artificial saliva faecal solution (X). A= rice straw(RS), B= corn stover (CS), C= napier grass (NG), D= pangola grass (PG).

Figure 1, also gave indication that faecal solution could be used as inoculant for replacing rumen fluid on determining *in vitro* organic matter digestibility as reported by Dhanoa *et al.* (2004).

There was a close correlation between inoculant of faecal solution (P1-P3) with rumen fluid (RF). Therefore, based on the results of this research, the use of distilled water as solvent for faeces could be recommended to replace rumen fluid for predicting *in vitro* digestibility of feeds. Based on the graph, the correlation of feed *in vitro* organic matter digestibilities among three kind of faecal solvent with rumen fluid, there were some interesting information, such as tendency of similarity of regression coefficient value (R^2) between napier and pangola grass, also between rice straw and corn stover. It was assumed to be correlated with the characteristic of the feeds. Napier grass had higher soluble nutrients than rice straw and corn stover. In contrary, both straw (rice straw and corn stover) had higher cell walls or neutral detergent fiber/NDF (Table 1).

CONCLUSION

Distilled water could replace artificial saliva as solvent for faeces and the dilution could be used as inoculant to replace rumen fluid to predict digestibility of tropical fibrous feeds (*in vitro*). However the faecal solvent as inoculants produce lower *in vitro* digestibility than that of rumen fluid.

ACKNOWLEDGMENT

We would like to express our thank to the Director of Directorate of Research and Community Services, Department of National Education, for funding the research through Hibah Bersaing XI 2004.

REFERENCES

- Abdullah, L. & Suharlina.** 2010. Herbage yield and quality of two vegetative parts of indigofera at different times of first regrowth defoliation. *Med. Pet.* 33: 44-49.
- Adesogan, A. T.** 2004. Effect of bag type on the apparent digestibility of feeds in ANKOM Daisy^{II} incubators. *Anim. Feed Sci. and Tech.* 119: 333-344.
- Akhter, S., E. Owen, M. K. Theodorou, E. A. Butler, & D. J. Minson.** 1999. Bovine faeces as a source of micro-organisms for the *in vitro* digestibility assay of forages. *Grass Forage Sci.* 54: 219-226.
- Bauer, E., B. A. Williams, M. W. Bosch, C. Voigt, R. Mosenthin, & M. W. A. Verstegen.** 2004. Differences in microbial activity of digesta from three section of the porcine large intestine according to in-vitro. *J. Sci. Food Agric.* 84: 2097-2104.
- Damiran, D., T. DelCurto, D. W. Bohnert, & S. L. Findholt.** 2008. Comparison of techniques and grinding size to estimate digestibility of forage based ruminant diets. *J. Anim. Feed Sci. and Tech.* 141:15-35
- Dhanoa, M. S., J. France, L. A. Crompton, R. M. Mauricio, E. Kebreab, J. A. N. Mill, R. Sanderson, R. Dijkstra, & S. Lopez.** 2004. Technical note: A proposed method to determine the extent of degradation of a feed in the *in vitro* gas production technique using feces as the inoculum. *J. Anim Sci.* 82: 733-746.
- Gomez, A. A. & A. A. Gomez.** 2007. *Prosedur Statistik untuk Penelitian Pertanian*. Edisi ke dua. Terjemahan: E. Sjam-suddin & J.S. Baharsjah. Penerbit Universitas Indonesia Press, Jakarta.
- Harris, L. E.** 1970. *Chemical and Biological Methods for Feed analysis*. Center For Tropical Agric. Feed Composition Project. Livestock Pavilion, University of Florida. Gainesville, Florida.
- Mahadevamma, S., T. R. Shamala, & R. N. Tharanathan.** 2004. Resistant starch derived from processed legumes: *in vitro* and in vivo fermentation characteristics. *Int. J. Food Sci. and Nutr.* 55: 399-405.
- Mauricio, R. M., E. Owen, F. L. Mould, I. Givens, M. K. Theodorou, J. France, D. R. Davies, & M. S. Dhanoa.** 2001. Comparison of bovine rumen liquor and bovine faeces as inoculum for an *in vitro* gas production technique for evaluating forages. *Anim. Feed Sci. Tech* 89: 33-48.
- Omed, H. M., D. K. Lovett, & R. F. E. Axford.** 2000. Faeces as a source of microbial enzymes for estimating digestibility. In: D. I. Givens, E. Owen, R. F. E. Axford, & H. M. Omed. (Editors). *Forage Evaluation in Ruminant Nutrition*. CAB International, pp. 135-154.
- Palić, D. V. & Klaas-Jan Leeuw.** 2009. Comparison of three *in vitro* methods for determining and predicting the organic matter digestibility of complete diets for ruminants. *Acta Periodica Technologica, APTEFF* 40: 79-82.
- Sudirman.** 2007. Kajian dan validasi faktor-faktor yang mempengaruhi efikasi penggunaan feses kerbau sebagai pengganti cairan rumen dalam penetapan pencernaan *in vitro* pakan di daerah tropik. Disertasi. Universitas Gadjah Mada, Yogyakarta.
- Utomo, R.** 2010. Modifikasi metode penetapan pencernaan *in vitro* bahan kering atau bahan organik. *Buletin Sintesis*. Yayasan Dharma Agrika Semarang 5: 1-11.
- Williams, B. A.** 2000. Cumulative gas-production Techniques for forage evaluation. In: D.I. Givens, E. Owen, R.F.E. Axford and H.M. Omed. (Editors), *Forage Evaluation in Ruminant Nutrition*. CAB International, pp. 189-213