SUPPORTING PAPERS
ISBN 979-97243-9-2

AN EVALUATION OF *IN VITRO* METHOD USING BUFFALO FAECES AS A SOURCE OF INOCULUM FOR THE MEASUREMENT OF TROPICAL FEED DIGESTIBILITY

Sudirman[1,3], R. Utomo[2], Z. Bachruddin[2], B. P. Widyobroto[2], Suhubdy[3]

[1]Postgraduate Student of Gadjah Mada University, [2]Faculty of Animal Science Gadjah Mada University, [3]Faculty of Animal Science Mataram University

ABSTRACT

The use of faeces as a source of inoculum has been reported to be a reliable method for measuring *in vitro* feed digestibility. However, the method was based on studies carried out in temperate or subtropik regions so its application to measure digestibility of tropical feeds may not be accurate. In the first experiment, a relationship between faeces density (g faeces/L distilled water) and *in vitro* digestibility was studied to establish an optimum level of faeces to be used in the inoculum. In the second experiment, *in vitro* digestibility of elephant grass, rice straw and pangola grass was measured using faeces liquor as inoculum. In this study, the faecal inoculum was dissolved in distilled water, not in artificial saliva as commonly used in previous studies, due to technical and economical considerations for application in Indonesia. The *in vitro* digestibility data obtained by using faecal inoculum were plotted agains those obtained by using rumen fluid as the inoculum to establish regression equations for the tested feeds. The results show that level of faeces in the inoculum did not significantly affect *in vitro* digestibility of dry matter. Relatively good regressions coefficient between *in vitro* digestibility using faecal inoculum and rumen fluid inoculum were obtained using fecal density of 350 and 400 g/L distilled water. However, the regression equations vary considerably between feed samples, indicating that the use of buffalo faeces as a source of inoculum requires further validation before the method can be used satisfactorily for Indonesian forages.

Keywords: *Faeces, in vitro, rumen fluid, tropical feeds, digestibility.*

INTRODUCTION

It has been a general knowledge that the actions of microbes in the rumen made it possible to use fibrous roughages, especially agricultural byproducts, as ruminant feeds. Sudirman et al. (2006) have evaluated the effectiveness of three feedstuffs i.e. rice straw, corn stover, and elephant grass, each supplemented with concentrate fed to buffalo and cattle feeds, as an indicator of feed value. The results showed that there was no significant effect of diet and animals in feed intake, *in vivo* digestibility, mocrobial colony, and CMC*ase* activity in the rumen fluid. However, the microbial colony and CMC*ase* activity were significantly lower in the faeces of similar animals.

The *in vivo* digestibility value is essential in selecting feed ingredients for diet formulation. However, *in vivo* digestibility trial is not economical because it requires a

SUPPORTING PAPERS
ISBN 979-97243-9-2

large number of animals and feedstuffs, and longer time. Many reports have shown that *in vivo* digestibility can be predicted by the corresponding *in vitro* values (Hvelplund *et al.*, 1999; Mahadevamma *et al.*, 2004). The *in vitro* digestibility values can be determined by Tilley and Terry (1963) method. In this methods, inoculum used for incubation always obtained from rumen fluid, which requires rumen fistulated animals. The disadvantages of using inoculum from rumen fluid include the high cost of maintaining fistulated animals, risk of infections (especially in the tropics), invasive and agains animal welfare principles (Thu, 2003; Mauricio *et al.*, 2001; Dhanoa *et al.*, 2004).

Many reports from subtropical environments have shown that rumen fluid can be replaced with faeces from various type of animals as the source of inoculum for *in vitro* digestibility determination (Omed *et al.*, 2000; El-Meadaway *et al.*, 1988; Akhter *et al.*, 1999). However, no information has been reported using buffalo faeces as the source of inoculum, even though there is an indication that faeces has similar microbial species as rumen fluid.

The type of microbes in the faeces depend on the type of donor animals and feeds consumed (Wannapat, 2001; Afdal *et al.*, 2003; Bauer *et al.*, 2004). For this reason, microbial species of buffalo faeces in the tropics is different from faecal microbes in the subtropics, therefore the *in vitro* methods based on using faecal inoculum should be evaluated before being applied in Indonesia or other tropical regions.

The purpose of this experiment was to evaluate the effectiveness of a modified *in vitro* method using buffalo faeces as inoculum. Results of this experiment is expected to provide a reference for further studies in an attempt to validate the method for determining *in vitro* digestibility of ruminant feeds in Indonesia.

MATERIALS AND METHODS

The experiment was carried out using two dry buffalo cows (average weight 306 kg) with rumen fistula. To obtain a stabile and optimum rumen ecology, a diet consisting of 70% elephant grass and 30% concentrate was fed (Table 1).

The animals were housed in individual concrete base pens. Feed and water were provided *ad libitum* for 45 days. The daily concentrate allowance was fed at 07.00 and 16.00 while the first portion of the grass was offered after the concentrate was consumed. The four forages evaluated were rice straw, corn stover, elephant grass, and pangola grass. All feeds were dried, milled, and sieved to obtain a particle size of 2 mm.

Rumen fluid and faeces as sources of inoculum were collected from the same animals. The rumen fluid was collected using an aspirator through rumen fistula and stored in a thermo flask which was previously warmed with 39°C distilated water. Grab samples of faeces was collected simultaneously and stored in a closed container prior to laboratory preparation.

Rumen fluid was strained using a four layer cheese cloth and then mixed with artificial saliva (McDaugals, 1948) with a 1 : 4 ratio according to the procedure of Tilley dan Terry (1963). Similar procedure was also applied to fresh faeces which was previously diluted with either artificial saliva (standard) or distilled water (350 g/L). Before filled into the tube containing 500 mg sample, the medium was placed in an incubator (at 39°C) and CO_2 gas was injected at the same time.

SUPPORTING PAPERS
ISBN 979-97243-9-2

Distilled water as a replacement of artificial saliva was evaluated in 5 levels, i.e. 150, 200, 250, 350, dan 500 g/L. After homogenized using a mixer for about 30 seconds, faecal solution was filtered and processed as the same produres as rumen fluid. 50 ml of medium was then filled into the tube containing 150, 200, 250, 350, dan 500 g/L.. After a 48 hours incubation period, content of the tubes was filtered with glass woll fitted crucible. The residues was oven dried at 105°C for 24 hours, then ashed at 550°C for 8 hours.

Data were tabulated and statistically analysed (anova: two-factor with replication) to determine the effect of faecal concentration on *in vitro* feed digestibility. Liniar regression Y = a + bX was applied to determine the relationship between *in vitro* digestibility obtained using rumen fluid and faeces as inoculum. All data analyses were carried out using Microsoft Excel®.

The assumption that drived this experiment was that the higher the concentration of faeces in the solution, the higher microbial cells available for digestion process and thus the *in vitro* digestibility of feeds improves. Table 2 shows that the relationship between faecal concentration and *in vitro* OM digestibility varies with type of feeds evaluated.

Table 1. Compositions of the experimental diet (% DM).

Feedstuff	Level	CP	CF	EE	NFE	Ash	NDF	ADF
Elephant 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

Notes: Calculated based on nutrient compositions determined at nutrition laboratory, Faculty of Animal Science Gadjah Mada University. CP = crude protein. CF = crude fibre. EE = extract ether. NFE = nitrogen free extract. NDF = neutral detergent fiber. ADF = acid detergent fiber.

RESULTS AND DISCUSSION

Variations in relationship between level of faeces and *in vitro* digestibility was probably due to the characteristics of the faecal inoculum, as indicated by the low density of bacteria (Thu, 2003; Wanapat, 2001) as shown in Table 3.

There was no consistent relationship between level of faeces and *in vitro* digestibility for all feeds evaluated. In a previous study an increase in level of faeces tended to increase the activity of CMC*ase*, the effect was not significant in this experiment. This inconsistent relationship can not be explained by the differences in the physical properties of feeds because elephant grass and pangola grass have similar characteristics so the *in vitro* digestibility can be expected to be similar.

For this reason, the variable responses to level of faeces in inoculum may be more related to technical aspects such as the use of distilled water as the diluter that can affect pH of inoculum and thus microbial growth. Most *in vitro* experiment in subtropical regions use artificial saliva as the solution (Tilley dan Terry, 1963; Mahadevamma *et al.*, 2004; Mauricio *et al.*, 2001; Omed *et al.*, 2000; Thu, 2003).

SUPPORTING PAPERS
ISBN 979-97243-9-2

Table 2. In vitro digestibility of organic matter of tropical feed using the inoculum faeces and rumen fluid (%)

Feedstuff	Faeces inoculum dilluted with		Rumen fluid inoculum
	distilled water	artificial saliva	
Rice straw	21.53	23.46	36.99
Corn stover	29.71	28.14	41.70
Elephant grass	25.18	23.09	49.11
Pangola grass	32.48	28.71	56.39
Average	27.23[a]	25.85[a]	46.05[b]

Notes: Mean values with different superscripts, differ significantly ($P < 0.05$).

Table 3. Total bacterial population of faeces liquor (grab sampling) and rumen fluid.

Variables	Faeces inoculum dilluted with		Rumen fluid inoculum
	distilled water	artificial saliva	
Total Plate Count (x 10^6/ml)	6.30 ± 0.40	7.80 ± 0.90	19.45 ± 0.35
Cellulolitic Count (x 10^6/ml)	1.45 ± 0.09	1.80 ± 0.21	4.47 ± 0.08

Notes: Samples analyzed at microbiology laboratory, Study Centre of Food and Nutrition, Inter-University Center, University of Gadjah Mada, Yoyakarta.

Tabel 4. In vitro digestibility of feeds using faeces liquor and rumen fluid inoculum of buffalo (%)

Feedstuffs	Level of faeces (g/L distiled water)					Rumen fluid
	150	200	250	350	500	
Rice straw	23.28	23.46	25.19	26.50	26.01	36.19
Corn stover	24.05	24.99	24.98	22.11	22.79	23.79
Elephant grass	26.43	25.69	26.52	25.88	29.35	48.40
Pangola grass	27.03	29.88	28.60	34.21	27.76	50.36
Mean	25.20[a]	26.01[ab]	26.32[bcd]	27.18[d]	26.48[c]	44.98[e]

Notes: Mean values with different superscripts, differ significantly ($P < 0.05$).

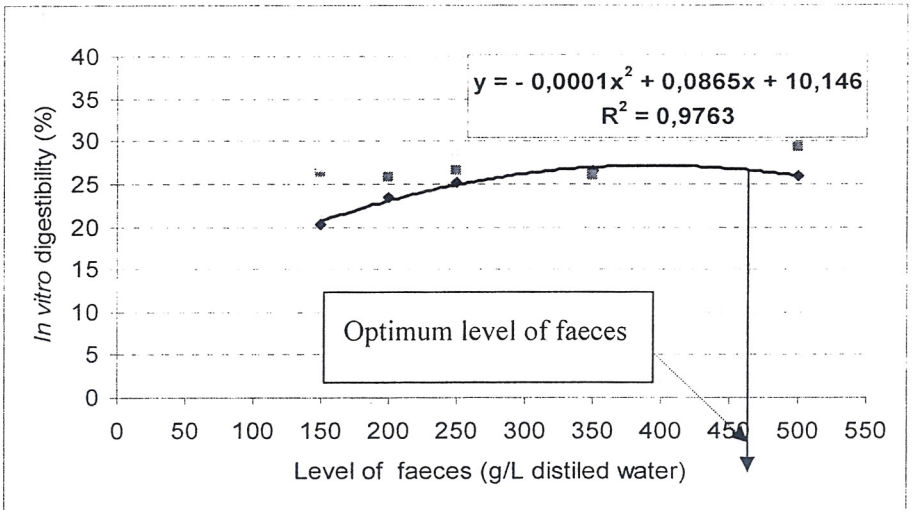

Figure 1. Assymtute of relationship between level of feces in inokulum (x) and *in vitro* digestibility of feedstuff (y).

SUPPORTING PAPERS
ISBN 979-97243-9-2

Table 6. Regression equation and coefisient of determination in vitro digestibility between faeces liquor levels (X) and rumen fluid (Y).

Level of faeces	Rice straw	Elephant grass	Pangola grass
150	Y = 6.13 + 2.09 X (R^2 = 0.89)	Y = 26.48 + 0.83 X (R^2 = 0.85)	Y = 38.88 + 0.42 X (R^2 = 0.88)
200	Y = 5.02 + 1.33 X (R^2 = 0.97)	Y = 18.31 = 1.17 X (R^2 = 0.84)	Y = 35.85 + 0.49 X (R^2 = 0.84)
250	Y = 11.52 + 0.98 X (R^2 = 0.95)	Y = 23.15 + 0.95 X (R^2 = 0.86)	Y = 34.85 + 0.54 X (R^2 = 0.87)
350	Y = 5.88 + 1.48 X (R^2 = 0.97)	Y = 33.66 + 0.57 X (R^2 = 0.80)	Y = 42.50 + 0.23 X (R^2 = 0.97)
500	Y = 4.11 + 1.23 X (R^2 = 0.97)	Y = 24.19 + 0.82 X (R^2 = 0.92)	Y = 29.98 + 0.97 X (R^2 = 0.85)

Similar regression equations have been reported by Akhter and Hossain (1998) and Thu (2003) i.e. Y = 184.87 + 0.954 X ($\mathbf{R^2}$ = 0.972) and Y = 3,26 + 1,34X ($\mathbf{R^2}$ = 0,86) respectively. The types of feeds evaluated in this experiment were more variable in digestibility compared to the feeds used by Akhter and Hossain (1998) and Thu (2003). This experiment also used distilled water, not artificial saliva which was used in the previous experiment.

As have been reported by previous researchers, it was evident in this experiment that the *in vitro* digestibility using faecal inoculum was much lower than that of *in vitro* digestibility obtained with rumen fluid as inoculum. This difference is due to the lower cellulolitic activity in faecal inoculum as a result of low population of fibrolitic bacteria compared to rumen fluid inoculum (El-Meadaway *et al.*, 1988; Akhter *et al.*, 1999; Omed *et al.*, 2000). This is also indicated by the lower bacterial colony in faeces compared to rumen fluid of the donor animals.

CONCLUSION

Faeces can be used as inoculum to evaluate *in vitro* digestibility of feeds but the results were lower than the values obtained using rumen fluid as inoculum. The values obtained with faecal inoculum were more variable and inconsistent for all feeds evaluated. The optimum level of faeces in the distilled water solution ranges from 350 - 400 g/L. Before this *in vitro* method can be used to evaluate *in vitro* digestibility of feeds in Indonesia, further studies are required on the time and site (intra rectum or defecated samples) of collection of faeces to be used as the source of inoculum and on the ratio of faeces inoculum relative to buffer in the *in vitro* method.

REFERENCE

Afdal, M., D.I. Givens, C. Rymer, E. Owen, and F. Mould. 2003. The relationship between diet and the chemical composition of sheep faeces. Proc. BSAS., p.150.

Akhter, S., E. Owen, M.K. Theodorou, E.A. Butler, and D.J. Minson. 1999. Bovine faeces as a source of micro-organisms for the *in vitro* digestibility assay of forages. Grrass and Forage Sci., 54 (3): 219-226.

ISBN 979-97243-9-2

Bauer, E., B.A. Williams, M.W. Bosch, C.Voigt, R. Mosenthin, and M.W.A. Verstegen. 2004. Differences in microbial activity of digesta from three section of the porcine large intestine according to *in vitro*. Jounal of the Science of Food and Agriculture. 84 (15): 2097-2104.

Dhanoa, M.S., J. France, L.A. Crompton, R.M. Mauricio, E. Kebreab, J.A.N. Mill, R.Sanderson, J. Dijkstra, and 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 iinoculum. J.Anim Sci., 82 :733-746.

El-Meadaway, A., Z. Mir, P.S. Mir, M.S. Zaman, and L.J. Yanke. 1988. Relatif efficacy of inocula from rumen fluid or faecal solution for determining *in vitro* digestibility and ga production. Canadian Journal of Animal Scinece. 78:673-679.

Hvelplund, T., M.R. Weisbjerg, and K. Soegaard. 1999. Use of *in vitro* digestibility methods to estimate in-vivo digestibility of straw. Diakses di internet 15/2/2005.

McDougal, E.I. 1948. Studies on ruminant saliva. 1. The composition and output of sheep's. Biochem. J. **43**: 99-109.

Mahadevamma, S., T.R. Shamala, and R.N. Tharanathan. 2004. Resistant starch derived from processed legumes: *in vitro* and in-vivo fermentation characteristics. International Journal of Food Sciences and Nutrition, 55(5):399-405.

Mauricio, R.M., E Owen, F.L. Mould, I. Givens, M.K. Theodorou, J. France, D.R. Davies, and M.S. Dhanoa. 2001. Comparison of bovine rumen liquor and bovine faeces as inokulum for an *in vitro* gas production technique for evaluating forages. *Animal Feed Science and Technology*, 89: 33-48.

Omed, H.M., D.K. Lovett, and R.F.E. Axford. 2000. Faeses as a source of microbial enzymes for estimating digestibility. In: D.I. Givens, E, Owen, R.F.E. Axford dan H.M. Omed. (Editors), Forage Evaluation in Ruminant Nutrition. CAB International, pp. 135-154.

Thu, N.V., 2003. Effect of different strategies of processing rice straw on *in vitro* digestibility using rumen fluid or faecal inocula of local cattle. In Proceedings of Final National Seminar-Workshop on Sustainable Livestock Production on Local Feed Resources (editor: Reg Preston and Brian Ogle).HUAF-SAREC, Hue City, 25 – 28 March 2003.

Tilley, J.M. and R.A. Terry. 1963. A two-stage technique for the *in vitro* digestion of forage crops. *Journal British Grassland Society*, **18**: 105-111.

Wanapat. M., 2001. Swamp buffalo rumen ecology and its manipulation. Dalam *Proceeding: Buffaloe Workshop in Vietnam*, 17 Desember 2001, pp. 1 – 6.-