

## RATION EVALUATION FOR *IN VITRO* DIGESTIBILITY APPLICATION USING CATTLE AND BUFFALO FAECES AS INOCULUM

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### ABSTRACT

An experiment has been conducted to evaluate three rations i.e 40% rice straw + 60% concentrate (A), 70% elephant grass + 30% concentrate (B), and 45% corn stover + 55% concentrate, using rumen fistulated cattle and buffalo. Variables measured were *in vivo* and *in vitro* organic matter intake and digestibility of these feeds. The kinetics of the rumen fermentation were also measured for 24 hours to compare biological conditions of rumen fluid in the two species. The results show that cattle and/or buffalo fed the three diets in this experiment are appropriate to be used as donor animals for *in vitro* experiment using faeces as an alternative to replacement rumen fluid. This is because intake and digestibility of organic matter (*in vivo* and *in vitro*), rumen ammonia concentration, pH, microbial population in the rumen and faeces and activity of cmc-ase enzyme activity were similar in buffalo and cattle.

(Key words: Cattle, Buffalo, Rice straw, Elephant grass, Corn stover, Digestibility)

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## EVALUASI RANSUM UNTUK PENERAPAN KECERNAAN *IN VITRO* DENGAN MENGGUNAKAN FESES SAPI DAN KERBAU SEBAGAI INOKULUM

### INTISARI

Evaluasi tiga jenis ransum campuran (A) 40% jerami padi + 60% konsentrat, (B) 70% rumput gajah + 30% konsentrat dan (C) 45% hijauan jagung + 55% secara bertahap telah dicoba pada sapi dan kerbau betina dewasa berfistula rumen. Penelitian ini bertujuan untuk mengetahui konsumsi dan nilai cerna bahan kering maupun bahan organik bahan pakan yang dievaluasi secara *in vivo* dan *in vitro*. Parameter kinetik rumen juga diamati selama 24 jam, dan membandingkan kondisi biologis cairan rumen kedua spesies ternak. Hasil penelitian menunjukkan bahwa ternak sapi dan/atau kerbau yang diberikan ransum dengan bahan pakan basal jerami padi, rumput gajah, atau jerami jagung, layak digunakan sebagai materi percobaan untuk penelitian kecernaan *in vitro* menggunakan feses sebagai sumber inokulum alternatif pengganti cairan rumen. Hal ini disebabkan karena konsumsi dan kecernaan bahan kering maupun bahan organik (*in vivo*, *in vitro*), kadar amoniak, pH, dan kadar NH<sub>3</sub> cairan rumen, populasi mikrobia cairan rumen dan feses, serta aktifitas enzim *cmc-ase* sapi dan kerbau relatif sama.

(Kata kunci: Sapi, Kerbau, Jerami padi, Rumput gajah, Jerami jagung, Kecernaan)

### Introduction

The *in vitro* method has developed rapidly and regularly applied because it uses small sample size, can be used to evaluate many samples at the same time, relatively accurate and its results positively correlate with *in vivo* values (Hervandez, 1984; Hvelplund, et al., 1999; Borba et al., 2001; Blummell et al., 2003; Mahadevamma et al., 2004). However, this technique has some disadvantages especially in preparation of inoculum, which is normally obtained from rumen fistulated animals (Tilley and Terry, 1963; Minson and McLeod, 1972; Baan et al., 2004). Obtaining rumen fluid through fistula tends to upset animals, requires high cost of maintenance, and high risk of infections especially under tropical environment (Mauricio et al., 2001; Baan, et al., 2004). In addition, the current animal welfare issue has further discouraged the use of fistulated animals for rumen fluid collection (Mauricio et al., 2001; Murray et al., 2003; Thu, 2003).

To avoid the use of invasive method in collecting inoculum, many researchers in

subtropical regions have successfully used faeces to replace rumen fluid (Ormed et al., 2000; Crompton et al., 2001; Borba et al., 2001; Mauricio et al., 2001; Afdal et al., 2003; Thu, 2003; Dhanoa et al., 2004; Bauer et al., 2004). This success has become a reference for other researchers all over the world despite the fact that it may not be applicable in tropical conditions. Apart from different environmental conditions, lack of appropriate laboratory equipment may contribute to possible inaccuracy of the technique if applied in developing countries. For these reasons, this technique should be verified before its application in the tropics.

To some extent, it can be assumed that that type, quality and quantity of microbes in faeces are similar with those exist in the rumen. Quantity and quality of rumen microbes depend on type of feeds and animal species (Akin, 1982; El-Meadaway et al., 1988; Ørskov, 2000; Afdal et al., 2003). The high lignocellulose content and low protein content in graminiae species (Haryanto and Djajanegara, 1993), result in low degradability of the feed and nutrient

deficiency for the rumen microbes (Webster, 1991; Ho and Abdullah, 1999; Wanapat, 1989, 2001). On the other hand, the high digestibility of subtropical plants is due to its high protein content and soluble carbohydrate (Preston and Leng, 1987). Therefore, it can be assumed that faecal microbes of animals in the tropics differ from faeces of subtropical animals. This assumption is thus the main reason for proposing a modified *in vitro* using faeces as the source of inoculum.

### Material and Methods

Two dry ongole cross cows (332 kg) and two dry swamp buffalo cows (321 kg) with rumen fistulated were individually housed and each fed either 40% rice straw + 60% concentrate (ration A), 70% elephant grass + 30 % concentrate (ration B), or 55% corn stover + 45% concentrate (ration C). Each diet was fed alternately for 17 days (51 days for the three rations).

Faecal output was collected and weighed soon after defecation to calculate total 24 h output. Rumen fluid samples were taken through the rumen fistulated. *In vivo* and *in vitro* digestibility, microbial colony, and activity of *carboxy methyl cellulase* were determined. 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). Before filled into the tube containing 500 mg sample, the medium was placed in an incubator (at 39°C) and CO<sub>2</sub> gas was injected at the same time.

Data were tabulated and statistically analysed (anova: two-factor with replication) to determine the effect of animal species and ration type on *in vivo* and *in vitro* feed digestibility. All data analyses were carried out using Microsoft Excel®.

### Results and Discussion

Table 2 demonstrates that the dry matter and organic matter intake of the three ration in cattle and buffalo did not differ significantly. Dry matter requirement in ruminants ranges from 2.5 - 4% of body weight (Kearl, 1982; Wanapat, 1989). In this experiment, the dry matter intake was 2.46% of body weight, which falls within these normal range. This lack of significant difference may be due to the relatively similar balance of nutrients consumed, particularly the ratio of protein to energy in the three rations tested (López, 2005). Ruminants will have high feed intake if the nitrogen content of the ration is sufficient, which is equivalent to crude protein content of 7 - 12% (Satter and Roffler, 1981; Kearl, 1982; NRC, 1984; Wannapat, 1989; Minson, 1990; Ørskov, 2000; Berger dan Merchen, 1995).

The constant digestibility values of the three ration by the two animal species were due to the similarity in the balance of nutrients in these diets. Protein content for example lies within the normal range of protein requirement for ruminants. Feed utilization by ruminants is affected by rumen ecology and protein to energy ratio (Preston and Leng, 1987; Armstrong, 1982; Hogan, 1982).

Table 1. Nutrient composition of experimental rations (% DM)

RATION	CP	CF	EE	BETN	ASH	NDF	ADF	TDN	CP/TDN
A	10.96	26.13	1.53	47.26	15.38	63.10	40.14	58.40	0.19
B	11.84	30.01	1.74	43.04	13.37	59.99	34.71	58.10	0.20
C	12.24	24.95	1.22	51.66	9.93	58.23	31.38	60.65	0.20

CP = crude protein, CF = crude fibre, EE = ether extract, NFE = nitrogen free extract, NDF = neutral detergent fibre, ADF = acid detergent fibre, TDN = total digestible nutrient.

Table 2. Dry matter (DM) and Organic Matter (OM) intakes (kg/d), *in vivo* and *in vitro* digestibility (%)

Ration	Parameter	Cattle		Buffalo	
		DM	OM	DM	OM
A	Feed Intake	8.52	7.60	7.47	6.65
	<i>In vivo</i> Digestibility	62.67	65.79	60.51	64.03
	<i>In vitro</i> Digestibility	42.36	44.86	43.39	46.19
B	Feed Intake	9.28	7.31	7.41	6.67
	<i>In vivo</i> Digestibility	57.79	62.12	51.92	53.46
	<i>In vitro</i> Digestibility	46.03	46.03	42.52	45.59
C	Feed Intake	7.48	6.66	7.00	7.03
	<i>In vivo</i> Digestibility	66.89	67.27	60.06	60.16
	<i>In vitro</i> Digestibility	43.31	45.76	43.29	46.65

Table 3. Total microbial, *cmc-ase* activity (rumen fluid and faeces solution), pH and NH<sub>3</sub> (ammonia) of rumen fluid (mg nitrogen/liter)

Parameter	Experimental Ration					
	A		B		C	
	Cattle	Buffalo	Cattle	Buffalo	Cattle	Buffalo
RF cfu	4.5x10 <sup>10</sup>	5.6x10 <sup>10</sup>	1.6x10 <sup>10</sup>	4.6x10 <sup>9</sup>	3.9x10 <sup>9</sup>	1.4x10 <sup>10</sup>
FS cfu	4.0x10 <sup>8</sup>	1.6x10 <sup>8</sup>	3.9x10 <sup>7</sup>	4.5x10 <sup>8</sup>	46x10 <sup>6</sup>	5.0x10 <sup>7</sup>
RF <i>cmc-ase</i>	3.51	3.59	3.26	3.62	3.43	3.23
FS <i>cmc-ase</i>	2.16	1.91	2.75	2.31	1.87	1.68
pH rumen fluid	6.41	6.61	6.85	6.72	6.65	6.53
NH <sub>3</sub> rumen fluid	102.6	96.05	84.88	104.9	92.44	111.42

RF = rumen fluid, FS = fecal solution, cfu = cell free unit/ml, *cmc-ase* = *carboxy methyl cellulose*

Table 3 shows that there was a variation in total microbial colony in the rumen fluid and faeces of two species fed the three diets. This variation was probably due to the differences in the level of concentrate and quality of the basal ration level (Akin, 1982; Jouany, 1989). Total bacterial population in rumen fluid and faeces of buffalo tended to be higher than that of cattle, but there was an interaction with *cmc-ase* activity in the two species (Wanapat, 1989, 2001; Fonty and Gouet, 1989).

Biological condition of the rumen of the two species throughout the day was within

normal range. Rumen pH for the three rations ranges from 6.1 to 7.4, which is within the normal range of 5.5 - 7.0 (Theodorou and France, 1993) and in fact very close to the normal values of 6.2 - 7.0 for fibrous feeds (Akin, 1982; Theodorou dan France, 2005). Cellulolytic activity was not disrupted because the rumen pH did not fall below 6.2 regarded as the starting point for declining cellulolytic activity (Givens and Moss, 1995). Rumen ammonia concentration increased several hours after morning feeding but it was not significantly higher than the values recorded in the following hours. The concentration was

within the normal Range of 50 - 250 mg ammonia N/L (Preston dan Leng, 1987; Maeng et al., 1997) so it can be assumed that the majority of rumen microbes had sufficient nitrogen supply for normal growth.

### Conclusion

All ration used in this experiment can support normal microbial growth in the rumen. Any of these rations can thus be used for *in vitro* digestibility determination. Similarly, cattle or buffalo can be used as donor animal in the *in vitro* analysis using faecal inoculum as replacement of rumen fluid.

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