Changes in Rumen Ecosystem and Feed Dry Matter Degradability of Buffalo which Received Rumen Content of Cattle through Cross Inoculation

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ABSTRAK

PAMUNGKAS, D., C.C. SEVILLA dan U.M. LUSTRIA. 2006. Perubahan ekosistem rumen dan kecernaan bahan kering pakan pada kerbau yang mendapat transfer isi rumen sapi melalui inokulasi silang. *JITV* 11(1): 24-33.

Suatu percobaan telah dilakukan untuk mengidentifikasi perubahan ekosistem rumen kerbau yang mendapat transfer isi rumen sapi. Sebanyak empat ekor kerbau (bobot hidup 450-550 kg) jantan berfistula dan empat ekor sapi betina berfistula (bobot hidup 250-380 kg) digunakan sebagai materi percobaan. Percobaan dilakukan dalam tiga tahap, yaitu: pra-inokulasi, inokulasi dan pasca-inokulasi. Pra-inokulasi, sampel isi rumen diambil 2 jam sebelum makan pagi dan langsung dilakukan pengukuran pH cairan rumen, sedangkan sampel cairan rumen diambil untuk analisis amonia nitrogen (NH_3-N) dan total asam lemak terbang (VFA). Tiga hari terakhir sebelum dan sesudah inokulasi dilakukan uji kecernaan bahan kering pakan secara *in situ*. Sampel pakan (rumput gajah, lamtoro dan dedak gandum) dalam kantong nilon diinkubasi selama 0, 3, 6, 9, 12, 24, 48, dan 72 jam dalam rumen. Proses inokulasi silang (total isi rumen) dilaksanakan kurang dari 10 menit. Pasca inokulasi, sampel isi rumen diambil dua jam sebelum makan pagi pada setiap dua hari selama dua minggu setelah proses inokulasi. Identifikasi perubahan terhadap parameter ekosistem rumen (pH, NH_3-N dan total VFA) diuji dengan menggunakan Rancangan Acak Lengkap. Hasil menunjukkan bahwa inokulasi silang isi rumen sapi secara nyata menurunkan pH rumen kerbau namun tidak menunjukkan pengaruh terhadap perubahan konsentrasi ammonia dan total VFA rumen. Keadaan ini berakibat menurunnya populasi bakteri ($P<0,05$), namun tidak mempengaruhi populasi protozoa dan jamur. Sementara itu nilai fraksi tidak terlarut dan fraksi potensi tercerna leucaena tampak menurun. Kecernaan efektif bahan kering rumput Gajah tampak meningkat ($P<0,05$) pada laju aliran 0,02 dan 0,04 jam^{-1}, sedangkan dedak gandum terjadi penurunan ($P<0,05$) pada laju aliran 0,04; 0,06 dan 0,08 jam^{-1}.

Kata Kunci: Ekosistem Rumen, Inokulasi Silang, Kecernaan

ABSTRACT

PAMUNGKAS, D., C.C. SEVILLA and U.M. LUSTRIA. 2006. Changes in rumen ecosystem and feed dry matter degradability of buffalo which received rumen content of cattle through cross inoculation. *JITV* 11(1): 24-33.

The research was done to identify changes in rumen ecosystem of buffalo which received rumen content of cattle. As much as three head of fistulated male buffaloes (live weight of 450-550 kg) and three fistulated female cattle (live weight 250-380 kg) were used. This experiment was done three stage as follows: pre-inoculation, inoculation and post-inoculation. In Pre-inoculation, the sample of rumen content was taken two hours before morning feeding and directly observed for pH rumen liquor, ammonia nitrogen (NH_3-N) and total volatile fatty acid (VFA). During the last three days of the first week of pre and post inoculation, the *in situ* dry matter digestibility was conducted. The samples were incubated for 0, 3, 6, 9, 12, 24, 48, and 72 hours in the rumen of the experimental animals. Napier grass, leucaena, and wheat pollard were incubated separately in the nylon bags to determine DM digestibility. The cross inoculation (total of rumen content) was done less than 10 minutes. In post-inoculation, the sample of rumen content was taken at two hours before morning feeding as long as two days of two weeks after inoculation. Changes in rumen ecosystem (pH, NH_3-N and total VFA) were tested by using Completely Randomized Design. Result showed that the transfer of rumen content from cattle to buffalo significantly reduced the pH level in the rumen of buffalo. However, it had no significant effect on the ammonia concentration in the rumen of buffalo and showed significantly decreased of bacteria but it was not affect on the total count of protozoa and fungi. The transfer of rumen content from cattle to buffalo significantly decreased the value of insoluble and potential digestible fraction of leucaena. The DM effective degradability of Napier grass was significantly increase ($P<0.05$) within outflow rate 0.02 and 0.04 h^{-1} after cross inoculation. In wheat pollard, a significantly decrease ($P<0.05$) was occurred when outflow rate was observed at 0.04, 0.06 and 0.08 h^{-1}

Key Words: Rumen Ecosystem, Cross Inoculation, Degradability

INTRODUCTION

The rumen serves as a fermentation vat where microorganisms (bacteria, protozoa, fungi, etc.) break down the feed. In a mature cow where rumen is very large, is capable of holding 40 to 50 gallons (BLEZINGER, 1998). Feeds such as hay, grass, silage, grain mix, water and saliva serve as food for these microbes. They break down the feed and produce certain by-products.

It is extremely important that either cattle or buffalo to be fed properly in order for the rumen to function at the optimum and maintain good rumen health. Rumen inoculations are effective in changing the nature of the microbiota if the ration remains different. There are factors that determine whether a particular microbe will occur in the rumen or not (HUNGATE, 1966); first, whether the conditions are favorable to its growth; and second, whether it gains access to these favorable conditions. Inoculation is essential only if the organism is absent from the rumen. However, if it is present in very small numbers, inoculation can hasten its development, particularly if the feed is changed. Meanwhile, JONES and MEGARRITY (1986) had successfully transferred bacteria culture from isolate of goat's rumen in Hawaii into cattle and sheep rumens in Australia, so the animals have an ability to break down 3-hydroxy-4 (IH)-pyridone (DHP) as the product of *Leucaena leucocephala* digestion in the rumen. This result had an advantage in overcoming the toxicity of leucaena, in relation to the role of bacteria for digesting DHP after it was synthesized from mimosine metabolism.

To better utilize such low quality feed, ways are now being intensely investigated by research groups around the world. These groups are focusing on a special characteristic of ruminant livestock, particularly those indigenous in the tropics, that enables them to utilize poor quality cellulosic feed as an energy source. The nutrition of ruminants is first and foremost the nourishment of the microorganism followed by the host animal (SEVILLA, 2002). The ruminant can only be productive if it maintains an optimized and efficient ecology. The rumen is a complex microbial ecosystem where fibrous feeds are digested and utilized for conversion into human food protein sources.

Understanding the factors controlling rumen microbial activity may allow scientists to modify the rumen ecology in order to create conditions that will optimize the use of poor quality feed by ruminant livestock. The fastest way to improve rumen function in an animal is to introduce digestion-enhancing microorganism species from other animals or to selectively increase populations of species that inhabit the rumen only at low levels. Bacteria from one ruminant species have been experimentally shown to colonize others successfully. It has been further demonstrated that the cross-inoculation of rumen fluid from wild to domestic ruminants alleviates tannin toxicity and enhances the productivity of livestock browsing on tannin-containing shrubs. The transfer of microbes between animal species is being facilitated by the precise molecular methods now available to track individual organisms within complex mixtures. Without precise and quantitative tracking systems, the effectiveness of any particular organism within the microbial community in the rumen cannot be clearly demonstrated (MCMILLLAN, 1996). In the past ten years, many approaches have been taken in attempts to transform rumen bacteria by introducing a foreign gene or genes into the bacteria.

The main objective of this study was to determine changes in buffaloe's rumen fermentation and microbial growth rate by manipulating the balance of organisms in the rumen through cross inoculation of ruminal contents from cattle.

MATERIALS AND METHODS

This study was the serial number of research which held in Institute of Animal Science Barns of University The Philippines at Los Banos, Philippines. It was the second part of the previous publication which published in the Proceedings of National Seminar on Livestock Production Technology and Veterinary, 2004.

Specifically, the study attempted:

a. To evaluate the *in sacco* digestibility of dry matter
b. To determine changes in ruminal pH, ammonia concentration and total volatile fatty acid in cross-inoculated animals; and
c. To determine changes in rumen microbial population.

Animal

Three male native buffaloes with the range of 450-550 kg and three female cattle with the range of 250-380 kg of live weight, fitted with permanent rumen cannula, were used. The animals were placed in individual pens during experiment.

Diet

Animals were fed a diet of 70% napier grass and 30% concentrate. The amount of dry matter offered was calculated at 2.5% of live weight. Feeding was done twice daily at 8.00 am and 2.00 pm. The concentrate mixture consisted of 60% wheat pollard, 36% copra meal, 2% urea, 1% salt and 1% dicalcium phosphate.

Experimental procedure

The experimental procedures consisted of pre-inoculation, cross inoculation and post-inoculation periods.

Pre-inoculation period

The pre-inoculation period lasted for one week. Samples of rumen content for microbial analysis were taken two hours before morning feeding via the rumen cannula. The pH of the rumen fluid was immediately taken, while samples of the rumen fluid for ammonia and VFA determination were kept in the freezer until it was ready for analysis.

Cross inoculation period

Cross inoculation (CI) was conducted before morning feeding. The transfer of rumen content from buffalo to cattle and vice versa was completed in 10 minutes for each pair of animals. It was conducted just once time.

Post-inoculation period

Samples of rumen content for microbial analysis were taken two hours before feeding. Every two days for two weeks starting at day-2 up to day-14 post-inoculation, the value of pH, VFA and NH_3 fluctuations were observed at 600, 900, 1200, 1500 and 1800 hours. The pH of the rumen fluid was immediately taken and observed, while the sample of the rumen fluid for ammonia and VFA determination were kept in the freezer until ready for analyzing.

Data gathering

Feed samples

Samples of napier grass and concentrate were taken daily, pooled at the end of pre-and post-inoculation periods for chemical analysis. Feed refusals were likewise sampled for chemical analysis.

Measurement of rumen ecosystem

Rumen fluid pH was determined by using a pH meter. Meanwhile, the determination of rumen ammonia nitrogen and VFA level in the rumen followed the methods of ABDULRAZAK and FUJIHARA (1999).

In sacco *digestibility of dry matter*

The digestibility characteristics of napier grass, leucaena and wheat pollard were defined by the Nylon Bag Methods. The nylon bag used had a size of 10 x 20 cm (White Polyester Monofilament), pore size of 50 um (+/- 10) and free nitrogen. All samples were inserted in the bags with approximately 5 g (DM basis) of each sample. Two bags per sample were used per incubation period (SUDEKUM and ANDREE, 1997). Zero time disappearance value (0 hours) was obtained by washing the empty nylon bag with distilled water. Every sample was placed in one bag and put into the rumen for the incubation process. On day one incubation, the bags were clamped and tied along main line tied outside the fistula. All bags were inserted in the ventral sac of rumen at early morning, immediately before morning feeding. Incubation period was done at 0, 3, 6, 9, 12, 24, 36, 48 and 72 hours (MADSEN and HVELDPLUND, 1994). After each incubation period, nylon bags were removed from rumen and washed with distilled water to remove the rumen fluid. These were then dried at 65^{o}C for 48 hours (TAGARI *et al*., 1986).

Statistical analysis

In sacco DM digestibility and feed intake were analyzed using the Analysis of Variance. To examine the difference within parameters, the t-test was used, as described by GOMEZ and GOMEZ (1984). The effect of time of collection on pH, ammonia concentration and total VFA in the rumen was analyzed using Completely Randomized Design (SAS, 1996).

RESULTS AND DISCUSSION

pH value in the rumen

The samples were taken at different times in a day to know the changes in rumen ecosystem either before or after cross inoculation. At day 0 by means the day before cross inoculation, the lowest pH level of 6.73 was recorded at 1200 hours, followed by 6.87 at 1800 hours, 6.93 at 900 hours and 6.98 at 600 hours, while the highest level of 7.00 was recorded at 1500 hours (Table 1). Differences in pH level taken at different times of the day were statistically significant.

Two days after CI, there was a significant decrease in pH level ($P<0.05$). Likewise, there were significant differences ($P<0.05$) in rumen pH levels taken at different times of days 4, 6, 8 and 10. The fluctuations in rumen pH reflect changes in the quantity of organic acids that accumulate in the ingesta and the amount of saliva that is produced. The pH levels in the buffalo rumen in this study were lower than those reported by THU and PRESTON (1999), which ranged from 7.01 to 7.32. The difference in levels may be due to the differences of feed offered. PRESTON and LENG (1986) added that buffalo could consume large amount of urea resulting increase in pH in the rumen. In buffalo, MUNIER (2001), however, reported the infusion of 0.0375% urea in the buffalo diets of 55% rice straw +

40% leucaena + 5% molasses, increased pH value in the rumen up to 6.8.

The lower pH values observed in rumen fluid taken at 600, 900 and 1500 hours, the more acidic occured in rumen ecosystem at those time. RUSSEL and WILSON (1996) stated that the effect of ruminal pH on cellulose digestibility often was confounded by changes in feed intake or by the concentration of fiber in the diet (ORSKOV and FRASER, 1975). In the *in vitro* study, MOULD *et al*. (1984) concluded that ruminal cellulolysis was totally inhibited at pH below 6.0.

The pH values in this study taken before and after CI were within normal levels of above 6.2. ORSKOV (1982) and LANA *et al*. (1998) reported that a rumen pH of less than 6.2 would seriously inhibit growth of cellulolytic bacteria. Low rumen pH can have a deleterious effect on fiber digestion, which in turn reduces feed intake and digestibility. In this study, the mean daily of pH value showed significantly diferrent over day observation. The lowest pH was reached at day 6. This reflected microbes activity apart due to the adjustment in term of either interrelationship among celulolytic bacteria or competition with fungi or protozoa. They did not significantly release VFA and NH3 at different level in a day although the rumen more acidic. However, the lowest pH at day 6 was set aside to normal fermentation.

Ammonia concentration

As presented in Table 2, at day 0, the lowest of ammonia concentration (78.56 mg/l) was recorded at 1500 hours, followed by 1800 hours (80.89 mg/l), 600 hours (109.67 mg/l) and 900 hours, while the highest (114.71 mg/l) was reached at 1200 hours. Compared with the results of ORDEN *et al*. (1999); GARCIA *et al*. (1994) and SATTER and SLYTER (1974), the levels ammonia in this study were within normal range.

Table 1. Effect of time of collection on the pH value of buffalo cross inoculated with rumen content from cattle

Days of CI	Collection time					Mean daily of pH
	600 hours	900 hours	1200 hours	1500 hours	1800 hours	
Day 0	6.98 [a]	6.93 [a]	6.73	7.00 [a]	6.87 [ab]	6.90
Day 2	6.50 [b]	6.47 [c]	6.90	6.68 [ab]	6.93 [a]	6.70
Day 4	6.80 [ab]	6.73 [ab]	6.67	6.80 [ab]	6.50 [b]	6.70
Day 6	6.67 [b]	6.37 [c]	6.53	6.57 [a b]	6.57 [ab]	6.54
Day 8	6.83 [ab]	6.83 [a]	6.63	6.67 [ab]	6.50 [b]	6.69
Day 10	6.80 [ab]	6.90 [a]	6.70	6.90 [ab]	6.67 [ab]	6.79
Day 12	6.87 [a]	6.77 [ab]	6.83	6.53 [b]	6.73 [ab]	6.75
Day 14	6.70 [ab]	6.70 [ab]	6.87	6.70 [ab]	6.73 [ab]	6.74
CV, %	2.14	2.96	1.89	2.38	2.40	

a-d = Means within columns with different superscripts were significantly different (P<0.05)
CI = Cross inoculation

Table 2. Effect of time of collection on the ammonia concentration (mg/l) in buffalo cross inoculated with rumen content from cattle

Days of CI	Collection time					Mean daily of NH_3
	600 hours	900 hours	1200 hours	1500 hours	1800 hours	
Day 0	109.67 [ab]	103.44	114.71 [a]	78.56	80.89	97.45
Day 2	122.89 [ab]	122.89	102.66 [ab]	108.89	80.89	107.64
Day 4	105.48 [b]	107.45	101.67 [ab]	101.50	84.00	100.02
Day 6	117.44 [ab]	113.09	92.40 [b]	94.13	84.32	100.28
Day 8	121.58 [ab]	119.65	100.99 [ab]	92.89	74.50	101.92
Day 10	118.65 [ab]	125.81	116.57 [a]	108.72	72.52	108.45
Day 12	135.56 [ab]	112.93	112.25 [ab]	96.08	76.15	106.59
Day 14	150.26 [a]	123.21	112.84 [ab]	95.93	82.82	113.01
CV, %	11.67	6.97	7.98	12.05	5.69	

a-d = Means within columns with different superscripts are significantly different (P<0.05)
CI = Cross inoculation

The mean daily of ammonia concentration as attached in Table 2 was the average all collection time. From this point, it can be shown that ammonia concentration tend to increase from 97.45 to 113.01 mg/l.

At day 2, there was a trend of increasing ammonia concentration but declined from day 4 to 8 and increased again at days 10 to 14. The levels for ammonia in this study were higher than those on the buffalo fed with 55% rice straw + 40% leucaena + 5% molasses, as reported by MUNIER (2001).

Total volatile fatty acid

At day 0, the lowest VFA concentration was recorded at 900 hours (35.02 m/mol), while the highest was at 1500 (80.75 m/mol). There was a trend of increasing total VFA at 600, 900 and 1800 hours. Conversely, there was a trend of decline at 1200 and 1500 hours. However, the total VFA concentrations before and after CI in the rumen of buffalo were not significantly different. Therefore, it can be concluded that transferring ruminal content from cattle did not change the total VFA concentration in the rumen of buffalo. WALLACE and MC PHERSON (1987) stated that a depression of microbial growth increases VFA, CO_2 and CH_4 production. An increased degradation of microbes within rumen also increases VFA production.

As expected, results of this study showed an inverse relationship between increasing pH value and decreasing total VFA concentration. LENG and LEONARD as cited by KANJANAPRUTHIPONG and LENG (1998) reported that fermentation rate and VFA concentration were correlated and thus the changing concentration probably reflects an increasing VFA production and may be an increase in the pool size of microorganism in the rumen.

Figure 1 shows the fluctuation in pH level, ammonia nitrogen and total VFA concentrations in the rumen of buffalo at different days of cross inoculation. It indicates that the transfer of rumen content from cattle to buffalo did not affect the fluctuation in pH level, ammonia nitrogen and total VFA concentrations. This might be due to the decrease in total count of bacteria.

Total counts of microbes

The two major classes of rumen microorganisms are bacteria and ciliated protozoa, although yeast-like organisms or fungi are occasionally found in appreciable numbers. Table 5 shows the total count of microbes in the rumen of cattle and buffalo.

Protozoa

Rumen protozoa have been observed to play a significant role in the primary degradation of plant fragment which derived from results of biochemical, cultural, microscopic and defaunation studies (WILLIAMS, 1988). Ciliates usually represent the bulk of the microfauna in the rumen fluid. Their numbers may exceed one million per gram of rumen contents and their mass roughly equals that of bacteria (WILLIAMS *et al*., 1963). SO (1982) reported that the total ciliate protozoal count in cattle was lower (8.56×10^4 cell/ml) than of buffalo (9.22×10^4 cell/ml) regardless of the ration. The decrease in total counts of protozoa by as much 42.41% after CI was not statistically significant. The total count of protozoa in buffalo rumen in this study was similar to the report of THALIB *et al*. (2000), which were 1.97×10^4, 1.7×10^4 and 3.02×10^4 cell/ml for holocellulose, cellulose and hemicellulose, respectively.

Table 3. Effect of time of collection on the total VFA (m/mol) concentration in buffalo cross inoculated with rumen content from cattle

Days of CI	Collection time					Mean daily of VFA
	600 hours	900 hours	1200 hours	1500 hours	1800 hours	
Day 0	65.44	35.02 ^{c}	77.42 ^{a}	80.75	58.52	63.50
Day 2	74.91	61.75 ^{b}	59.91 ^{c}	68.20	41.47	61.25
Day 4	79.81	72.35 ^{ab}	75.57 ^{a}	83.05	60.15	74.19
Day 6	72.97	69.14 ^{b}	68.14 ^{abc}	80.66	59.08	69.99
Day 8	75.90	90.48 ^{b}	74.04 ^{ab}	77.31	54.41	74.43
Day 10	74.87	77.41 ^{ab}	62.40 ^{abc}	81.00	58.89	70.91
Day 12	73.60	69.27 ^{b}	70.81 ^{abc}	68.63	60.40	68.54
Day 14	75.31	68.05 ^{b}	61.42 ^{c}	70.54	53.83	66.83
CV, %	5.47	23.22	9.94	8.07	11.30	

a-d = Means within columns with different superscripts are significantly different (P<0.05)
CI = Cross inoculation

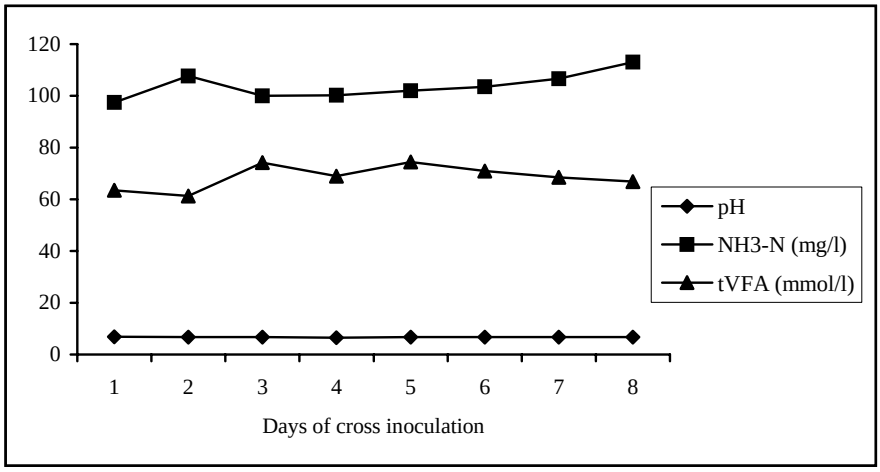

Figure 1. The fluctuation on pH value, ammonia nitrogen and total VFA concentrations in the rumen of buffalo at different days of cross inoculation

Table 5. Total counts of microbes in the rumen of buffalo

Animal	Protozoa (cell/ml)		Bakteria (cell/ml)		Fungi (sporangia cm^2)	
	Before CI	After CI	Before CI	After CI	Before CI	After CI
Buffalo-1	3.6×10^4	1.5×10^4	3.3×10^9	2.2×10^7	5	0.9
Buffalo-2	1.4×10^4	1.5×10^4	3.7×10^9	1.7×10^7	4	27.9
Buffalo-3	3.7×10^4	2.0×10^4	1.9×10^9	2.5×10^7	25	0.4
Average	2.9×10^4	1.67×10^4	2.97×10^9	2.1×10^7	11.33	9.73
CV, %	44.83	17.32	31.86	18.94	104.52	161.66
t-test	ns		*		ns	

CI = Cross inoculation, * P<0.05, ** P<0.01, ns = not significant

Bacteria

The decrease of closed to100 % in the total counts of bacteria after CI was found to be statistically significant. This indicates that the rumen environment of buffalo is not conducive to the growth of the rumen bacteria found in cattle. It can be assumed that this happened temporarily as long as the observation period and those situation could be change due to the balance in rumen ecosystem. For those reason that there were marked interactions between bacteria and protozoa. Protozoa ingest and digest bacteria and reduce the bacterial biomass that is floating free in the rumen liquid (HUNGATE, 1966; COLEMAN, 1975). However, the population of protozoa were lower after CI than those before CI although it wasn't significant. Therefore, the further observation will be highly needed to answer this phenomenon. The total counts of bacteria in this study were lower than those indicated in the report of SO (1982). He observed that the total bacterial count in buffalo (4.76×10^{10} cell/ml) was significantly higher than that of cattle (3.94×10^9 cell/ml) regardless of the rations. HUNGATE (1966) stated that bacterial colonization of grass and straw fragments took place soon (by 15 minutes) after they were introduced into the rumen. Within one hour, micro-colonies (of single or mixed morphological types) were present both in the inter and intra-cellular compartments of the plant fragments.

Fungi

Large population of fungi occurs in animal fed with fibrous stalky diets, but fungi could be absent low in number when the animals are fed with soft leafy diets or high starch diets (BAUCHOP, 1979; GRENET *et al*., 1989). There was no significant difference in total counts of fungi after CI (Table 5). The nutritional status of the diets may influence the population density of the fungi in the rumen. *In vitro* studies of one of the rumen showed that it requires sulphur for growth (ORPIN and GREENWOOD, 1986). In colonizing the plant tissues,

aerobic rumen fungi show a preference for lignified thick wall tissues. Within six hours, thick-walled tissues such as *Schlerenchyma* and the vascular elements are extensively colonized (HO *et al*., 1988). As more hyphae colonize and penetrate the cells, degradation of cell walls occurs. Moreover, USHIDA *et al*. (1997) reported that anaerobic *Chytridiomycetes* fungi are now well recognized as one of the major components of rumen microflora. *Chytriodiomycetes* fungi appear in the rumen of young animals about ten days after their birth. Forage-based diets promote the development of fungal population and generally, diets rich in fiber promote a larger population of *Chytridiomycetes* fungi in the rumen of adult animals.

In Sacco Dry Matter Digestibility of Feed

The *in sacco* feed DM digestibility characteristics of napier grass, leucaena and wheat pollard before and after CI are presented in Table 6. The soluble fraction (a) of napier grass, leucaena and wheat pollard were not affected by cross inoculation. The soluble fraction values of napier grass and leucaena were higher compared with those reported by MGHEN *et al*. (1994), which were 18.00 and 24.4%, respectively. For wheat pollard, the (a) value (48.98%, after CI) was higher than that of rice bran, soybean meal and flake corn, which was 21.1, 31.4 and 34.9%, respectively (ISLAM *et al*., 2002).

Cross inoculation of rumen content from cattle to buffalo significantly reduced the insoluble DM fraction of leucaena, but not of napier grass and wheat pollard. The slowly soluble DM fraction of napier grass (24.87%) was lower than the 63.0% reported by MGHEN *et al*. (1994). While the slowly DM fractions of wheat pollard (24.05%) was lower than that of rice bran, soybean meal and flake corn, which was 59.4, 70.1 and 77.9% respectively (ISLAM *et al*., 2002). In this study, the slowly soluble DM fractions of leucaena was 29.06% which was lower than that reported by SERRA *et al*. (1997) of 58.9%.

The potential digestible DM fraction (a+b) of leucaena was significantly reduced after CI but not in napier grass and wheat pollard. In this study, the slowly soluble DM fraction of leucaena was 74.39%, close to 77.6% as reported by BEJO and SEVILLA, (2001). However, this value for leucaena was lower than those of *Glricidia* spp. (KHAMSEEKIEW *et al*., 2001; BEJO and SEVILLA, 2001). The difference in the potential digestible DM fraction may be due to tannin in leucaena (SEVILLA, 2002). There was a negative correlation between condensed tannin and digestibility of the different nutrients in leucaena (DALZELL *et al*., 1998).

The amount of DM degradability of wheat pollard degraded significantly decreased towards the end of the incubation period. The lower DM degradability of wheat pollard in the rumen indicates the possibility of higher amount being degraded in the lower gastrointestinal tract.

Generally, rate of DM degradation of b (c) of napier grass, leucaena and what pollard was not affected by cross inoculation. These results may be related to the decreasing total number of protozoa, bacteria and fungi after CI. In this study, the rate degradation of napier grass after CI (0.0541%/h) was faster than that reported by MGHEN *et al.* (1994), which was 0.027%/h. The c value of leucaena (0.1605%/h) was also faster than that reported by MUNIER (2001), which was 0.1118%/h. Some workers reported that the rate degradation of b for leucaena was 0.04%/h (SERRA *et al*., 1997) and the average of degradation b for leucaena (species *Bahru* and *Rendang*) 40% mixed 60% oil palms fronds was 0.05%/h (KHAMSEEKHIEW *et al*., 2001).

Table 6. *In sacco* dry matter digestibility (%) characteristic of feeds before and after cross inoculation in buffalo

Feed	Degradation (%)			
	a	b	(a+b)	c
Napier grass				
Before CI	38.21	41.54	79.75	0.0374
After CI	53.58	24.87	78.45	0.0541
t-test	ns	ns	ns	ns
Leucaena				
Before CI	36.38	48.70	85.08	0.0430
After CI	36.37	29.06	74.39	0.1605
t-test	ns	P<0.05	P<0.05	ns
Wheat pollard				
Before CI	67.55	21.38	88.93	0.0506
After CI	48.98	24.05	73.03	0.0311
t-test	ns	ns	ns	ns

a = Soluble fraction
b = Water insoluble fraction
(a+b) = Potential digestible fraction
c = Rate of degradation of b (h^{-1})
CI = Cross inoculation
ns = Not significant

The values for effective degradation (ED) of DM of napier grass, leucaena and wheat pollard before and after CI are presented in Table 7. After CI, the ED of DM napier grass was significantly higher (P<0.05) for outflow rates 0.02 and 0.04 h^{-1} than those of before CI, rendering an increasing value of 21.18 and 20.11%, respectively. The ED of napier grass for outflow rate 0.02 h^{-1} in this study (71.58%) was higher than that reported by MGHEN *et al.* (1994) of 64.00%.

Table 7. Dry matter effective degradability of napier grass, leucaena and wheat pollard before and after cross inoculation in buffalo

Feed	Effective degradability (%)			
	0.02	0.04	0.06	0.08
Napier grass				
Before CI	50.40	47.55	45.88	44.72
After CI	71.58	67.66	65.18	63.46
t-test	P<0.05	P<0.05	ns	ns
Leucaena				
Before CI	52.36	48.44	46.09	44.52
After CI	59.43	55.99	53.70	52.04
t-test	ns	ns	ns	ns
Wheat pollard				
Before CI	82.17	78.99	76.95	75.54
After CI	62.83	59.28	57.22	55.85
t-test	ns	P<0.05	P<0.05	P<0.01

CI = Cross inoculation
ns = Not significant

The ED of DM of leucaena was not affected by cross inoculation. These values were lower than those reported by SERRA *et al.* (1997) on the diet of leucaena without urea infusion. These were 67.77, 57.95, 52.06 and 48.13% in outflow rates of 0.02, 0.04, 0.06 and 0.08 h^{-1}, respectively. In the study on buffalo, MUNIER (2001) reported that urea infusion reduced the DMED of leucaena. After CI, the ED of DM of wheat pollard after CI decreased significantly when the outflow rates were 0.04, 0.06 and 0.08/h.

CONCLUSION

Through cross inoculation of rumen content from cattle, significantly changes were occurred in the rumen of buffalo in reducing pH level, but no effect on rumen ammonia concentration and the total volatile fatty acid. However, the transfer had significantly decreased in bacteria population though there was no affect in the total count of protozoa and fungi.

The transfer of rumen content from cattle to buffalo had significantly decreased the value of water insoluble and potential digestible fraction of leucaena. However, the dry matter effective degradability of napier grass was significantly increased (P<0.05) within outflow rate 0.02 and 0.04 h^{-1} after cross inoculation.

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