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The utilization of faecal inoculum in in vitro technique: The investigation of the digestibility of NDF and ADF of Napier Grass (Pennisetum purpureum)

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ABSTRACT--*The aim of this study was to reveal the effect of faecal inoculum on the in vitro digestibility of acid detergent fibre (ADF) and neutral detergent fibre (NDF) on Napier grass (Pennisetum purpureum) (PP). Samples of Napier grass were incubated for 96 h following the first step of the Tilley and Terry in vitro technique. The treatments included liquor inoculum, faecal inoculum (FI), FI added with sugar of 2.5 % (w/v), FI added with sugar of 2.5 % (w/v) and urea of 2.5 % (w/v) and FI added with urea of 2.5 % (w/v) for treatment A, B, C, D and E respectively. The design of this experiment was a Completely Randomized Design (5 x 4) with five treatments and four replications. Results of this study showed that faecal inoculum significantly (P<0.05) decreased the digestibility of Dry matter (DM), ADF and NDF of Napier grass. The use of faecal inoculum (treatment B, C, D and E) showed the lower digestibility of ADF and NDF than those of using the rumen liquor. It might be conclude that the addition of sugar or and urea in the faecal inoculum could not increase the digestibility of DM, ADF and NDF of Napier grass.*

Keywords -- *faecal inoculum, in vitro, napier grass, digestibility, ADF, NDF*

I. INTRODUCTION

Faeces has a good potency as a substitute of rumen liquor in *in vitro* technique for feed evaluation. Microbes in fresh faeces or in rectum could still be utilised as in rumen inoculum. Balfe (1985) used faeces on the Tilley and Terry two-step Method to evaluate feed in her final task as undergraduate student. Mauricio (1999) also used faecal inoculum in *in vitro* gas method to evaluate some tropical grasses in Brazil. Afdal (2003) reported that the gas production was higher when using rumen liquor compared to those using rectal inoculum in studying of the *in vitro* degradation of cellulose, washed hay and hay while it was not any differences of gas production from incubating of starch and glucose. A study in Gajah Mada University done by Sudirman *et al* (2006) using buffalo faecal inoculum in evaluating the *in vitro* degradation tropical feed. However there was not much information concerning the use of faecal inoculum in evaluating of the *in vitro* degradation of tropical forage especially Napier grass (Pennisetum *purpureum*) or the gas production and gas profile from it.

The shortage of using faecal inoculum is lower microbial population than those the microbial population in rumen liquor. Todar (1998) reported that the total number of bacteria in colon was almost one tenth of the total

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microbial number in rumen liquor. Efforts to enhance the total microbial in faeces had been done by Harris (1998). Increment of energy in the inoculum could increase the microbial population number. Ørkov *et al* (1972) reported that the addition of sucrose post ruminal could increase the total microbial population in saecum. In addition Garcia *et al* (1992) reported that increment of free fatty rice meal in ration can increase the microbial population in the Rumen Simulation Technique.

In vitro technique is one of methods of feed evaluation. This technique will image the degradation of ration in the digestive tract from rumen, reticulum, omasum and abomasum. In principal, this technique can evaluate the chemical digestibility of feed like DM, organic matter, crude protein, crude fibre, ether extract, neutral detergent fibre (NDF), acid detergent fibre (ADF) and other nutrients. The gas produced from the incubation would also create the fermentation process and the rate of digestibility in ferment bottle during incubation. This shows the image of fermentation in rumen.

Napier grass or *Pennisetum purpureum* (PP) is one of forage, generally consumed by ruminant. This grass is usually a prime grass and grown by farmer in Indonesia and other tropical places. Hartadi *et al* (1997) mentioned that the chemical composition of PP is 29.3, 3.2, 11.5 % for crude fibre, crude fat and crude protein respectively. There is lack information concerning the digestibility, degradability or profile of fermentation of PP and other properties. It is therefore needed to study the *in vitro* of this grass.

The aim of this study was to reveal the *in vitro* digestibility of DM, ADF and NDF of PP in term of the effect of using faecal inoculum as replacement of rumen liquor.

II. MATERIALS AND METHODS

One two-year old, weighted of 250 kg, canulated cow was used to provide rumen liquor and faeces collected from rectum. Some chemical material for medium preperation and chemical analysis, urea and commercial sugar were used. A unit of Tilley and Terry (1963) apparatus of *in vitro* technique was prepared.

Rumen liquor was collected from cow one hour before morning feeding at 07.00 and followed with faecal collection from the same cow. Cow was fed with 100 % field grass *ad libitum*.

Rumen inoculum was colleted from rumen, squeezed, transferred into a thermos adjusted for 39 ^{0}C and transported soon to the laboratory. Then it was be filtered using two layers muslin into container placed in the water bath. Inoculum was always flushed by CO_2 for anaerobic condition until inoculating started.

Faecal inoculum was prepared according to method of Afdal (2003). Faeces was collected from rectum, transferred into rice thermos adjusted at 39 ^{0}C and transported soon to the laboratory. It then was prepared by mixing of faeces and medium solution of 500 : 500 ml ratio, blended using food blender for 20 seconds. The mixer was next filtered with one layer muslin into container placed and flushed as rumen liquor inoculum preparation.

Grass samples were incubated according to procedure set up by Tilley dan Terrie (1963) with some modification as available facility at Laboratory of Ruminant Nutrition, Jambi University.

This study was designed in Completely Randomized Design (5x4) with five treatments and four replications in which the treatment were as below:

A. Rumen inoculum (control)

B. Faecal inoculum

C. Faecal inoculum added with 2,5 % (w/v) of sugar

D. Faecal inoculum added with 2,5 % (w/v) of sugar and 2,5 % (w/v) of urea

E. Faecal inoculum added with 2,5 % (w/v) of urea

w/v: weight/volume

Parameters measured included the digestibility of acid detergent fibre (ADF) and neutral detergent fibre (NDF) of PP. All data were statistically analysed using anova and followed with Duncan test (Steel dan Torry, 1991)

III. RESULTS AND DISCUSSION

Digestibility of Dry matter

The dry matter digestibility of PP was significant different (P<0.05) among five treatment (Table 1). Using only rumen inoculum and only faecal inoculum looks higher digestibility of DM than using additional sugar or and urea within faecal inoculum. The addition of energy and nitrogen source, sugar and urea, into faecal inoculum might probably influence the rumen environment so that it could induce on the microbial population inbalance in the rumen. Franzolin et al (2010) mentioned that the supplementation of energy and nitrogen in diet provided the different results onto the rumen environment in their in vivo study. The addition of the amount of sugar and urea into the faecal inoculum in this experiment might not balance as result that it did not generate good rumen environment for microbial growth. This might possibly inhibit the microbial population till this would decrease the digestibility of DM.

Table 1: *In vitro* digestibility of Dry matter, ADF and NDF of PP

Treatment	Dry Matter	ADF	NDF
A	35.39^{a}	$37,46^{a}$	$33,28^{a}$
B	36.28^{a}	$31,53^{b}$	$33,53^{a}$
C	14.41^{b}	$35,99^{ab}$	$31,11^{ab}$
D	11.43^{b}	$22,52^{c}$	$26,00^{b}$
E	19.04^{b}	$23,20^{c}$	$27,45^{b}$

Different superscript within the same column was significant different (P<0.05)

IV. DIGESTIBILITY OF ACID DETERGENT FIBRE

The ADF digestibility of PP can be seen in Table 1. It showed that it was significantly (P<0.05) different among all treatments. Treatment A using rumen inoculum was the highest digestibility among other treatments. This might be due it was much more microbes existing within this inoculum than others. Todar (1998) mentioned that the total microbial number in the rumen liquor is ten times higher than in faeces. Then the microbial activity to degrade nutrient like ADF in the ferment bottles would also high. Increment of sugar and urea as sources of energy and protein into the faecal inoculum affected the microbial activity. Ørskov *et al*

(1972) reported that the addition of sucrose in saecum could enhance the microbial population and then increased the sample degradation in the bottles. It might significantly ($P<0.05$) influence the digestibility of PP in treatment B, C, D and E. Addition of sugar into faecal inoculum looks not significant effect on the degradation of PP compared to rumen liquor (A) whereas treatment B, D and E are much lower than A and C. The phenomenon might possibly not optimal or not sincrone of utilization of energy and protein from sugar and urea respectively by microbes on treatment D and E so reduce the microbial growth. Kaswari dan Dianita (2006) stated that the availability and sincronization of energy and protein in rumen strongly affected the microbial population and ration degradation.

V. THE DIGESTIBILITY OF NEUTRAL DETERGENT FIBRE

It is significantly ($P<0.05$) different the digestibility of NDF among all treatment (Table 1). Treatment A as control using rumen liquor still shows highest in the digestibility of NDF among all treatment. Apart from control, treatment C using addition of sugar was higher than the rest of treatment. Addition of faecal inoculum with sugar and urea in treatment D shows the lowest digestibility of PP while treatment E is slightly higher. It looks like that the digestibility of NDF is almost similar to the digestibility of ADF. Overall the intensity of sample degradation is still high in control as the microbial population is intense at this treatment. Addition of sugar and urea in treatment C, D and E is not significant to increase the digestibility of NDF.

Apart from this, the addition of sugar and urea into the faecal inoculum like in treatment B, C, D and E did not significantly increase the digestibility of NDF. This was shown with the lower digestibility of NDF in comparison with treatment A, rumen inoculum. This might be possibly due to that the addition of sugar and urea into faecal inoculum was not optimal or not synchrony for microbial growth. More possibility, the addition of sugar and urea into faecal inoculum would create the accumulation of short chain fatty acid production and ammonia within fermenter bottle that inhibits the microbial activity as the reducing buffer capacity (Kohn and Dunlap, 1998). In this experiment the addition of sugar and urea as energy and protein source into the inoculum did not increase the microbial population but it inhibit as because of no absorption of SCFA from bottle wall. This might probably decrease the *in vitro* digestibility of ADF and NDF. Treatment D, faecal inoculum added with sugar and urea was expected to be the same with treatment A but in fact it shows the lowest digestibility of ADF and NDF.

VI. CONCLUSSION

It might be concluded that the addition of sugar and urea into faecal inoculum in *in vitro* technique could not equal to the rumen inoculum. This can be seen that the low digestibility of DM, ADF and NDF of PP using faecal inoculum compared to using rumen liquor.

It recommend to do more study such as identification of the microbial population, species of microbe, viability of microbe in each rumen liquor and faecal inoculum. These results would be hoped to create or modify faeces as inuculum source in *in vitro* technique of feed evaluation.

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