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Use of Swamp Grass and Agricultural Waste as Materials for Total Mixed Fiber (TMF) in Rations and its Effect on Methane Gas Production and Production Efficiency of Beef Cattle

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Abstract: The use of total mixed fiber (TMF) in feed production involves mixing of forages from agricultural byproducts that have different fiber fractions, nutritional values and availabilities. The forage materials used in this study were rice straw, palm oil fronds and swamp grass. Here we tested: (1) TMF composition of single samples and (2) TMF of different rations based on the forage materials they contained. The study used *in vitro* methods and randomized group design to evaluate 5 replicates of 4 different feed rations. The ration compositions were: Ration 1 (R1) = 40% *kumpai tembaga* swamp grass + 20% rice straw; Ration 2 (R2) = 40% swamp grass + 20% palm oil frond; Ration 3 (R3) = 20% swamp grass + 20% rice straw + 20% palm oil frond; Ration 4 (R4) = 30% rice straw + 30% palm oil frond, which were then added to a standard feed concentrate. *In vitro* methods were used to measure methane gas production, digestibility of dry matter (DMD), organic matter (OMD) and neutral detergent fiber (NDF, NDFD), as well as NH_3-N and partial volatile fatty acid (VFA) concentrations. The results showed that, relative to rations with rice straw and palm oil frond, rations with *kumpai tembaga* grass produced higher concentrations of acetic, propionic and butyric acid (16.10 mM, 6.05 mM and 5.99, respectively) as well as 9.68 mM methane gas. The optimal TMF composition was 20% swamp grass, 20% rice straw and 20% palm oil frond, which produced 36.32% DMD, 35.96% OMD, 17.86% NDFD and 10.84 mM NH_3-N, as well as 12.39 mM acetic acid, 4.39 mM propionic acid, 4.36 mM butyric acid VFAs and 6.91 mM methane gas.

Key words: Agricultural byproducts, animal feed, rice straw, palm oil fronds, swamp grass

INTRODUCTION

Total mixed fiber (TMF) in animal feed production involves the incorporation of forage derived from agricultural or plantation waste that contains different amounts of fiber and nutritional values into livestock feed. TMF is also dependent on the availability of these wastes. The use of TMF derived from agro-industrial and agricultural wastes such as corn cobs, corn bran and rice straw, as well as waste from pineapple plantations, in feed for dairy cattle significantly improved milk production but did not affect milk composition (Maneerat *et al*., 2013).

The use of TMF in beef cattle feed and how it impacts methane gas production by beef cattle has not been evaluated, but is an important question given the important role that fiber plays in ruminant feed as both an energy source and promoter of rumen microbial growth.

To date, there are few studies describing the use of different agricultural waste materials such as palm fronds, palm fiber and rice straw as TMF constituents to increase the feed efficiency of cattle and affect methane gas produced during feed digestion by ruminants. This study used *in vitro* approaches to examine how the use of several types of agricultural waste and swamp grass, as well as different forages, in TMF can increase livestock productivity and affect methane gas production.

MATERIALS AND METHODS

The study was conducted using an *in vitro* method described by Theodorou and Brooks (1990). Raw materials used were rice straw, palm oil fronds and swamp grasses. A randomized group design consisting of 4 types of rations based on the TMF material composition with 5 replications was used. Treatments were described according to the fiber source and composition: Ration 1 (R1) = 40% *kumpai tembaga* (swamp grass) + 20% rice straw; Ration 2 (R2) = 40% swamp grass + 20% palm oil frond; Ration 3 (R3) = 20% swamp grass + 20% rice straw + 20% palm oil frond; Ration 4 (R4) = 30% rice straw + 30% palm oil frond. To obtain 100% rations, the above rations were added to the concentrate with constituent ingredients, including corn flour, rice bran, soybean meal, tofu waste and cassava waste at a 60:40 proportion of forage: constituents.

Parameters measured with *in vitro* techniques were methane production, digestibility of ration nutrients consisting of dry matter (DMD), organic matter (OMD) and neutral detergent fiber (NDF, NDFD). The media

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characteristics that were assessed with *in vitro* measurements included the concentration of NH_3-N and partial volatile fatty acids (VFA), which comprises acetic acid, propionic acid and butyric acid concentrations.

Implementation research: Procedures for determining nutrient digestibility and rumen conditions are described below.

Preparation of McDougall's artificial saliva: To prepare 6 liters of McDougall's artificial saliva, $NaHCO_3$ (58.8 g), $Na_2HPO_4 \cdot 7H_2O$ (42 g), KCl (3.42 g), NaCl (2.82 g), $MgSO_4 \cdot 7H_2O$ (0.72 g) and $CaCl_2$ (0.24 g) were dissolved in ~5 L distilled water in a 6 L flask. $CaCl_2$ was added after the other ingredients were completely dissolved. The neck of the flask was then washed with distilled water and the volume was adjusted to 6 L. The mixture was shaken slowly with CO_2 gas to lower the pH to 6.8
Preparation of Pepsin 0.2%
Pepsin (2.86 g) was dissolved in 850 mL deionized water to which 17.8 mL HCl was added. The mixture was put into a flask and the volume was adjusted to 1 L with distilled water.

Making the indicator red boric acid:

Solution A: Boric acid (4 g, H_3BO_3) was dissolved in 70 ml distilled water with heating and the volume was adjusted to 100 mL

Solution B: Bromocresol green (66 mg, BCG) and 33 mg methyl red (MR) was dissolved in 95% EtOH and the volume was adjusted to 100 mL

Making the solution A and solution B: Solution B (20 mL) was mixed into a solution A (20 mL) that had been chilled in a flask and the final volume was adjusted to 100 mL with distilled water. The *in vitro* digestion assay followed the method described by Tilley and Terry (1963) modified according to Goering and Van Soest (1970). Rumen fluid obtained from slaughter houses was first filtered through four layers of cheese cloth. Rumen fluid and media were mixed at a 1:4 ratio (10 mL rumen fluid with four parts (40 mL) media)to yield a solution consisting of buffer, macro- and micro minerals, resazurin and reduction solution. One gram of sample was inserted into the venoject of 100 mL, then added with 50 mL of the mixture. CO_2 gas was flowed across the mixture for 30 sec before the tube was closed and incubated for 24, 48, or 72 h. Two drops of $HgCl_2$ were added at the end of the indicated incubation period. Samples and incubation media were then centrifuged at 4,000 rpm for 10 min. The supernatant was used for further analysis of partial VFA and NH_3-N concentrations, as well as quantification of cellulolytic bacteria and protozoa. Then, the residue was added with 50 mL pepsin-HCl 0.20% and incubated for 48 h. The resulting solution was filtered using Whatman filter paper No. 41, then dried for 48 h at 60°C to analyze nutrient levels.

Determination of dry matter digestibility (DMD) and organic matter digestibility (OMD):

$$DMD\ (\%) = \frac{\left[\begin{array}{c}\text{Sample weight} \times \text{DM} - \\ (\text{residue weight} \times \text{DM} - \text{control})\end{array}\right]}{\text{Sample weight} \times \text{DM}}$$

$$OMD\ (\%) = \frac{\left[\begin{array}{c}\text{Sample weight} \times \text{OM} - \\ (\text{residue weight} \times \text{OM} - \text{control})\end{array}\right]}{\text{Sample weight} \times \text{OM}}$$

Determination of ammonia nitrogen (NH_3-N) concentration: NH_3-N concentration was determined using the Conway micro diffusion technique. In brief, 1 mL supernatant obtained as described above was placed in the outer ring of a Conway diffusion cup and 1 mL saturated Na_2CO_3 solution was added. The smaller center cup was filled with boric acid and bromocresol green indicators (1 mL). The Conway cup was then tightly sealed with petroleum jelly, shaken to mix the solution with the saturated Na_2CO_3 solution and allowed to stand for 24 h at room temperature. In this reaction, N bonded with boric acid was titrated with 0.005 N H_2SO_4 until the solution color began to turn from blue to red. The NH_3-N concentration was calculated using the formula:

$$NH_3\text{-N} = \text{mL titration} \times \text{N } H_2SO_4 \times 14 \times 1.00\ (\text{mg}/100\ \text{mL})$$

Determination of total VFA: VFA levels were measured using distillation method. The supernatant obtained as described above was inserted into a Markham still and 1 mL 15% H_2SO_4 was added to the distillation tube before the assembly was closed. The outlet tube was connected to an Erlenmeyer flask containing 5 mL 0.5 N NaOH. When ~200 mL distillate was collected, phenolphthalein (1-2 drops) as an indicator was added to the distillate, which was titrated with 0.5 N HCl until the solution color changed. Total VFA concentrations were calculated using the formula:

$$VFA = \frac{(a\text{-}b) \times \text{N HCl} \times (1.000)}{5\ \text{mM}}$$

Description, a: mL titrated control, b: mL titrated sample.

Determination of methane gas production: The production of methane gas was estimated from the concentration of volatile fatty acids (VFA) that comprised the concentration of acetic acid, propionate acid and

butyric acid in rumen fluid. VFA concentrations were analyzed using gas liquid (GC) chromatography (Hewlett-Packard, 3700, USA). The formula of Owen and Goetsch (1988) was used to calculate methane gas production:

$$CH_4 = 0.5\ [acetic] + 0.5\ [butyric] - 0.25\ [propionic]$$

where, (acetic): concentration of acetic acid, (butyric): concentration of butyric acid, (propionate): concentration of propionic acid.

RESULTS AND DISCUSSION

Statistical analysis showed that the total mixed fiber (TMF) constituents in the rations significantly affected ($p<0.05$) dry matter digestibility (DMD), organic matter digestibility (OMD), NDF digestibility (NDFD) and NH_3-N concentrations. Overall, the DMD and OMD values for Rations R1-R4 were lower than that for Ration R0 (control, king grass), while the values for R3 and R4 were higher than those for R1 and R2, which had the lowest DMD and OMD values.

Meanwhile, R0 and R4 had higher NDFD than R1, R2 and R3, with R2 again being the lowest. Differences between DMD, OMD and NDFD were related to the different nutritional values associated with the varying TMF sources, which yielded different crude fiber contents.

The use of swamp grass in TMF decreased DMD, OMD and NDFD values, likely because the crude fiber content of swamp grasses was higher than the two other forage sources used, namely rice straw and palm frond. For digestibility parameters, Ration R2 yielded the lowest level of digestibility in terms of DMD, OMD and NDFD. Ration R2 had TMF composed of 40% swamp grass and 20% palm fronds, which produced a higher crude fiber content of 31.7% (Table 1). This outcome is consistent with earlier findings that high crude fiber contents in livestock feeds are associated with lower digestibility. Indeed, Mapato *et al*. (2010) found that levels of dry matter and organic matter are negatively correlated with the NDF and acid detergent fiber (ADF) of the ration. Meanwhile, Davidson *et al*. (2003) and Dahiya *et al*. (2004) both showed that a high content of crude fiber in feed contributes to a decrease in DMD and OMD. Griswold *et al*. (2003) also demonstrated that increased OMD was associated with dry matter content, with ash content being an important factor.

Further tests showed that feeding R1 yielded a significantly higher NH_3-N concentration ($p<0.05$) compared with other treatments, while R0 and R2 showed no significant effect ($p>0.05$), but both were significantly ($p<0.05$) higher than that for R3 and R4, with R3 being higher than R4. Differences in NH_3-N concentration generated from all treatments were likely due to the different TMF compositions that had different proteins that affected the protein content of the ration. The fact that R1 had the highest dietary protein content of all the rations is consistent with this ration producing higher NH_3-N concentrations. The high levels of dietary protein in TMF containing grass swamp grass could be attributed to the high levels of kumpai copper protein. An analysis of a single sample showed that the concentration of NH_3-N generated from a single sample of copper kumpai grass was higher than that of rice straw and palm fronds. A report by Orskov (1992) stated that some proteins in the rumen would be metabolized to ammonia and the amount would depend on dietary protein solubility, the amount of dietary protein, the time that the ration remained in the rumen and rumen pH. From this study, NH_3-N concentration levels ranging between 10 and 12 mM were sufficient to meet the needs of livestock to produce NH_3-N, as was defined in a report by Sutardi (1980) that stated the ammonia levels required to support the optimal growth of rumen microbes is between 4 and 12 mM. *In vitro* measurements of NH_3-N can be used to estimate degradation of proteins that can then be used by microbes. The maximum number of microbes in the rumen was associated with ammonia levels of 10-25 mg/100 ml (Leng, 1990; Orskov, 1992). Meanwhile, the NH_3-N concentration required for optimal microbes growth was between 5 and 15 mg N/dl (Satter and Slyter, 1974; Alcaide *et al*., 2003) and higher NH_3-N

Table 1: Composition of nutrients in the ration (%)

	Treatments				
Nutrient	R0	R1	R2	R3	R4
Crude protein	12.12	12.41	12.37	10.21	8.02
Crude fiber	25.39	28.85	31.70	29.19	28.12
TDN	72.29	68.50	72.30	65.74	61.08

Table 2: Digestibility of dry matter (DMD), organic matter (OMD), NDF (NDFD) and NH_3-N concentration of treatment rations (%)

Treatments	DMD	OMD	NDFD	NH_3-N
R0	47.09^a	45.85^a	29.01^a	11.03^b
R1	34.46^c	33.12^c	14.05^c	12.93^a
R2	31.23^d	30.31^d	10.19^d	11.46^b
R3	36.32^b	35.96^b	17.86^b	10.84^c
R4	36.77^b	35.95^b	23.89^a	8.50^d

Different superscripts in the same column represent significant differences ($p<0.05$)

Table 3: Partial VFA concentration and production of methane gas (%)

Treatment	Acetic acid	Propionic acid	Butyric acid	Methane gas
R0	5.53^a	2.8^a	3.75^a	3.39^a
R1	12.69^b	4.49^b	5.49^b	7.97^{bc}
R2	10.93^c	4.37^b	4.06^c	9.86^c
R3	12.39^b	4.39^b	4.36^c	6.91^b
R4	10.93^c	5.48^c	5.33^b	7.61^b

Different superscripts in the same column represent significant differences ($p<0.05$)

concentrations (19 mg N/dl) were needed for fermentation *in vivo* (Mehrez *et al*., 1977). A study by Nagadi *et al*. (2000) found that the NDF fermentation rate increased in accordance with increasing NH_3-N concentrations, which is consistent with our results. Dettmann (2009) reported that the optimum concentration of ruminal NH_3-N for degradation and consumption of NDF ranged from 8-15 mg N/dl, while Islam *et al*. (2000) demonstrated NH_3-N concentrations as high as 6.4 mg N/dl in rumen fluid from animals fed diets containing 60% palm fronds and 40% concentrate. Our statistical analysis showed that TMF composition affected partial VFA concentration and methane production. R1 and R3 produced higher levels of acetic acid ($p<0.05$) compared to R2 and R4, while R4 produced the highest amount of propionic acid ($p<0.05$) relative to R1, R2 and R3. Furthermore, R1 and R4 produced a higher butyric acid concentration ($p<0.05$) than did R2 and R3, which produced the lowest amount of methane gas. Although R3 showed no significant difference ($p>0.05$) compared to R1 and R4, it did differ significantly ($p<0.05$) from R2. Based on the composition of partial VFA generated by different fiber sources and composition of TMF, our results showed that overall the proportion of the acetic acid concentration that was produced exceeded that for propionic acid and butyric acid.

The methane content increased with increasing NDF and hemicellulose contents. An increased NDF content will in turn increase methane levels by shifting the proportion of VFAs toward increased amounts of acetic acid, which produces hydrogen gas (H_2) as a substrate for methanogenesis reactions (Jayanegara *et al*., 2008). Methane gas production is closely related to the amount of acetic acid and butyric acid produced during feed fermentation in the rumen, but is independent of propionic acid production. This relationship is due to the dependence of methane gas production on the availability of H_2 and CO_2 in the rumen. H_2 and CO_2 are released during production of acetic acid and butyric acid that occurs during feed fermentation in the rumen. In contrast, propionate acid production is not accompanied by H_2 and CO_2 production (Church, 2002). In this study we showed that R3 resulted in lower levels of methane gas (6.91 mM), but high concentrations of acetic acid and butyric acid relative to the other treatments. This outcome is likely due to the high protein content of R3, which would affect the use of acetic acid and butyric acid that resulted from NDF metabolism to produce amino acid precursors in the rumen. In turn, H_2 availability for bacterial methanogenesis is reduced to promote further reductions in methane gas production. This outcome is consistent with a study by Widyawati *et al*. (2010), which stated that diets with increased protein content can reduce the production of methane following consumption of legumes.

Conclusion: The optimal TMF composition for cattle was 20% *kumpai tembaga* grass (swamp grass), 20% rice straw and 20% palm fronds. This composition yielded 36.32% DMD, 35.96% OMD, 17.86% NDFD and 10.84 mM NH_3-N concentration. The partial VFA concentration was 12.39 mM, 4.39 mM and 4.36 mM acetic acid, propionic acid and butyric acid, respectively, while 6.91 mM methane gas was produced.

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