

## Nutrient Digestibility of Beef Cattle Ration with Total Mixed Fiber Ammoniation (TMFA) as Basal Feed and Organic Mineral Supplementation by In Vitro

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### Abstract

The research was aimed to obtain the level of organic minerals supplementation with Total mixed fiber Ammoniation (TMFA) as basal feeding for the improvement of nutrient digestibility in rumen. Technique of research conducted by In Vitro technique. The method used in this research is a Completely Randomly Designed (CRD) with 4 treatments and 4 replications. P1: 60% ammoniation TMF + 0 x Organic macro minerals (Ca, P, and S) NRC (2000). P2: 60% ammonia TMF + 0.75 x Organic macro minerals (Ca, P, and S) NRC (2000), P3: 60% ammonia TMF + 1 x Organic macro minerals (Ca, P, and S) NRC (2000) + 15% Legume Swamp P4: 60% ammoniation TMF + 1.5 x Organic macro minerals (Ca, P, and S) NRC (2000). Each treatment was added with an organic micro-mineral consisting of 40 ppm organic Zn, 10 ppm organic Cu, 0.10 ppm organic Se, and 0.30 ppm organic Cr. The parameters measured in the in-vitro technique were nutrient digestibility including dry matter and organic matter, crude protein, crude fiber, NDF and ADF. The results showed that organic mineral supplementation had significantly different effect ( $P < 0.05$ ) on nutrient digestibility. The conclusion of this study was that organic mineral supplementation of Ca, P and S 1.5 times of requirement according to NRC (2000) gave the best results for in vitro nutrient beef digestion with Total mixed fiber ammoniation (TMFA) as basal feed and supplementation of 15% legume swamp (Kemon air).

Keywords: Ammoniation, total mixed fiber, organic mineral, nutrient digestibility

### Abstrak (Indonesian)

Penelitian ini bertujuan untuk mendapatkan tingkat mineral organik dengan pakan basal amoniasi TMF yang tepat bagi peningkatan pencernaan nutrisi dalam rumen. Teknik penelitian yang dilakukan adalah tehnik *In Vitro*. Rancangan yang digunakan yaitu Rancangan Acak Lengkap (RAL) dengan 4 Perlakuan dan 4 ulangan. P1 : 60 % amoniasi TMF + 0 x Mineral makro organik (Ca, P, dan S) NRC (2000). P2: 60 % amoniasi TMF + 0.75 x Mineral makro organik (Ca, P, dan S) NRC, P3 : 60 % amoniasi TMF + 1 x Mineral makro organik (Ca, P, dan S) NRC, P4 : 60 % amoniasi TMF + 1.5 x Mineral makro organik ( Ca, P, dan S) NRC. Parameter yang diukur adalah, Pengukuran pencernaan nutrisi yaitu bahan kering dan bahan organik, protein kasar, serat kasar, NDF dan ADF. Hasil penelitian menunjukkan bahwa suplementasi mineral organik memberikan pengaruh berbeda nyata ( $P < 0.05$ ) terhadap pencernaan nutrisi. Kesimpulan dari penelitian ini adalah suplementasi mineral organik Ca, P dan S 1,5 kali dari kebutuhan menurut NRC (2000) memberikan hasil yang terbaik untuk pencernaan nutrisi sapi potong secara in vitro dengan pakan basal amoniasi total mixed fiber (TMF) dan suplementasi 15% kemon air.

Kata Kunci: Amoniasi, Total campuran serat, mineral organik, pencernaan

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## INTRODUCTION

Utilization of agricultural and plantation waste is considered to be potential as an alternative to the use of ruminant animal feed. Agricultural and plantation wastes that can be utilized as materials for Total Mixed Fiber (TMF) such as oil palm frond and rice straw. Total mixed fiber (TMF) is a combination of several sources of fiber, both from forage and from agricultural waste [1]. Research of [2] has obtained the best composition from TMF that is 20% Grass Kumpai Tembaga, 20% Rice Straw and 20% oil palm frond, This composition produces dry matter digestibility rate 36.32%, organic matter digestibility 35.96% by in vitro technique. Application of ammoniation technology for total mixed fiber resulted dry matter digestibility of 52.36%, VFA concentration of 65.41 mM [3]. The dry matter digestibility and VFA concentration from the use of 60% of the total ammonium mixed fiber is still low.

Another thing that can be done to improve the ration digestibility is increase of the bioprocess in the rumen with mineral and protein supplementation. Bioprocess in the rumen and post-rumen should be supported by the adequacy of macro and micro minerals. Minerals play a role in optimizing bioprocess in the rumen and metabolism of feed substances. Provision of minerals in organic form can increase the availability of minerals so that it can be higher absorbed in the body of cattle [4]. Based on the description above it is necessary to do research to see the effect of supplementation kemon air (*Neptunia Oleracea* Lour) as a source of protein supplements and the level of organic mineral supplementation as mineral supplements in rations based on ammonia TMF and its effect on increasing digestibility of rations.

## MATERIALS AND METHODS

### Research Method

The method used in this research is a Completely randomly designed (CRD) with 4 treatments and 4 replications, Treatment consists of: P1: 60% TMF Ammoniation + 0 x Organic macro minerals (Ca, P, and S)P2: 60% TMF Ammoniation + 0.75 x Organic macro minerals (Ca, P, and S)P3: 60% TMF Ammoniation + 1 x Organic macro minerals (Ca, P, and S)P4: 60% TMF Ammoniation + 1.5 x Organic macro minerals (Ca, P, and S). The macro minerals are tailored to the needs of beef cattle based on NRC 2000. Each treatment adds 40% concentrate to sufficient 100% ration requirement and addition of 15% swamp legume and organic micro mineral comprising 40mg /

kg organic Zn, 10mg / kg Organic Cu, 0.10mg / kg organic Se and 0.30mg / kg Cr organic

### Preparation for TMF ammoniation

The first stage is the processing of TMF with ammoniation method. The ammoniation process was performed using 4% urea of 1 kg TMF. TMF (20% kumpai tembaga grass+20% oil palm fronds+20%rice straw) was chopped to a theoretical cut length 2-3 cm by machine. Urea-treated TMF was prepared by using 4 kg urea of fertilizer grade (46% N) plus 100 kg water, sprayed onto 100 kg TMF and then covered up and then incubated for 21 days before directly feeding to the animals [5].

### Preparation of Organic Macro Minerals (Muhtarudin et al., 2003)

The process of making macro organic mineral supplements consists of stages: dissolving 10M NaOH 400 g then dissolved into distilled water until 1Liter further mixed into cooking oil 912 g (Initial solution). Furthermore, for Ca organic mineral  $\text{CaCO}_3$  5M required 680.33g dissolved in Distilled water to 1Liter, this solution is mixed with the initial solution and composed until homogeneous. The organic mineral P is prepared by dissolving the initial solution with aqueous  $\text{KH}_2\text{PO}_4$  601.84 diluted in distilled water to 1 Liter. The organic mineral S is carried out by dissolving the starting solution into a solution of  $(\text{NH}_4)_2\text{SO}_4$  5 M of 601.84 g dissolved in distilled water to 1liter.

### Preparation of Organic Micro Minerals (Muhtarudin et al., 2003)

The process of making organic micro mineral supplement consists of stages: dissolving 43,823 g amino acid lysine HCl with distilled water 100 ml, then mixing with  $\text{ZnSO}_4$  solution 16,139 g dissolved with distilled water 100 ml to obtain Zn-Lisinate ; dissolved 43,823 lysine HCl dissolved with distilled water of 100 ml; then mixing with 15,955 g of  $\text{CuSO}_4$  solution dissolved with distilled water of 100 ml to obtain a Cu-lysine; dissolved Cr from  $\text{K}_2\text{Cr}_2\text{O}_7$  29.4 g then dissolved with distilled water to 500 ml, then added  $\text{H}_2\text{O}_2$  8.79 ml and HCl 100.69 ml then dissolved again with distilled water up to 1 liter of solution, then mixed with lysine solution HCl 65.73 g dissolved with distilled water of 1 liter to obtain a mixture of Cr-Lisinate; dissolved Se from  $\text{SeO}_2$  2,1096 g then dissolved with distilled water 100 ml then added  $\text{HNO}_3$  4.34 ml then dissolved with distilled water up to 1 liter, then mixed with lysine HCl solution 4,38 g dissolved with distilled water 1 liter to obtain a Se-Lisinate .

Mixing micro mineral in ration with concentration of 40 ppm Zn-Lisinate, 10 ppm Cu-Licinate, 0.10 ppm Se-lysine and 0.30 Cr-Lisinate in ration.

### Concentrate Preparation

Preparation of concentrate was done by mixing the feed ingredients i.e. rice bran 60%, milled corn 12%, tofu waste 26%, urea 1%, 0.5% salt into container then stirring, mixing from material size (%) smallest to largest.

### Research implementation

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<sub>3</sub> (58.8g), Na<sub>2</sub>HPO<sub>4</sub>·7H<sub>2</sub>O (42g), KCl(3.42g), NaCl (2.82g), MgSO<sub>4</sub>·7H<sub>2</sub>O (0.72g), and CaCl<sub>2</sub> (0.24g) were dissolved in ~5 L distilled water in a 6 L flask. CaCl<sub>2</sub> 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<sub>2</sub> 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<sub>3</sub>BO<sub>3</sub>) was dissolved in 70ml distilled water with heating and the volume was adjusted to 100 mL.

Solution B. Bromo cresol green (66 mg, BCG) and 33 mg methyl red (MR) was dissolved in 95% Ethyl alcohol 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 [6] modified according to [7]. Rumen fluid obtained from slaughterhouses was first filtered through four layers of cheesecloth. 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 microminerals, resazurin, and reduction solution. One gram of sample was inserted into the venoject of 100mL, then added with 50 mL of the mixture. CO<sub>2</sub> gas was flowed across the mixture for 30 seconds before

the tube was closed and incubated for 24, 48, or 72 hours. Two drops of HgCl<sub>2</sub> were added at the end of the indicated incubation period. Samples and incubation media were then centrifuged at 4,000 rpm for 10 minutes. The supernatant was used for further analysis of partial VFA and NH<sub>3</sub>-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 hours. The resulting solution was filtered using Whatman filter paper No. 41, then dried for 48 hours at 60 °C to analyze nutrient levels

### Data Analysis

1. Determination of dry matter digestibility (DMD)

$$\text{DMD (\%)} = \frac{SxDM - (RxDM - \text{Control})}{SxDM} \times 100$$

2. Determination of organic matter digestibility (OMD)

$$\text{OMD (\%)} = \frac{SxOM - (RxOM - \text{Control})}{SxOM} \times 100$$

3. Determination of crude protein digestibility (CPD)

$$\text{CPD (\%)} = \frac{SxOM - (RxOM - \text{Control})}{SxOM} \times 100$$

Where,

S: Sample weight

R: Residue weight

DM: Dry Matter

OM: Organic Matter

CPD: Crude Protein Digestibility

The same formula was used for determination of crude fiber, ADF and NDF digestibility

## RESULT AND DISCUSSION

### Effect of organic mineral supplementation of Ca, P and S on nutrient digestibility

Dietary digestibility is influenced by several factors, according to [8], which is the composition of feed ingredients used in rations with other feed ingredients, feed composition, dietary supplementation, feeding, livestock and feeding levels. Nutrient digestibility data of Total Mixed Fiber Ammoniation (TMFA) with organic mineral supplementation and swamp legume are presented in Table 1.

**Table 1.** Nutrient digestibility value (%) TMFA with organic mineral Ca, P and S supplementation

Nutrient digestibility	Treatment			
	P0	P1	P2	P3
Dry matter	54.44a	57.25ab	60.25b	72.59c
Organic Matter	52.41a	52.98ab	55.77b	65.23c
Crude Protein	38.15a	44.65b	48.83b	52.21c
Crude Fiber	30.52a	37.49b	46.18c	51.51c
NDF	27.46a	29.96a	37.43b	49.78c
ADF	26.71a	31.71b	35.30b	48.91c

Note: different superscript on the same row showed the treatment gave influence significantly different ( $P < 0.005$ )

Based on statistical analysis, it was found that organic mineral and swamp legume supplementation in TMFA as basal feed had significant effect ( $P < 0.05$ ) on dry matter digestibility value, organic matter, crude protein, crude fiber, ADF and NDF. Further test results on dry matter digestibility and organic matter showed that organic mineral supplementation of Ca, P and S 1.5 times from the recommended [9] requirement gave higher dry matter and organic matter digestibility value compared with 1 and 0.75 times supplementation from which is recommended by the NRC and from controls without supplementation, while for supplementation 1 and 0.75 of the NRC recommendations have the same effect while the supplemented treatment has the same effect with supplementation of 0.75 times the NRC recommendation but gives the dry matter digestibility and the lower organic matter of treatment with a 1-time supplementation recommended by the NRC.

The organic mineral supplementation also gave a significantly different effect ( $P < 0.05$ ) on the digestibility of crude protein, where from the further test it was found that Ca, P and S mineral supplementation 1.5 times from the NRC recommendation resulted in higher crude protein digestibility rate than the other 3 treatments, while the supplementation treatment of 0.75 and 1 times of the NRC recommendations gave the same effect but compared to the control treatment (without supplementation) 0.75 and 1 times supplementation of the NRC recommendations resulted in higher levels of the crude protein digestibility.

Data of nutrient composition feed stuff showed in Table 2. In fiber digestibility parameter, crude fiber, ADF and NDF, treatment of supplementation also gave significant different effect ( $P < 0.05$ ).

**Table 2.** Nutrients composition of feed stuff (%)

No	Feed Stuff	CP	CF	TDN
1	Rice brans	11,2	18,5	65
2	Milled corn	10,82	2,61	83
3	Tofu west	11,6	7,79	70
4	Salt	0	0	0
5	Urea	2,61	0	0
6	TMFA	6,65	27,91	66,99
7	Kemon Air	28,02	17,25	44,86

Source: Laboratory of Agriculture Product Technology Sriwijaya University (2016)

**Table 3.** Material and nutrient concentrate composition

No	Feed Stuff	Compo-sition	CP %	CF %	TDN %
1.	Rice brans	60,5	6,77	11,19	39,32
2.	Milled corn	12	1,29	0,31	9,96
3.	Tofu waste	26	3,01	2,02	18,2
4.	Salt	0,5	0	0	0
5.	Urea	1	2,61	0	0
Total		100	13,68	13,52	67,48

Source: Calculated based on Table 2 with concentrate composition

**Table 4.** Nutrients value in Ration

Ration	Composition (%)	Nutrient		
		CP	CF	TDN
TMFA	60	3,99	16,75	40,19
concentrate	40	5,47	5,40	6,72
Total		5,47	22,15	67,18

Note: Calculated based on Table 2 and Table 3

Based on further tests it was found that Ca, P and S mineral supplementation 1.5 times from NRC recommendations gave a significantly higher effect than other treatments for the digestibility of crude fiber, ADF and NDF, although on the digestibility of crude fiber gave the same effect as the 1 times supplementation of recommendations NRC. The treatment of supplementation of Ca, P, and S organic minerals from the NRC recommendation gave the crude fiber digestibility value, and the ADF was higher than the control treatment and the supplementation treatment 0.75 times from the NRC recommendation, while for NDF digestibility of organic mineral supplementation treatment 1 times from recommendation NRCs have the same effect as 0.75 times supplementation of NRC recommendations but these treatments provide significantly higher levels of digestibility than control treatments. The results of this

study are in line with those stated by [10] that P and S mineral supplementation on ammonia rice straw increased NDF, ADF, cellulose, and hemicellulose digestibility

Based on the research data can be seen that the utilization of TMFA 60% in beef cattle ration with organic mineral supplementation Ca, P and S and supplementation of 15% swamp legume (Kemon Air) can increase the digestibility of feed stuff. The amount of organic mineral supplementation 1.5 times from the recommendation of NRC (P3) gives the best digestibility value of the substances in vitro. Dry matter digestibility increased 33.34% and organic matter by 24.46% resulting from 60% TMFA with organic mineral supplementation Ca, P and S 1.5 times NRC recommendations compared with no mineral supplementation. The results of this study are in line with the results of [11] research which states that the digestibility of dry matter and organic materials with organic mineral supplementation gives better results than without supplementation, this is because rumen microbes require Ca and Mg minerals for their metabolic activity.

Increasing of dry matter and organic matter digestibility in the presence of organic mineral supplementation is closely related to the increase of digestibility in crude protein, crude fiber, ADF and NDF in this study. Supplementation of Ca, P and S organic mineral minerals in the TMFA as basal feeding has a positive effect on the improvement of rumen conditions, especially for the activity and growth of rumen microbes, with increased growth and activity of rumen microbes, this will also affect the increase of feed digestibility. Aurora [12] states that cellulolytic bacteria require minerals Ca, P, Mg, S, Zn, Co, and Mn for their activity.

Further explained by other author [13] that Ca mineral has a role in maintaining the stability of cell wall structures, this mineral deficiency can cause growth and metabolic processes that require disturbed Ca. Mineral P is essential for all microorganisms as it is an integral part of the nucleotide and some coenzymes. Mg minerals are essential for various cellular processes that are required by all microorganisms. Many S minerals are present in amino acids containing S in microbial proteins. Ca, P, Mg and S minerals are also involved in activating enzymes needed to ferment food substances in the rumen, so that by direct or indirect means this mineral adequacy can affect the rumen microbial population. The availability of macro and micro minerals is quite supportive in the process of metabolism in the rumen and post-rumen. In optimal bioprocess in the rumen, minerals play an

important role, especially minerals in organic form other than other food substances [14, 5]. Organic minerals have advantages over inorganic minerals, among others, more soluble because of the solubility of organic compounds that bind them, more easily absorbed and prevent antagonism with other minerals [12,15].

## CONCLUSION

Based on the results of this study it can be concluded that the use of 60% TMFA on beef cattle ration with organic mineral supplementation of Ca, P and S with level of 1.5 times from NRC recommendation can improve dry matter, organic matter, crude protein, crude fiber, NDF and ADF digestibility.

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