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## The effect of organic mineral supplementation in the ration with total mixed fiber ammoniation as basal feed to rumen environment

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**Abstract.** This study aims to evaluate the effect of organic mineral supplementation on ration with total mixed fiber as basal feed to rumen environment. This study consists of four organic mineral level on ration and four replicates of each treatment. The treatments are : P1: 60% ammoniation TMF + 0 x Organic macrominerals (Ca, P, and S) NRC (2000) + 15% Swamp Legumes. P2: 60% ammonia TMF + 0.75 x Organic macrominerals (Ca, P, and S) NRC (2000) + 15% Swamp Legum. P3: 60% ammonia TMF + 1 x Organic macrominerals (Ca, P, and S) NRC (2000) + 15% Legum Swamp P4: 60% ammoniation TMF + 1.5 x Organic macrominerals (Ca, P, and S) NRC (2000) + 15% Swamp Legum. Each treatment is 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 Cr organic. Parameters measured are N-NH<sub>3</sub> concentration, total VFA concentration, SCFA concentration, pH rumen, methane gas production and microbial population. The results show that organic mineral supplementation had a significantly different effect (P <0.05) on rumen condition characteristics (N-NH<sub>3</sub>, total VFA, SCFA and methane), and bacterial cellulolytic populations but did not affect the total bacterial rumen and protozoan population. It can be concluded that organic mineral supplementation of Ca, P and S 1.5 times of requirement according to NRC (2000) gives the best result for improving the rumen environment with Total mixed fiber ammoniation (TMFA) as basal feed and 15 supplementation % legume swamp (kemon air).

### 1. Introduction

The low intake and utilization of feed ingredients of rumen ecosystems and unbalance products from the rumen fermentation so it decreases livestock production [1]. Some studies of supplementation for low-quality forages such as the use of rumen-degradable protein (RDP) or non-protein nitrogen (NPN) shows the increase of feed intake [2]. Digestibility increasing and rate of digestive in the rumen [3]. NPN supplementation such as urea to 30 g / kg Dry matter ration significantly increases consumption, digestibility of nutrients, volatile fatty acid (VFA) production, Nitrogen balance and contribution of microbial nitrogen and substantially will be increasing for body weight gain [4]. The efficiency of rumen ecosystems is also influenced by minerals. Mineral supplementation in rations can fulfil mineral deficiency, it can also support optimization of bioprocess in the rumen.

Forage with low digestibility is usually from the agricultural waste product such as oil palm fronds and rice straw. Total Mixed Fiber is a combination of several forages that serve as a source of fiber. In the previous study, the best composition of TMF was 20% Grass Kumpai Copper, 20% Rice Straw and 20% palm stem. Composition result in dry matter digestibility rate 36.32%. Organic material digestibility 35.96%. NDF Digestibility 17.86%. N-NH<sub>3</sub> 10.84mM. Partial VFA Concentration (12.39mM acetic acid, 4.39mM propionic acid and 4.36mM butyric acid) and 6.91mM methane gas



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production in Vitro [5]. The utilization of TMF 60% ammoniation in Balinese cow ration gave the result of 0.47kg / head /day weight gain. but the low VFA and N-NH<sub>3</sub> rumen concentrations were 61.07mM and 38.75mM. Therefore. it is necessary to conduct research related to the utilization of TMF ammoniation with Swamp Legum as supplementation of protein and organic mineral in rations on beef cattle production. The use of organic minerals can increase the using value of minerals and it can also protect the bound the mineral organic material from degradation in the rumen. Metabolism of micro and macro minerals have interactions each other. The interaction can be a negative and positive interaction in the digestive system of livestock, especially in the rumen. Other things that affect the working of minerals in the rumen such as phytic acid, crude fiber, and other substances. This factor can reduce the availability of minerals in the rumen [7].

Based on the previous study. it is necessary to conduct study to obtain the level of swamp legume supplementation as a source of protein supplementation and the implementation of organic minerals in a ration with TMF Ammoniation as basal feed and its effect on increasing bioprocess in the rumen and increasing beef production.

## 2. Material and Method

### 2.1. Preparation for TMF ammoniation

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

Preparation of Mineral Ca. P and S Organic. Organic Mineral Preparation by [7]. Preparation of initial solution: 10 M NaOH of 400 g dissolved into adistilled water up to 1 Liter. NaOH solution mixed into cooking oil as much as 912 g. Preparation of Ca Organic mineral: CaCO<sub>3</sub> 5M 680.33g dissolved in Aquades to 1Liter. this solution is mixed with the initial solution and composite until homogeneous. P organic Mineral: KH<sub>2</sub>PO<sub>4</sub> 601.84 is diluted in aquadest to 1 Liter. then mixed with the initial solution. Making organic mineral S: initial solution into solution (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> 5 M 601.84 g and dissolved in aquades to 1 liter.

### 2.2. Method

The method used in this study is an experimental method with Completely Random Design (CRD) 4 treatments and 4 replications. The treatment consists of:

P1 : 60 % TMFA + 0 x organic macrominerals (Ca. P. dan S ) NRC (2000) + 15% swamp legum

P2 : 60 % TMFA + 0.75 x organic macro minerals (Ca. P. dan S ) NRC (2000) + 15% swamp legum

P3 : 60 % TMFA + 1 x organic macrominerals (Ca. P. dan S ) NRC (2000) + 15% swamp legum

P4 : 60 % TMFA + 1.5 x organic macrominerals (Ca. P. dan S ) NRC (2000) + 15% swamp legum

Each treatment is 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. Parameters measures characteristic of rumen conditions: N-NH<sub>3</sub> concentration, total VFA concentration, partial VFA concentration, pH rumen. methane gas production and rumen microbial population.

**Table 1.** Nutrition content of feedstuff for Ration (%)

No	Feed Stuff	Crude Protein	Crude Fiber	TDN
1	Rice brands	11.2	18.5	65
2	Milled Corn	10.82	2.61	83
3	Tofu Waste	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 (Swamp Legum	28.02	17.25	44.86

Source: Laboratorium Teknologi Hasil Pertanian Universitas Sriwijaya (2016)

**Table 2.** Material dan Nutrition of Concentrate

No	Fedd Stuff	Percentage on concentrate	CP %	CF %	TDN %
1.	Rice Brands	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 using of feedstuff on concentrate

**Table 3.** Nutrition of Ration

Ration	(%)	Nutrition Content		
		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 by Table 2 dan 3

### 2.3. The procedure for determining the characteristics of rumen conditions with in vitro techniques [8]

The rumen fluid is extracted using four layers of cheesecloth. Ten milliliters of rumen fluid is fed into incubation tubes and added with 40 ml of buffer solution, macro and micro mineral solutions, reduction and resazurin solutions [9]. Fifty milliliters of solution have been subsequently added 1g sample and CO<sub>2</sub> gas for 30 seconds, closed and incubated for 24, 48 and 72 hours. Each incubation time ends then incubation tube plus HgCl<sub>2</sub> 2 drops to kill microbes. After all incubation period is completed, the media is centrifuged at 4000 rpm for 10 minutes. The media will separate into residues and supernatant. The residue was added with 50 ml of 0.2% pepsin solution and incubated for 48 hours. The incubation results were filtered using Whatman no 41, then dried in an oven at 60°C for 48 hours. Centrifugation supernatants were used to determine the characteristics of rumen conditions (concentrations of N-NH<sub>3</sub> and VFA, SCFA) and populations of cellulolytic bacteria and rumen protozoa.

#### 2.3.1. Determination of In-vitro Media Characteristics

##### 2.3.1.1. Determination of ammonia concentration of nitrogen (NH<sub>3</sub>-N)

The NH<sub>3</sub>-N concentration is determined by the Micro Conuse Diffusion technique. One ml of supernatant is placed in a partition of Conway's plate. On the other side is 1 ml of saturated Na<sub>2</sub>CO<sub>3</sub>

solution. In the center of small plate. it is filled boric acid with red indicator and green Brom cresol 1 ml. The Conway plate is tightly closed with a baseline and then mixed with  $\text{Na}_2\text{CO}_3$ . After it is left for 24 hours at room temperature. N bounded to boric acid is titrated with  $\text{H}_2\text{SO}_4$  0.005 N until the initial point of discoloration from blue to reddish. The  $\text{NH}_3\text{-N}$  concentration is calculated using the formula:

$$\text{NH}_3\text{-N} = \text{ml titration} \times \text{N H}_2\text{SO}_4 \times 14 \times 1.00 \text{ (mg/100ml)}$$

### 2.3.1.2. Determination of total VFA concentration

Determination of VFA concentration is conducted by steam distillation method. A total of 5 ml of in vitro vitality of supernatant was inserted into Markham's tube added 1 ml of 15%  $\text{H}_2\text{SO}_4$ . The distillation tube is immediately closed. The tube is connected to an Erlenmeyer flask containing 5 ml of  $\text{NaOH}$  0.5 N. The distillation process ends until the distillate is accommodated to a volume of approximately 200 ml. Then add 1-2 drops of the phenolphthalein indicator and in titers with  $\text{HCl}$  0.5 N until the color change occurs.

The total VFA concentration is calculated using the formula:

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

Note:

a= ml titrant blanko

b= ml titrant sample

### 2.3.1.3. Determination of Methane Gas Production

The determination of methane gas production is carried out by using short-chain fatty acid concentration data, consisting of concentrations of acetic acid, propionic acid, and butyric acid, while the analysis of short-chain fatty acid concentrations is carried out by using Gas Liquid Chromatography (GLC, HEWLETT PACKARD, 3700, USA). Estimated methane gas production is calculated using the formulas of Owens and Goetsch (1988) [10]:

$$\text{CH}_4 = 0.5 [\text{acetyc}] + 0.5 [\text{butyric}] - 0.25 [\text{propionic}]$$

[acetyc] : acetic acid concentration  
 [butyric] : butyric acid concentration  
 [propionic]: propionic acid concentration

### 2.3.2. Protozoa and Cellulolytic Bacteria Population

Calculation of Protozoa Population

Protozoa populations are calculated using counting chamber and formal saline solution. The formal saline solution is a mixture of 100 ml of 4% formalin with 8 g  $\text{NaCl}$  in 900 ml of solution and trypan blue 0.6 gr. The ratio of formal saline solution and rumen fluid sample is 1: 1. Two drops of the mixture are placed on the counting chamber with a thickness of 0.1 mm from 16 boxes that are read as many as 5 boxes. Protozoa population calculations are performed using a microscope at 40 times magnification.

$$\text{Protozoa/ml cairan rumen} = \frac{1}{(0,1 \times 0,0625 \times 16 \times 5)} \times 1000 \times C \times \text{FP}$$

Note:

C = Protozoa population in a counting chamber

FP = Dilution Factor

Bacterial population calculations are performed by the Hungate method (Ogimoto and Imai, 1981)[11]. The population of cellulolytic bacteria is calculated using bacterial growth medium in the form of Carboxy Methyl Cellulose (CMC). The rumen fluid to be identified by the cellulolytic bacteria is diluted by 4 dilution levels. From each series, the dilution tube is taken 0.1 ml and is transferred to CMC media to be rotated while the water is fed, so that the medium can solidify evenly on the inner tube wall. The tube incubated for 2-3 days. The bacterial population can be calculated by the formula  
 Bacterial Population =  $n \times 10 \times 0.05 \times 0.1$  cfu/ml

#### 2.4. Data analysis

The data of this study are analyzed statistically according to the design used. the treatment effect continued with Duncan's Multi-Range Test [12].

### 3. Results and Discussions

#### 3.1. The effect of organic mineral supplementation of Ca, P and S on rumen fermentation condition

The condition of rumen fermentation is an indicator of the occurrence of metabolism in the rumen. Indicators of rumen fermentation conditions are illustrated from the concentrations of N-NH<sub>3</sub>, total VFA, partial VFA, pH and methane gas production. Based on the results of statistical analysis, it indicates that the use of TMF ammoniation as basal feed with organic mineral supplementation of Ca, P and S gave significant different effect ( $P < 0.05$ ) to N-NH<sub>3</sub> concentration, the total concentration of VFA, acetate and propionate concentration and methane gas production. While the concentration of butyrate and pH rumen indicate that there is no significant different effect ( $P > 0.05$ ) (Table 4 and Figure 1)

Further tests of N-NH<sub>3</sub> concentration parameters result in the use of 60% ammoniation of TMF in rations with organic mineral supplementation of Ca, P and S 1.5 times from NRC recommendations resulting in significantly higher N-NH<sub>3</sub> concentrations compared with treatment without supplementation and organic mineral supplementation of Ca, P and S 0.75 times from the recommendation of NRC, but it does not give different effect to the organic mineral supplementation of Ca, P and S according to recommendation of NRC. Supplementation of Ca, P and S organic minerals in accordance with NRC recommendations in this study resulted N-NH<sub>3</sub> Concentration significantly higher ( $P < 0.05$ ) than without supplementation, but gave the same effect as 0.75 times supplementation of NRC recommendations, while supplementation organic minerals Ca, P and S 0.75 times from NRC recommendations have the same effect as those without supplementation.

**Table 4.** The effect of organic mineral Ca, P dan S supplementation to rumen condition

The Condition of Rumen Fermentation	Treatments			
	P0	P1	P2	P3
N-NH <sub>3</sub>	5.24 <sup>a</sup>	7.55 <sup>ab</sup>	9.53 <sup>bc</sup>	13.91 <sup>c</sup>
Total VFA	80.18 <sup>a</sup>	105.99 <sup>b</sup>	133.76 <sup>c</sup>	165.31 <sup>d</sup>
Acetate	5.35 <sup>a</sup>	4.15 <sup>ab</sup>	4.14 <sup>ab</sup>	3.32 <sup>b</sup>
Propionate	1.08 <sup>a</sup>	0.98 <sup>ab</sup>	0.86 <sup>ab</sup>	0.45 <sup>b</sup>
Butyrate	0.25	0.25	0.15	0.16
Methane gas	2.53 <sup>a</sup>	1.95 <sup>b</sup>	1.93 <sup>b</sup>	1.63 <sup>b</sup>
pH	6.96	6.86	6.71	6.71

Note: Different superscripts on the same line indicated that the treatment gave a significantly different effect ( $P < 0.005$ )

At the total concentration of VFA the further test indicates that the organic mineral supplementation of Ca, P and S 1.5 times from the NRC recommendation is significantly higher ( $P < 0.05$ ) than the other three treatments, as well as the supplementation of NRC recommendations resulting in total concentration of real VFA ( $P < 0.05$ ) is higher than 0.75 times supplementation of the NRC recommendations and those without supplements, while organic mineral supplementation 0.75 times from the recommendation resulting the same total VFA concentration with no supplementation.

The result of Further test for partial VFA concentrations indicates that 1.5 times the organic mineral supplementation of the NRC recommendation result in higher concentrations of acetate and propionate ( $P < 0.05$ ) compared with no supplementation but it gives the same effect as 0.75 supplementation and NRC-recommended supplementation. An increase in N-NH<sub>3</sub> concentration with an increase in organic mineral supplementation of Ca, P and S is up to 1.5 times from NRC recommendation. This increase is related to the increase of crude protein digestibility in the rumen which

also increased with the presence of organic mineral supplements <sup>1</sup>Ca, P and S 1.5 times from the recommendation of NRC. N-NH<sub>3</sub> is the result of feed protein degradation, so if the protein digestibility value increases then N-NH<sub>3</sub> concentration will also increase. [13] explains that the degradation of protein sources is derived from feed ingredients and NPN (Nitrogen Non-protein) will produce N-NH<sub>3</sub> in the rumen. The N-NH<sub>3</sub> concentration in the rumen is an indication of the ability of rumen microbes to degrade proteins in the ration [14]. Based on the average N-NH<sub>3</sub> concentration obtained in this study is between 5.24-13.91 mM. The result of N-NH<sub>3</sub> concentration is sufficient for the N-NH<sub>3</sub> requirement for growth of rumen microbes and for livestock consumption. [15] state that the N-NH<sub>3</sub> concentration requirement for rumen microbial synthesis is 3.57-7.14mM, while Ref. [16] states that the optimum N-NH<sub>3</sub> rumen concentration for NDF degradation and consumption between 8-15 mg N / dl.

Total VFA concentrations are also increasing with the use of basal 60% TMF ammonia ration supplemented with Ca, P and S organic minerals in the ration. Increased supplementation of Ca, P and S organic minerals in rations to 1.5 times from Ref. [17] recommendations gives the highest total VFA concentration level of 165.31mM. This increase in total VFA concentration in the presence of organic mineral supplements Ca, P and S are associated with the increased digestibility of crude fiber, ADF, and NDF. This occurs because VFA is the final result of fibre digestibility in the ration. [18] states that the carbohydrate component in the ration of cellulose, hemicellulose, starch, and pectin digested with the final result of VFA

Pentosan is the main result of hemicellulosic degradation in the rumen. The hemicellulase enzyme hydrolyzes the hemicellulose into xylose and uronic acid. The uronic acid is also produced from the decomposition of pectin by pectinase and polygalactonidase. The second stage is simple sugar metabolism by rumen microbes intracellularly into pyruvic acid. Furthermore, pyruvic acid is converted to VFA [19]. [20] state that the activity of microbes in degrading fiber in the rumen will produce VFA. VFA is the final product of carbohydrate fermentation and is the main source of energy for ruminant livestock [21]. The total VFA concentrations obtained in this study ranged from 80.18mM-165.31mM were sufficient for ruminants, as [22] found that VFA needs for ruminants ranged from 70-130mM, while [23] VFA concentrations for rumen microbial growth are 80-160mM.

<sup>1</sup> In this study, there is decreased concentration of acetate and propionate by increasing 1.5 times organic mineral supplementation of Ca, P and S from [17] recommendation with basal diet 60% ammoniation of TMF, but the supplementation does not affect butyric acid concentration (Figure 1).

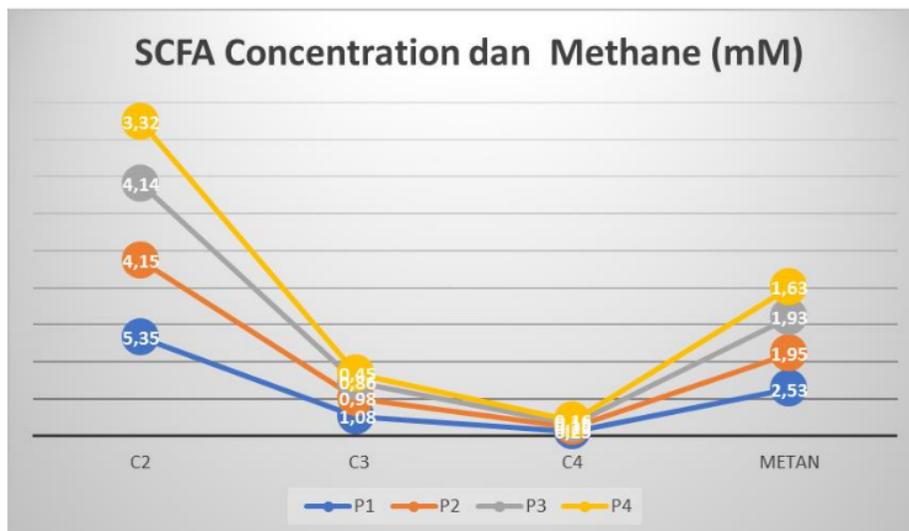


Figure 1. SCFA Concentration dan Methane production

The results of this study are in line with the study conducted by [24] which states that vitamin-mineral supplementation in beef cattle ration with grass basalt feed Gajah suppresses concentrations of acetate and propionate acids but not yet affect the concentration of butyrate. Decreased concentrations of acetate and propionate acids also result in a decrease in methane gas production. This is related to the population of rumen protozoa that is not significantly different, while in the metabolic process of methane production, protozoa play a big role. Ref. [25] states that fermentation of fiber with the acetic end result is closely related to methane gas production. The production of acetic acid and propionate has a major effect on methane gas. Methane gas production is closely related to the amount of acetic acid and butyric acid production. While propionate acid production is not followed by the production of H<sub>2</sub> and CO<sub>2</sub> [26].

### 3.2. Effect of organic mineral supplementation of Ca, P and S on Rumen Microbial Population

Rumen microbes play an important role in feed metabolism in the rumen, rumen microbes produce enzymes that play a role in the degradation of crude fiber and can also be used as a source of protein and vitamin synthesis for the livestock itself. Based on the research that has been done, the use of 60% ammonia TMF with organic mineral supplementation Ca, P and S on rumen microbes presented in table 5.

Based on statistical analysis, it is found that 60% ammonia of TMF as a basal diet with organic mineral supplementation of Ca, P and S in ration gave significant different effect ( $P < 0.05$ ) to cellulolytic bacteria population but did not give significant effect ( $P > 0.05$ ) to total bacteria and rumen protozoal populations

**Table 5.** The rumen microbial population receives 60% ammonia TMF basal feed with Ca, P and S organic mineral supplementation

Treatment	Cellulolytic Bacteria	Log cell/mn rumen liquid	
		Total bacteria	Protozoa
P0	7.46 <sup>a</sup>	9.09	5.78
P1	7.16 <sup>a</sup>	9.25	5.45
P2	8.55 <sup>b</sup>	9.40	5.63
P3	8.62 <sup>b</sup>	9.22	5.34

note: Different superscripts in the same column indicates significantly different treatment effect ( $P < 0.05$ )

Based on the further tests on parameters of cellulolytic bacteria population, it indicates that the use of a 60% TMF basal ration with Ca, P and S organic mineral supplementation in rations with 1 and 1.5 times of NRC recommendations is significantly higher in the population of the cellulolytic bacteria compared with those not supplemented and 0.75 times supplementation from NRC recommendations. The utilization of 60% ammonia of TMF in rations with organic mineral supplementation of Ca, P and S has not affected the total population of rumen microbes, but for cellulolytic bacteria, the population increased with organic mineral supplementation of Ca, P and S to 1.5 times recommended by NRC. This indicates the growth and positive response of organic mineral supplementation to cellulolytic bacteria. According to Ref. [27], P and S minerals are needed to digest cellulose. Further Ref. [28] states that minerals such as Ca, Mg, and S are proven by in vitro can stimulate cellulolytic bacterial activity. Minerals needed for cellulolytic bacteria activity are Ca, P, Mg, S, Zn, Co, and Mn [25]

Increased populations of cellulolytic bacteria can affect the digestibility of crude fiber and the production of VFA. It can be seen in Table 1 and 2 that there is an increase in the digestibility of crude fiber, NDF, ADF and VFA production by organic mineral supplementation of Ca, P and S in rations up to 1.5 times from NRC recommendations. Ref. [21] states that an increase in the population of cellulolytic bacteria can stimulate the digestion of complex carbohydrates better and faster. Complex carbohydrates from high-fiber feeds are converted to VFA by cellulolytic microbes and absorbed to fulfill energy requirements.

The total population of rumen bacteria from this study ranges from 109 cells /ml of rumen fluid. The total number of rumen bacteria obtained is still within the normal range of rumen bacteria in cattle. This is consistent with that stated by Ref. [25] the total bacteria in the rumen can reach 21 x 10<sup>9</sup> cells/ml of rumen fluid. Similarly, the population of cellulolytic bacteria obtained using TMF 60% ammoniation in the ration with organic mineral supplementation of Ca, P and S ranges from 107 to 108 cells/ml of rumen fluid

The rumen protozoa populations obtained from the use of TMF 60% ammoniation in rations with organic mineral supplementation of Ca, P and S ranges from 5.34 to 5.88 (log cells/ml of rumen fluid) or 105 cells/ml of rumen fluid. The results of this study are still within the normal number of population in cattle. as [26] suggests that the population of protozoa is in the range of 10<sup>5</sup>- 10<sup>6</sup>sel / ml of rumen fluid or about 40-50% of rumen biomass, but this may be affected by the composition of the feed consumed by the livestock itself. Protozoa is important for digestion to maintain pH. Usually, rapid rumen pH decreases when non-structural carbohydrates are fermented rapidly. This happens when the amount of bacteria is high enough. In the presence of protozoa, some bacteria are eaten so that the fermentable substance is somewhat slowly fermented and the pH does not decrease drastically [29]. Protozoa play a role in digesting low-quality forage and its contribution reaches 12-20% [30].

#### 4. Conclusion

Based on the research it can be concluded that: The use of 60% TMF ammoniation in the ration with organic mineral supplementation of Ca, P and S in the ration can improve the fermentative condition in rumen and population of cellulolytic bacteria, but non-significantly affect the total population of bacteria and protozoa and can decrease methane gas production in vitro. The organic mineral supplementation level of Ca, P and S 1.5 times from NRC recommendation with 60% of TMF ammoniation gives the best result to the nutrition digestibility, rumen fermentative condition and rumen microbe.

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