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S and P Mineral Supplementation of Ammoniated Palm Leaves as Ruminant Feed

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Abstract: This study aims to obtain the best combination of mineral S and P levels on ammoniation palm leaves in order to improve the digestibility of palm leaves as ruminant feed. The study was conducted starting on June 17, 2013 until October 17, 2013. Ammoniated palm leaf samples supplemented with mineral sulfur and phosphorus are subjected to Proximate and Van Soest analysis in Ruminant Nutrition Laboratory of the Faculty of Animal Science Andalas University, Padang. The method used in this study is an experimental method using a factorial randomized block design (3×3) with 3 replications. The first factor is the treatment of mineral Sulfur with 3 levels: S_O = 0.0%, S_1 = 0.2% and S_2 = 0.4% of DM. The second factor is the mineral P in 3 levels, ie P_0 = 0.0%, P_1 = 0.27% and P_2 = 0.54% of the DM. Parameters measured were dry matter digestibility (IVDMD) organic matter, crude protein, NDF, ADF, cellulose, hemicellulose and characteristic rumen fluid: pH of the rumen fluid, the levels of VFA, NH_3-N content of rumen fluid.

Key words: Palm leaves, mineral S and P supplementation, ammoniation, *in vitro*

INTRODUCTION

Availability of palm leaves quite a lot and has great potential to be used as forage, but its utilization as feed is still very limited. This is partly due to the low biological quality palm leaf. The results of the analysis showed palm leaf nutrient content dry matter 54.12%; organic matter 89.86%, crude protein 8.51%, crude fiber 28.48%; NDF 59.11%; ADF 42,87%; cellulose 24.69%; hemicellulose 16.24% and lignin 14.21%. The high lignin content causes low digestibility and palatability (Widjaja and Utomo, 2001). Efforts to optimize feed utilization of palm waste have focused on processing techniques, physical, chemical, biological, or a combination thereof. Treatment alone was only a small response to the increase in digestibility. Therefore, efforts to increase the digestibility of fibrous feeds also should be combined with efforts to optimize bioprocess in the rumen through the rumen microbial population increase (Warly *et al*., 1998).

Mineral sulfur (S) and phosphorus (P) is a mineral that is essential for microbial protein synthesis. The content of both minerals is very low and often deficient in the waste feed. This will negatively affect microbial protein synthesis and degradation of nutrients. Both mineral supplementation is expected to support the growth and development of optimal rumen microbes, which in turn will improve feed digestibility in the rumen and increase the supply of microbial protein to ruminants. S and P mineral is an essential component for the synthesis of amino acids that lack the S (methionine, cystine and cysteine), in addition to the S also play a role in the formation of vitamins and biotin. In *in-vivo* S inorganic form of ammonium sulphate can improve rumen microbial growth, growth in cows and digestibility of the ration (Erwanto, 1995).

In addition to the S minerals, mineral P is also needed for the synthesis of microbial protein. Phosphorus is needed by all cells to maintain the integrity of microbes mainly from the cell membrane and cell wall, a component of nucleic acids and part of the high-energy molecules (ATP, ADP, etc.). Specifically, P is required as an essential element for cell wall especially celulolisis who seem to have a higher P requirement than hemicelulolisis and amylolyses (Komisarczuk and Durrand, 1991).

This study aimed to obtain a combination level of sulfur minerals (S) and phosphorus (P) is the best for improving the digestibility of processed palm leaves as ruminant feed. The benefits of this research is to increase the diversity of feed materials by utilizing oil palm waste potentially abundant as well as a ruminant feed alternative solutions to address the problem of inadequate forage and environmental improvement. In addition, the results of this study may also improve the welfare of society and the improvement of workforce expansion. It is expected that the results of this study for the development of science in general and science in particular farm.

MATERIALS AND METHODS

Materials: The research material is ammoniated palm leaf from the best results of phase 1 study, sulphur as a source of mineral S, SP-36 fertilizer as a source of mineral P, as donors of rumen fluid microbes and Mc Dougall's solution as a buffer. Equipment used is: machetes, balance, autoclave, plastic bags, oven for drying materials, milling machines for grinding material prior to analysis, *in-vitro* devices, digital pH meter to

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measure the pH of the rumen fluid and set of laboratory equipment for Proximate analysis, Van Soest, VFA and NH_3-N.

Methods: The method used in this study is an experimental method using a factorial Randomized Block Design (3×3) with 3 replications. As the first factor is the treatment of mineral S with 3 levels: S_O = 0.0%, S_1 = 0.2% and S_2 = 0.4% of DM. The second factor is the mineral P in 3 levels, ie P_O = 0.0%, P_1 = 0.27% and P_2 = 0.54% of DM. Design model used according to Steel and Torrie (1991) as follows:

$$Yijk = \mu + Ai + Bj + Kk + (AB)\ ij + ijk\epsilon$$

where, differences between treatments were tested by Duncan's Multiple Range Test (DMRT).

Research Procedures: Samples were prepared in Erlenmeyer and add mineral S and P, respectively as 0% for S_1, 0.2% for S_2 and 0.4% for S_3 and 0% for P_1, 0.27% for P_2 and 0,54% for P_3. Minerals and sample were mixed evenly. Furthermore, mixture of Mc.Dougall solution and rumen fluid were added flushed with CO_2 and incubated for 2×24 h in a water bath shaker. After fermentation completed, filtrate and precipitate were separated and measured for its characteristics of rumen fluid while residue were subjected for analysis of Dry Matter, Organic Matter, Crude Protein, Neutral Detergent Fiber (NDF), Acid Detergent Fiber (ADF), cellulose and hemicellulose.

Parameters observed:

a) Digestibility of dry matter, organic matter, crude protein and fiber fractions: neutral detergent fiber, acid detergent fiber, cellulose and hemicellulose *in vitro* by the method of Tilley and Terry (1963).
b) Characteristics of rumen fluid (rumen fluid pH was measured using a digital pH meter, VFA concentration of rumen fluid by gas chromatography and distillation vapor and NH_3-N concentration was measured by the method of distillation according to Sthill Markhan (General Procedures Laboratories).

RESULTS AND DISCUSSION

Feed digestibility: Nutrients digestibility of ammoniated palm leaves supplemented with mineral S and P are presented in Table 1. Results of analysis of variance showed that there were significant interaction effect ($p<0.05$) between the S and P levels of minerals to organic matter digestibility, dry matter and crude protein of ammoniated palm leaves. Digestibility of ammoniated palm leaves supplemented with mineral S and P significantly increased compared to control (S_OP_O). It is a reflection that the supplementation of minerals could stimulate microbial growth and its development, so that the rumen microbial population and activity increased, resulting in increased digestibility of feed ingredients.

Table 1: Effect of mineral S and P supplementation on nutrient digestibility of ammoniated palm leaf (%)

		Treatments				
Digest.	P (S)	P_0	P_1	P_2	Av.	S.E.
DM	S_0	34.66^{Bc}	38.85^{Ac}	40.10^{Aab}	37.87	
	S_1	39.58^{Ab}	42.90^{Ab}	41.81^{Aa}	41.43	1.13
	S_2	43.70^{Ba}	47.40^{Aa}	38.01^{Cb}	43.04	
	Av.	39.31	43.05	39.97		
OM	S_0	44.41^{Bc}	48.20^{ABb}	51.64^{Aa}	48.08	
	S_1	48.60^{Ab}	52.14^{Ab}	51.71^{Aa}	50.82	1.32
	S_2	54.30^{Aa}	57.74^{Aa}	47.80^{Ba}	53.28	
	Av.	49.10	52.69	50.38		
CP	S_0	46.92^{Ab}	47.30^{Ab}	49.87^{Aa}	48.03	
	S_1	47.04^{Ab}	49.10^{Ab}	47.56^{Aa}	47.90	1.59
	S_2	52.17^{Aa}	55.81^{Aa}	46.85^{Ba}	51.61	
	Av.	48'71	50.74	48.09		

Values with different superscripts in the row (capital letters) and columns (lower case) are significantly different ($p<0.05$)
Av: Average, Digest: Digestibility

The highest digestibility obtained in the treatment of S_2P_1 that is supplementation of 0.4% S and 0.27% P of dry matter in the amount of 47.40, 57.74 and 55.81%, respectively for the digestibility of dry matter, organic matter and crude protein. In this treatment increased digestibility of dry matter, organic matter and crude protein respectively 36.75, 30.01 and 18.95% compared to controls (S_OP_O). This illustrates the presence of an optimal balance of nutrients to support the growth and activity of rumen microbes, which in turn increases the digestibility of feed. The lowest digestibility of nutrients were obtained in the control treatment (= without supplementation S_OP_O) that is equal to 34.66, 44.41 and 46.92%, respectively for the digestibility of dry matter, organic matter and crude protein.

Sulfur (S) and phosphorus (P) is a mineral that is essential for microbial growth. According to Hungate (1966) microflora in the digestive tract requires nutrients including minerals. Sulfur is an important component of rumen bacteria and is required for the synthesis of microbial protein. The P minerals are needed by all cells to maintain the integrity of microbes, especially the cell membrane and cell wall (Komisarczuk and Durrand, 1991).

In this study, increased levels of S from 0 to 0.4% of the dry matter able to increase the digestibility of nutrients. However, increased levels of P from P_1 (0.27%) to P_2 (0.54%) no longer increases the digestibility even decreasing so that the equivalent level of control in combination S_2P_2. S showed increased levels of mineral nutrient digestibility is higher than the increase in the level of P. This is due to the effect of ammoniation which causes high levels of N in the material thus increasing the need for synthesis of microbial protein S. Supplementing P also increases the digestibility of nutrients. S and P minerals play an important role in the digestion of fiber fraction. S and P mineral supplementation in this study were able to increase fiber digestibility fractions. Effect of mineral supplementation on the S and P digestibility of fiber fractions are presented in Table 3.

Results of analysis of variance showed that there were significant interaction ($p<0.05$) between the levels of the mineral sulfur and phosphorus on digestibility of the fiber fraction. The highest digestibility of fiber fractions obtained at S_2P_1 treatment that is a combination of the level of S = 0.4 and P = 0.27% of the dry matter in the amount of 47.40, 45.73, 53.01 and 57.88%, respectively for NDF digestibility, ADF, cellulose and hemicellulose, while the lowest one was obtained in the control treatment (S_0P_0) equal to 34.02, 29.32, 40.82 and 49.61%, respectively for the digestibility of NDF, ADF, cellulose and hemicellulose. At S_2P_1 treatment increased the digestibility of fiber fractions of 39.37, 55.97, 29.86 and 16.67%, respectively for NDF, ADF, cellulose and hemicellulose compared to the control treatment (S_0P_0).

Increased digestibility of fiber fractions in this study reflects that sulfur and phosphorus mineral supplementation has a positive effect on the growth and activity of fiber-digesting microbes in the rumen. This is in accordance with the opinion of Komisarczuk and Durrand (1991) explains that sulfur is important for fiber digestion in the rumen, which is sufficient to optimize the sulfur supply digestibility of cellulose through a specific stimulation of cellulolytic bacteria, ciliate protozoa and fungi activity of anaerobic rumen. While phosphorus is specifically required for the digestibility of the cell wall constituent, especially for cellulolysis who seem to have higher phosphorus requirements than hemicellulose and amylolysis. This study shows the digestibility of the fiber fraction mainly ADF increased by 55.97% from 29.32 to 45.73%.

Characteristic of rumen fluid: In general, three main factors can be used as a criterion in assessing the condition that the rumen pH, total VFA and NH_3 concentrations of rumen fluid. The results of measurements of pH, total VFA and NH_3 concentrations in this study are shown in Table 2.

Optimum rumen conditions for microbial activity and growth is a necessary condition that must be met to support high livestock production. Results of analysis of variance showed that sulfur and phosphorus mineral supplementation did not affect significantly ($p<0.05$) on pH and total VFA production but significantly ($p<0.05$) on rumen fluid NH_3-N. Rumen fluid pH values obtained ranged from 6.60 to 6.87. Near-neutral pH value is obtained due to the use of artificial saliva as buffer still able to maintain stable conditions that influence the activity of rumen fermentation. This is in accordance with the opinion of Church (1988) that saliva acts as a buffer to maintain the stability of rumen fluid. In addition, this value is due to the balance of fermentation products that VFA and NH_3-N. High or low pH of the rumen fluid is determined by the type of feed, the time available for the rumen microbes to do the fermentation of feed, the capacity of the buffer system and the levels of NH_3-N and total VFA rumen fluid, so that the pH of the rumen may differ at times before and after eating. PH values obtained in this study are in optimal condition to support the growth and activity of rumen microbes. This is in accordance with the opinion of Church (1997) that range from 5.5 to 7.2 and the pH of the rumen can normally is around 6.0 to 7.0.

Production of total VFA in this study ranged from 98.14 to 119.51 mM. Sulphur and phosphorus mineral supplementation did not affect significantly ($p>0.05$) on total VFA production, although an increase in the digestibility of nutrients. VFA is a dynamic element fermentability amount depends on the material,

Table 2: Effect of supplementation of mineral S and P on ammoniated palm leaves on characteristic of rumen fluid

Char. of Rumen Fluid	Treatment P (S)	P_0	P_1	P_2	Av.	S.E.
	S_0	6.61	6.60	6.65	6.62	
pH	S_1	6.79	6.84	6.84	6.82	0.04
	S_2	6.65	6.87	6.67	6.73	
	Av.	6.68	6.77	6.72		
	S_0	119.51	113.91	109.98	114.47	
VFA (mM)	S_1	98.14	105.13	106.35	103.21	6.41
	S_2	106.58	108.87	107.54	107.66	
	Av.	108.08	109.30	107.96		
	S_0	56.81^{ABa}	53.52^{Ba}	62.89^{Aa}	57.74	
NH_3-N	S_1	58.91^{Aa}	58.97^{Aa}	57.85^{Aab}	58.58	2.83
(mg/100	S_2	63.57^{Aa}	59.73^{ABa}	52.51^{Bb}	58.60	
mL)	Av.	59.76	57.41	57.75		

Values with different superscripts in the row (capital letters) and columns (lower case) are significantly different ($p<0.05$)

Chara: Characteristic, Av: Average

Table 3: Effect of S and P supplementation on digestibility of fibre fractions of ammoniated palm leaves

Digst.	Treatments P (S)	P_0	P_1	P_2	Av.	S.E.
N D F	S_0	34.01^{Bc}	39.71^{Ac}	41.45^{Aab}	38.39	
	S_1	39.23^{Bb}	44.53^{Ab}	42.88^{Ba}	42.21	1.41
	S_2	44.09^{Aa}	47.40^{Aa}	28.99^{Bb}	40.16	
	Av.	39.11	43.88	37.77		
A D F	S_0	29.32^{Bc}	34.89^{Ab}	36.41^{Aa}	33.54	
	S_1	33.57^{B}	39.98^{Ab}	38.17^{Ba}	37.24	1.64
	S_2	39.38^{Aa}	45.73^{Aa}	34.25^{Ba}	39.79	
	Av.	34.09	40.20	36.28		
Cellulose	S_0	40.82^{Bb}	47.56^{Ab}	49.04^{Aa}	45.81	
	S_1	46.25^{Bb}	56.91^{Ab}	50.51^{ABa}	51.22	1.89
	S_2	50.51^{Aba}	53.01^{Aa}	46.85^{Ba}	50.12	
	Av.	45.86	52.49	48.80		
Hemi	S_0	49.61	54.93	57.91	54.15	
Cellulose	S_1	57.82	58.76	58.54	58.37	2.26
	S_2	59.77	57.88	55.04	57.56	
	Av.	55.73	57.19	57.16		

Values with different superscripts in the row (capital letters) and columns (lower case) are significantly different ($p<0.05$)

Av: Average, Digest: Digestibility

absorption and utilization in the rumen wall by rumen microbes (Warly *et al*., 1998). VFA production is highly dependent on a variety of carbohydrates contained in a material. The increased total VFA production also means increased carbohydrate fermentation in the rumen (Baldwin and Denham, 1979). Total VFA production obtained in this study could support the growth and activity of rumen microbes, according to that described by Van Soest (1982) total VFA required for rumen microbial growth and activity ranged from 80-160 mM.

NH_3-N is the result of the digestibility of protein or NPN compounds by rumen microbes. NH_3-N concentration obtained in this study ranged from 52.51 to 63.57 mg/100 mL rumen fluid. NH_3-N concentration is strongly influenced by the type of feed, the solubility of nitrogen and protein digestibility levels, the concentration of nitrogen ration, time after feeding, the rate of use of nitrogen for the rumen microbial biomass, absorption of NH_3-N or urea recycling and bacterial nitrogen rumen (Djajanegara, 1979).

In this study, the concentration of NH_3-N control treatment (S_0P_0) that ammoniated palm leaves without mineral supplementation was 56.81 mg/100 ml of rumen fluid, this value was consistent with the results of phase 1 that was 58, 72 mg/100 ml rumen fluid. Overall concentrations of NH_3-N in this study were above the optimal concentration for rumen microbial growth as reported by Satter and Slyter (1974) that the concentration of NH_3 in the rumen varies between 0-130 mg/100 mL rumen fluid, whereas the minimum levels for synthesis optimal rumen microbial protein was 5 mg/100 mL of rumen fluid. While Mehrez *et al*. (1977) and Perdoks *et al*. (1988) reported that concentrations of NH_3-N is required for consumption, digestibility and microbial protein synthesis is the maximum range between 10-23 mg/100 mL of rumen fluid.

Conclusion: Results showed that mineral supplementation of S: 0.4% and mineral P: 0.27% of dry matter digestibility which gives the highest compared to the other. The in-vitro digestibility: dry matter (47.40%), organic matter (57.74%), crude protein (55.81%), NDF (47.40%), ADF (45.73%), cellulose (53.01%), hemicellulose (57.88%). Rumen fluid characteristics: pH (6.87), VFA (108.87 mM) and NH_3-N (59.73 mg/100 mL).

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