

# ***In vitro* Fermentation and Bacterial Protein Synthesis in the Different Diets Supplemented with Lerak Extract plus Mineral (Ca, P, Mg, S)**

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## **Abstract**

*Bacterial protein supply is usually low in animals fed with high forage ration because of the lack of sources of minerals in the rumen. The aim of this study was to evaluate the use of lerak extract plus mineral (Ca, P, Mg, S) on fermentation and bacterial protein synthesis in the in vitro fermentation with different ratios of forage and concentrate. The design of experiment was factorial block design (3x3) with 2 factors which were: ratio of forage and concentrate (70:30, 50:50, 30:70) as the first factor and the type of supplements (0, 1mg/ml lerak extract and 1mg/ml lerak extract + minerals) as the second factor. Total volatile fatty acid (VFA), NH<sub>3</sub> concentration, population of protozoa, and bacterial protein synthesis were measured at 4 h incubation. Dry matter and organic matter digestibility were evaluated after 48 h incubation. The result showed that there was no interaction effect between ratio of forage to concentrate and the type of supplements. The different ratios of forage and concentrate had no effect on dry matter and organic matter digestibility, and NH<sub>3</sub> concentration. The increase of concentrate ratio in the diet reduced population of protozoa, but increased total VFA and bacterial protein synthesis. The addition of 1 mg/ml lerak extract without minerals significantly decreased ( $P < 0.05$ ) population of protozoa and increased ( $P < 0.05$ ) bacterial protein synthesis but no effect on dry matter and organic matter digestibility, NH<sub>3</sub> concentration, and total VFA production compared to the control. However, the addition of lerak extract plus mineral (Ca, P, Mg, S) had no effect on all parameters measured. In conclusion, bacterial protein synthesis increased by supplementation of lerak extract without mineral addition.*

*Keywords: bacterial protein synthesis, fermentation, mineral, Sapindus rarak*

## Introduction

Rumen microbial population has an important role in the digestion of feed fiber by ruminants. The rumen has a lot of variety of microbial communities such as protozoa, bacteria, fungi, and viruses. Interaction between protozoa and bacteria sometime has disadvantage because of predation of bacteria by protozoa. This predation can cause the reduction of bacterial population and affect the growth of ruminants. It has been known that microbial protein in the rumen, especially bacterial protein, is the major good quality protein resource for ruminants (Pathak, 2008).

Bacterial protein supply is usually low in animals fed by high forage ration because of the nitrogen/protein deficiency in the diet. In addition, forage based diets often lack of some minerals required for the synthesis of microbial protein in the rumen. To overcome this problem, a strategy is required to improve the quality of forage with mineral supplementation and to reduce the population of rumen protozoa. Defaunation by using whole fruit lerak (*Sapindus rarak*) extract modified bacterial composition and increased the growth of some bacteria, especially *Prevotella ruminicola* and *Ruminococcus albus*, and stimulated propionate production (Suharti *et al.*, 2011). Our previous *in vivo* study showed that the use of lerak extract for beef cattle with high forage diets increased VFA production without a significant increase in microbial protein synthesis (Suharti *et al.*, 2010) which may be due to mineral deficiency in the high forage based diet, especially Calcium (Ca), Phosphor (P), Magnesium (Mg) and Sulfur (S).

This study was conducted to evaluate the use of lerak extract plus mineral (Ca, P, Mg, S) on fermentation and bacterial protein synthesis in the *in vitro* fermentation with different ratios of forage and concentrate.

## Materials and Methods

### *Whole Fruit Lerak Extract Preparation*

The lerak fruits (including seed) were harvested from Central Java Indonesia. Whole fruit lerak extract was extracted by using maceration method (Wina *et al.*, 2006). The extract was freeze-dried the freeze-dried extract was dissolved in distillate water just before being used as extract of whole fruit lerak

### *In vitro Fermentation*

*In vitro* fermentation was conducted according to Tilley and Terry method (1963). The rumen fluid for this experiment was obtained before morning feeding from the rumen of fistulated Ongole crossbred beef cattle fed commercial concentrate and elephant grass (50:50, DM). The rumen fluid was squeezed through a double-layer cheesecloth for *in vitro* experiment. The commercial concentrate consisted of rice bran, molasses, bread industry waste, groundnut meal, cassava waste,

and wheat pollard. Elephant grasses were harvested from the Faculty of Animal Science Farm, Bogor Agricultural University, and then were dried in the oven and milled. The design of experiment was factorial block design (3x3) with 2 factors i.e., ratio of elephant grass and concentrate (70:30, 50:50, 30:70) as the first factor and type of supplements (0, 1mg/ml lerak extract and 1mg/ml lerak extract + minerals mix) as the second factor. Mineral mix was composed of Ca 0.54%, P 0.37%, Mg 0.23%, and S 0.1%. The levels of lerak extract used in this experiment were based on our previous study showing that the addition of 1 mg/ml lerak extract increased fermentation activities. Five hundred milligrams of substrate (Concentrate, elephant grass and supplement) according to the treatments were put into a 100 ml fermentation tube. Forty milliliters of McDougall buffer was added, followed by 10 ml of rumen fluid. The McDougall buffer contained, per 6 liters, NaHCO<sub>3</sub> (58.8 g), Na<sub>2</sub>HPO<sub>4</sub>·7H<sub>2</sub>O (42 g), KCL (3.42 g), NaCl (2.82 g), MgSO<sub>4</sub>·7H<sub>2</sub>O (0.72 g), CaCl<sub>2</sub> (0.24 g) and H<sub>2</sub>O. The mixture was stirred and flushed with O<sub>2</sub>-free carbon dioxide. The tubes were then sealed with rubber corks fitted with the gas release valve. All fermentation tubes were incubated in a shaker water bath at 39°C.

#### *Sampling and measurement*

Total volatile fatty acid (VFA), NH<sub>3</sub> concentration, protozoal population, and bacterial protein synthesis were measured from liquid sample taken at 4 h incubation. Dry matter and organic matter digestibility were evaluated after 48 h incubation. After 4 h of incubation, samples of rumen aliquot were taken for protozoa counting under a microscope (Ogimoto & Imai, 1981). The contents of fermentation tubes were shaken and 0.5 ml aliquot was mixed with 0.5 ml methyl green formaldehyde saline solution containing 35% formaldehyde, distilled water, methyl green and NaCl. The stained sample was kept at room temperature and the population of protozoa was counted directly by using a counting chamber under a microscope (40×). Ammonia concentration was analyzed by using micro diffusion Conway and total volatile fatty acid production was analyzed by steam distillation (General Laboratory Procedure).

Microbial protein synthesis was determined based on Lowry assay according to Makkar *et al.* (1981). The strained rumen liquor was shaken in a magnetic stirrer (400 rpm) for 45 s to remove the microbes adsorbed on the feed particles. It then was centrifuged at 408 × g for 5 min for removing protozoa and remaining feed particles. Aliquots of 10 ml of rumen liquor, obtained after removing the feed particles and protozoa, were taken, and 2.5 ml of 64.5% TCA and 3.8 ml of 2/3 N sulfuric acid were added to each sample. Aliquots were then centrifuged at 15000 rpm for 20 min. Supernatants were discarded, and cells obtained were washed with McDougall's buffer and the mixtures were then centrifuged. The precipitates obtained after washing with distilled water were suspended in 30 ml of .25 N NaOH, heated in boiling water bath for 10 min, and protein was estimated according to Lowry method (Lowry's *et al.*, 1951).

### *Statistical Analysis*

Statistical analysis of the data was carried out by ANOVA using General Linear Procedure. Computation was performed using SPSS 13.0 for windows evaluation version.

## Results and Discussion

### *Population of Protozoa*

The population of protozoa were significantly reduced ( $P < 0.05$ ) with the addition of lerak extract. Supplementation of mineral mix to the lerak extract did not affect the population of protozoa as compared to the control treatment at 4 h incubation. The different ratios of forage to concentrate did not affect the population of protozoa (Table 1). There was no interaction effect between the addition of supplement (lerak extract or lerak extract plus mineral supplementation) and ratio of forage to concentrate on the population of protozoa.

The reduction of protozoal population was caused by saponin content in the lerak extract. It is known that saponin could inhibit the growth of protozoa due to binding activity of saponin to sterol that composed protozoal membrane (Patra *et al.*, 2006). Among all rumen microbes, protozoa are almost susceptible to saponin-induced changes in cell membrane properties (Moss *et al.*, 2000). Hu *et al.* (2005) reported the reduced protozoal numbers was due to the saponin treatment. The addition of mineral mix (Ca, P, Mg, & S) to lerak extract did not significantly reduce protozoal population compared with lerak extract alone. These may be due to the presence of mineral that could stimulate the growth of protozoa and improve protozoal survival.

### *Bacterial Protein Synthesis (BPS)*

There was no interaction effect between the addition of lerak extract supplementation and the different ratios of forage to concentrate on bacterial protein synthesis. The addition of lerak extract or lerak extract + mineral increased bacterial protein synthesis significantly ( $P < 0.05$ ). The different ratios of forage to concentrate also affected bacterial protein synthesis significantly ( $P < 0.05$ ). Bacterial protein synthesis increased when the level of concentrate was increased in the ration (Table 1).

The increased bacterial protein synthesis may be due to the reduced protozoal population in the presence of lerak extract supplementation. Protozoa has an important role in the turnover of microbial biomass. The digestion of bacteria by protozoa leads to a direct decline of efficiency of microbial growth. It was known that protozoa often digested bacteria to fulfill their protein requirement (Guiterez, 2007). Inhibition of protozoal growth, allows some bacteria to grow and increase bacterial protein supply in the rumen.

Table 1. Protozoal population, bacterial protein synthesis and fermentation characteristic with the addition of lerak extract or lerak extract + mineral in different diets

Parameters	Type of Supplementation			Diet Ratio (F:C)		
	0	lerak extract	lerak extract + mineral mix	70:30:00	50:50:00	30:70
Protozoa (Log 10 CFU)	4.37±0.09 <sup>a</sup>	4.18±0.15 <sup>b</sup>	4.24±0.05 <sup>a</sup>	4.34±0.06	4.27±0.09	4.19±0.06
BPS* (mg/10 ml)	95.90± 9.57 <sup>a</sup>	137.49±20.92 <sup>b</sup>	114.39±24.61 <sup>ab</sup>	92.38±29.12 <sup>a</sup>	119.66±24.14 <sup>ab</sup>	135.70±12.18 <sup>b</sup>
NH <sub>3</sub> (mM)	8.76±1.07	9.82±0.78	10.08±1.22	8.60±0.29	9.58±1.11	10.48±1.22
Total VFA (mM)	158.08±14.16	148.55±8.87	138.51±15.27	148.88±5.84 <sup>a</sup>	128.81±7.98 <sup>b</sup>	167.45±8.87 <sup>a</sup>
DMD (%)	57.86±1.73	55.48±3.11	55.26±1.4	54.16±3.27	56.90±1.14	57.53±3.36
OMD (%)	60.75±1.44	60.20±2.23	54.27±0.88	54.89±3.39	55.99±0.91	59.23±1.28

\*BPS=Bacterial Protein Synthesis, F= Forage, C= Concentrate

Means in the same row with different letters are significantly different (P<0.05).

The different diets also affected the bacteria protein synthesis. Bacterial protein synthesis tended to increase in line with the increase in concentrate ratio in the rumen. This may be due to the sufficiency of energy protein ratio (C/N) in the higher concentrate ratio that stimulates bacterial protein synthesis.

#### *Fermentation Characteristic*

**Ammonia Concentration.** At 4 h of incubation, there was no interaction between the addition of supplement (lerak extract or lerak extract plus mineral supplementation) and the ratio of forage to concentrate on ammonia concentration. Ammonia concentration was not affected by either lerak extract or lerak extract + mineral supplementation or different ratios of forage to concentrate (Table 1). The addition of saponin from lerak extract did not affect protein degradation in the rumen.

**Total VFA Production.** The addition of lerak extract or lerak extract + mineral did not affect total VFA production. However, total VFA production was significantly affected by forage:concentrate ratio in the diet. Total VFA production increased when the level of concentrate in the ration was 70%. There was no interaction effect between lerak extract supplementation and different ratios of forage to concentrate (Table 1). The higher concentrate ratio in the diets could stimulate VFA production by rumen microbe as concentrate feed contains high carbohydrate that a major substrate for VFA production.

**Dry Matter and Organic Matter Digestibilities.** There was no interaction effect between the addition of lerak extract supplementation and the different ratios of forage to concentrate on dry matter and organic matter digestibilities. The addition

of lerak extract or lerak extract + mineral did not affect dry matter and organic matter digestibilities. The different ratios of forage to concentrate also did not affect dry matter and organic matter digestibilities (Table 1). These results showed that the reduction of protozoa caused by saponin did not alter the digestibility of feed in the rumen. Although protozoa have a role in feed degradation, inhibition of protozoa by saponin did not affect *in vitro* dry matter and organic matter digestibilities.

## Conclusion

There was no interaction effect between ratio of forage to concentrate and the type of supplements on protozoal population and bacterial protein synthesis. The addition of 1 mg/ml lerak extract with or without minerals decreased protozoal population and increased bacterial protein synthesis without effect on dry matter and organic matter digestibilities, NH<sub>3</sub> concentration, and total VFA production as compared to control. The different ratios of forage and concentrate had no effect on dry matter and organic matter digestibilities and NH<sub>3</sub> concentration. The increased concentrate ratios in the diet reduced protozoal population but increased total VFA production and bacterial protein synthesis. Bacterial protein synthesis increased by supplementation of lerak extract without mineral addition.

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