Proceeding

The 2nd International Seminar

Feed Safety for Healthy Food

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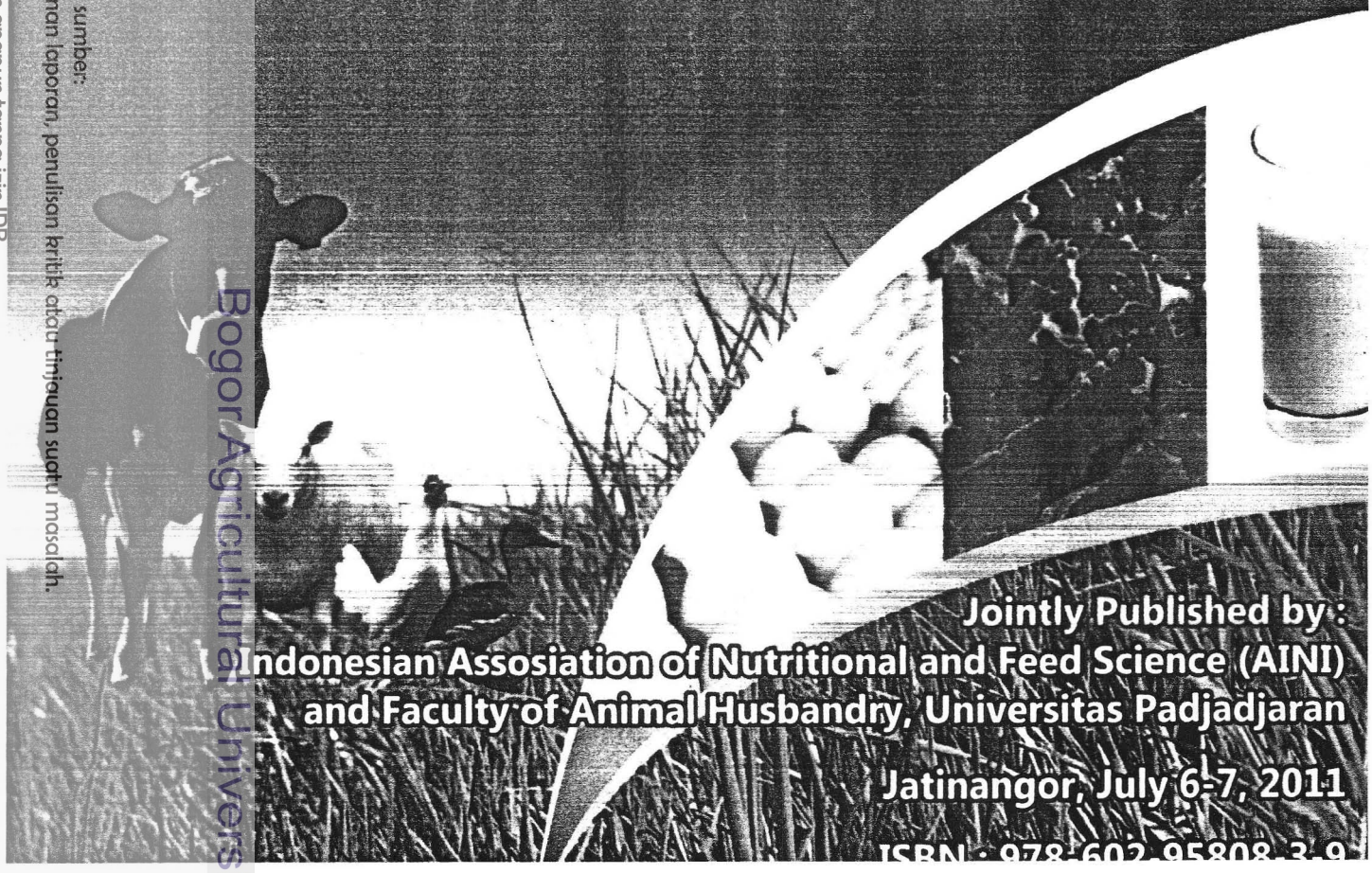

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"Feed Safety for Healty Food"

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Proceeding

The 2nd International Seminar
"Feed Safety for Healty Food"

Keynote Speaker :

Director General of Animal Husbandry and Animal Health

Main Speakers :

Prof. Fr. Jurgen Zentek (Berlin, German)

Prof. Abdul Razak Alimon (Malaysia)

Dr. Kevin Liu (Singapore)

Prof. E. R. Ørskov, Ph D., FPAS, FRSE (Scotland)

Proceeding

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FOREWORD

We thank the Almighty Allah, the Most Gracious and the Most Merciful that the proceedings of the 2nd International Seminar, the 8th Biannual Meeting and 3rd Congress and Workshop of AINI with the theme "Feed Safety for Healthy Food" organized by Indonesian Association of Nutrition and Feed Science, Faculty of Animal Husbandry, Universitas Padjadjaran on 6 - 7 July 2011 have been completed.

These activities were to collect variety of scientific information with the purpose to collect scientific information about feed for a healthy food, to produce a draft policy on a national feed system and to make a scientific forum for Academics, Researchers, Practitioners of animal husbandry, Health and Policy makers. Scientific papers that were presented either in oral or poster stated in the proceedings.

Thanks go to all those who have provided both moral support or material so that this seminar can be carried out and the proceeding can be issued.

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Committee

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In vitro RUMEN ENZYME ACTIVITIES ON DIFFERENT RATIO OF FORAGE AND CONCENTRATE SUPPLEMENTED BY LERAK (*Sapindus rarak*) EXTRACT[1]

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ABSTRACT

Defaunation using lerak (*Sapindus rarak*) extract containing saponin showed that the depression of rumen protozoa and could alter bacterial composition and rumen enzyme activities. This experiment was designed to investigate the effect of lerak extract on the activities of rumen enzyme in the *in vitro* fermentation with different ratios of forage and concentrate. *In vitro* fermentation was conducted according to the method of Tilley and Terry (1963). The design of experiment was a factorial block design with 2 factors. The first factor was the ratio of forage and concentrate (90:10, 80:20, and 70:30 w/w) and the second factor was the level of lerak extract (0, 0.6, and 0.8 mg/ml). Specific rumen enzyme activities such as carboxymethylcellulase (CMCase), amylase and xylanse were measured at 4 h and 24 h incubations. The result showed that in the 4 h incubation, the addition of lerak extract at level 0.6 and 0.8 mg/ml increased ($P<0.05$) xylanase activity and tend to increase ($P<0.1$) carboxymethylcellulase activity but decreased ($P<0.05$) amylase activity with different ratio of forage to concentrate. In contrast, all rumen enzyme activities measured were similar at 24 h incubation. There was no interaction between ratio of forage to concentrate and level of lerak extract on rumen enzyme activities.

Keywords : *Lerak extract, ruminal fermentation, carboxymethylcellulase, xylanase, amilase*

INTRODUCTION

The growth of ruminants are influenced by rumen microbial in which nitrogen and energy are very important nutrients. Rumen microbes need nitrogen for protein synthesis and cell multiplication, while the microbes use energy from lignocelluloses and celluloses cell plant wall as energy sources. The presence of rumen microbes such

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as bacteria, fungi and protozoa allows ruminants to utilize grasses, legumes and agricultural wastes that have high lignocelluloses or cellulose.

Hydrolysis process of feed cellulose in the rumen involving bacteria, protozoa and fungi activities. Protozoa have important role in the providing of hydrolytic enzymes in the rumen. However, the protozoa are active proteolysis and engulf some bacteria as a nitrogen source (Gutierrez, 2007). *In vitro* studies suggested that engulfment and digestion of bacteria by protozoa is by far the most important cause of microbial protein turnover in the rumen (Wallace and McPherson, 1987). Therefore, suppression of protozoa population has been suggested to be an attractive way to optimize bacterial growth in the rumen and prevent the recycling of N between bacteria and protozoa, thus increase the efficiency of N metabolism in the rumen and stimulate the flow of microbial protein from the rumen.

Therefore, some researchers tried to reduce the population of protozoa in the rumen by using secondary metabolite such as saponin or use some acidic compounds. Saponins or saponin-like substances have been reported to have potency of suppressed growth of protozoa and change fermentation patterns in the rumen system (Makkar and Becker; 1997; Wang *et al.*, 1998; Benchaar *et al.*, 2008).

Previous study showed that the whole fruit lerak (*Sapindus rarak*) extract have high saponin (84.5%) and have activity to inhibit the growth of rumen protozoa (Suharti *et al.*, 2009; 2011). Even though, rumen protozoa also produce enzymes that essential for feed degradation so inhibition of protozoa may affect rumen enzyme activities such as amylase, cellulase and xylanase. However, few reports about effect of saponin on rumen enzyme activities are available. In this experiment, different ratio of forage and concentrate in the diets were used as substrates and were incubated in the presence of different levels of whole lerak fruit extract.

METHODS

In vitro Fermentation

The rumen fluid for this experiment was collected just before morning feeding from a ruminally fistulated Ongole crossbred beef cattle. The animal fed a diet consisted of native grass and commercial concentrate (50:50, DM). The rumen fluid was filtered through a double layer cheesecloth for *in vitro* experiment. The substrate for *in vitro* rumen fermentation was a mixture of concentrate feed and dried milled native grasses at different ratios. The concentrate mix (self mixing) comprised of soybean meal, coconut meal, cassava waste, wheat pollard, mollases, Dicalcium Phosphate (DCP), NaCl and $CaCO_3$. Native grasses were harvested from the surrounding area of Bogor Agricultural University farm (Indonesia), were dried in the oven and milled.

In vitro fermentation was conducted according to Tilley and Terry (1963) method. The design of experiment was Factorial Block Design with 2 factors. Factor 1 was the ratio of forage and concentrate (90:10, 80:20, 70:30) and factor 2 was the level of lerak extract (0, 0.6, 0.8 mg/ml). Level of lerak extract used in this experiment based on our previous study that showed an increased of feremntation characteristic at level 1 mg/ml. For this experiment, we evaluated some moderate level (0.6 and 0.8 mg/ml) for optimalization the used of lerak extract. The substrate (500 mg) was put into a 100 ml

fermentation tube. Forty ml of McDougall buffer was added, followed by 10 ml of rumen fluid. The McDougall buffer containing, per 6 liters, $NaHCO_3$ (58.8 g), $Na_2HPO_4.7H_2O$ (42 g), KCL (3.42 g), NaCl (2.82 g), $MgSO_4.7H_2O$ (0.72 g), $CaCl_2$ (0.24 g) and H_2O. The mixture was stirred and flushed with O_2-free carbon dioxide and the tubes were then sealed with a rubber cork fitted with the gas release valve. All fermentation tubes were incubated in a shaker waterbath at 39°C.

Sampling and measurement

The rumen liquor after 4 and 24 h incubations was transferred to 1.5 ml tube and mixed with 0.25 ml lysozyme solution (0.4 g/100 ml phosphate buffer, 0.1 M, pH 6.8) for enzyme extraction (Agarwal *et al.*, 2006). The contents were incubated at 40°C for 3 h. The samples were centrifuged at 24 000×*g* for 20 min at 4 °C and clear supernatant was used for the estimation of enzyme activities. The reaction mixture contained 1 ml phosphate buffer (0.1 M, pH 6.8), 0.5 ml carboxymethylcellulose (1.0 g/100 ml phosphate buffer) in 0.1 M phosphate buffer (pH 6.8) and 0.5 ml extracted supernatant for the estimation of CMCase. For xylanase activity, the reaction mixture contained 1 ml phosphate buffer, 0.5 ml xylan (0.25 g/100 ml phosphate buffer) and 0.5 ml extracted supernatant. The reaction mixtures were incubated for 60 min (CMCase) and 15 min (xylanase) at 39 °C. The reducing sugars thus released were estimated according to Miller (1959) using glucose and xylose (Sigma Chemical Company, USA) as standards. The enzyme activities were expressed as mol of reducing sugars released per min per ml under assay conditions (Patra *et al.*, 2006).

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

The addition of lerak extract 0.6 and 0.8 mg/ml increased ($P<0.05$) xylanase activity and tend to increase ($P<0.1$) carboxymethylcellulase (CMCase) activity, but decreased ($P<0.05$) amylase activity at 4 h incubation (Table 1). Different ratio of forage to concentrate did not affect rumen enzyme activities tested. There was no interaction between level of lerak extract and ratio of forage to concentrate. In contrast, at 24 h incubation there was no different of rumen enzyme activities in the presence of lerak extract up to level 0.8 mg/ml.

The increasing of xylanase and CMCase activities at 4 h incubation due to the change of bacterial composition in the presence of lerak extract. The result of denaturing gradient gel electrophoresis (DGGE) in our previous study of the effects of saponin (under process of publication) showed a new band and two other bands increased their intensity indicating the increase in several specific bacterial populations. Some of those bacteria, stimulated by lerak extract had high similarity to *Prevotellas* sp. Quantification of bacterial populations with real time PCR showed that the population of *Ruminococcus albus* seems to increase in the presence of lerak extract. *Prevotella*

ruminicola significantly increased in a dose dependent manner with the addition of lerak extract but did not show any significant increase with different types of diets (Suharti *et al.*, 2011).

The possibles role of *P. ruminicola* in the rumen ecosystem include the degradation and utilization of starch and of plant cell wall polysaccharides such as xylans and pectin (Hobson and Stewart, 1997). This may cause an increase in xylanase activity in this experiment. The tendency of increase in CMCase may be due to the increase of *R. albus* population in the addition of lerak extract (Suharti *et al.*, 2011). *Ruminococcus* albus is the most celullolytic bacteria in the rumen that degrade celullose from the diet.

The decreased of amylase activity in the presence of lerak extract may be associated with a reduced protozoal population due to saponin contained in the lerak extract. It has been known that protozoa also have important role in the starch digestion and produce amylase in the rumen. The reduction of protozoal population may affect amylase activity in the rumen.

All rumen enzyme activities measured were similar with control treatment at 24 h incubation in the presence of lerak extract at it may be due to the length of *in vitro* incubation time. The earlier reports indicate that at 24 h incubation. The specific activities of CMCase were not affected by saponin from some plant extracts, whereas specific activity of acetylesterase was reduced significantly ($P<0.05$) by the extracts (Patra *et al*, 2006). Wina *et al.* (2006) also reported that Sapindus (lerak) extract significantly depressed rumen xylanase activity in both trials and carboxymethylcellulase activity in the long-term (98 days) trial with sheep ($P < 0.01$). Hanim *et al.* (2011) observed that a decrease in cellulase activities was not affected by saponin at 72 h incubation. Eugene *et al.* (2004) observed that both CMCase and xylanase activities were reduced in the absence of protozoa.

CONCLUSIONS

The addition of lerak extract at level 0.6 and 0.8 mg/ml could increase xylanase activity and tend to increase carboxymethylcellulase activity but decreased amylase activity with different ratio of forage to concentrate at 4 h incubation. In contrast, all rumen enzyme activities measured were similar at 24 h incubation. There was no interaction between ratio of forage to concentrate and level of lerak extract on rumen enzyme activities.

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Table 1. Rumen enzyme activities of different ratio of forage and concentrate in the presence of lerak extract at 4 and 24 h incubation

Parameter	Forage:concentrate ratio			Level of lerak extract (mg/ml)			SEM
	90:10	80:20	70:30	0	0.6	0.8	
Amylase (µmol/ml/h)							
• 4 h	10.34	9.49	9.70	10.86[a]	9.37[b]	9.30[b]	0.28
• 24 h	11.61	11.38	10.75	11.77	11.27	10.70	0.44
CMCase (µmol/ml/h)							
• 4 h	5.21	5.09	4.96	4.73	5.12	5.41	0.19
• 24 h	5.63	5.87	5.30	5.56	5.50	5.74	0.18
Xylanase (µmol/ml/h)							
• 4 h	12.48	13.32	12.23	11.65[b]	12.65[a]	12.15[a]	0.48
• 24 h	12.30	12.52	12.75	12.59	13.09	11.90	0.55

Different superscripts on the same row represents a significant difference ($p<0.05$). CMCase=Carboxymethylcellulase. SEM =Stándar Error of Means