THE INFLUENCE OF KAPOK (*Ceiba pentandra*) SEED OIL SUPPLEMENTATION ON CELLULOLYTIC ENZYME AND RUMEN MICROBIAL FERMENTATION ACTIVITY OF LOCAL SHEEP

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ABSTRACT

This research was conducted to study the influence of kapok seed oil (KSO) supplementation on cellulolytic enzyme and microbial fermentation activity. Sheep rumen fluid was used as enzyme source and inoculant, whereas carboxymethylcellulose (CMC) was used as the substrate. There were 4 levels of KSO supplementation as treatment, i.e. : 0% (T0), 5% (T1), 10% (T2), and 15% (T3). Two measured variables were reduced sugar production rate and gas fermentation production. The data were analyzed by analysis of variance in completely randomized design. The result showed that reduced sugar production rate in T0, T1, T2 and T3 treatment groups were 2.58; 2.93; 2.08 and 1.58 mg/g CMC/minute, respectively, whereas gas production were : 15.97; 13.26; 10.54 and 7.57 mg/g CMC, respectively. Kapok seed oil supplementation up to 5% DM of cellulose substrate (CMC) did not influence the ruminal cellulolytic enzyme activity. The KSO supplementation level 10% - 15% decreased the ruminal cellulolytic enzyme activity.

Keywords : kapok seed oil, fermentation, rumen, polyunsaturated fatty acid, local sheep.

INTRODUCTION

The productivity and quality of sheep meat could be increased by polyunsaturated fatty acid (PUFA) source supplementation. Polyunsaturated fatty acid have two main roles in enhancing animal productivity, namely nutritional and biological roles (Jenkins, 1992; Sardesai, 1992). Polyunsaturated fatty acids were absorbed preferentially, by forming the chollesterylester and phospholipid. Absorption of those over the requirement for structural component, allows the PUFA bounded as triglyceride and available for energy source with high availability, indicated by short retention time in circulation system. Those also distributed and deposited as fat reservoir in adipose tissue, especially intramuscular adipose tissue, which can be mobilized when required (Christie, 1979; Palmquist and Jenkins, 1980). Plant oil as PUFA source was not bulky and rich in energy content, therefore it can be used as supplement to increases the feed energy density. The PUFA also can increase the energy efficiency via its effect on ruminal fermentation. Unsaturated fatty acid (UFA) depresses methanogenic bacteria, so that it reduces methane production and then increases the ruminal propionic acid proportion (Baldwin and Allison, 1983; Van Soest, 1994).

Several sources of PUFA can be used as feed supplement for ruminant, for example: kapok seed oil (KSO). Kapok seed oil is one of the potential source of UFA. According to Sarosa (1990), the proportion of PUFA in total lipid of KSO is 71.95%. Amount of 54.29% of those is linoleic acid, whereas oleic and linoleic acids are 43.50% and 2.21%, respectivelly.

Polyunsaturated fatty acid source supplementation (in this case is KSO) is an alternative technology which is expected can improve the ruminal energy metabolism efficiency as well as intermediary metabolism. On the other hand, PUFA supplementation can also inhibit the cellulolytic enzyme activity as well as fibrolytic microbes fermentation activity, as shown on decreasing of reducing sugar and gas fermentation production (Dijkstra *et al*., 2000). Based on both reasons, this research was conducted to obtain the information about the effect of KSO supplementation on ruminal fiber metabolism, through determination of cellulolytic

enzyme activity and microbial fermentation activity.

MATERIALS AND METHODS

This research included 2 experiments, namely cellulolytic enzyme activity assay through determination of reduced sugar production rate, and microbial fermentation activity assay which reflected by gas fermentation production. Prior to the experiment were conducted, the KSO was analyzed in fatty acids (FA) relative compostion, iodine number, and saponification number (Cabatit, 1979).

Ruminal Cellulolytic Enzyme Activity

This experiment consisted of 4 supplementation levels of unsaturated fatty acid source (KSO), namely 0% (T0); 5% (T1); 10% (T2), and 15%(T3), with 6 replications for each treatment group. The ruminal cellulolytic enzyme assay was conducted according to Bachruddin (1996). Carboxymethylcellulose (CMC) (10 mg/1.8 ml) as substrate was obtained from "Indrasari" chemicalia shop, Semarang. Rumen fluid of local sheep fed field grass was used as enzyme source, whereas KSO as supplement obtained from UD THT Pati, Central Java. Cytrate phosphate buffer solution pH 6, 0.2M was prepared at Biochemistry Nutrition Laboratory, Faculty of Animal Science, Gadjah Mada University, Yogyakarta. Four kinds of tube were prepared, namely ES (enzyme + substrate) tube with 4 levels of KSO supplementation, E (enzyme) tube, B (blank) tube, and S (substrate) tube. The ES tube filed with 0.2 ml enzyme source; 0.9 ml buffer solution, and 0.9 mg CMC. The E tube filed by 0.2 ml enzyme source; 0.9 ml buffer solution, and 0.9 ml aquadest. The B tube filed with 0.9 ml buffer solution and 1.2 ml aquadest, wheareas the S tube filed by 0.9 ml buffer solution; 0.9 mg substrate, and 0.2 ml aquadest. All of tubes were incubated on 50ºC for 30 minutes then were boiled for 10 minutes. Filtrate were analyzed by reducing sugar method according to Nelson – Somogyi (Tranggono *et al.*, 1989).

The collected data (reduced sugar production rate), was analyzed by analysis of variance, in completely randomized design. Difference test of mean between treatment was conducted by Duncan's Multiple Range Test (Astuti, 1981; Sugandi and Sugiarto, 1993).

Rumen microbial fermentation activity

The gas fermentation production which reflected rumen microbial fermentation activity, was measured according to Bachruddin(1996). The experimental material consist of CMC as substrate, KSO as supplemen, local sheep rumen fluid as innoculant, and cytrate phosphate buffer solution pH 6.50. There were 4 levels of KSO supplementation as treatment in this experiment, namely : 0; 5; 10 and 15%. Substrate and rumen fluid were filed into fermentor equipped by syringe. Each treatment group consist of 6 replications. Flushing for each fermentor with CO2 gas were conducted before covered, so that the fermentor tubes in anaerobe condition. Fermentor tubes were placed in incubator at 39oC for 36 hours. Gas production was observed in syringe each 6 hours.

RESULTS AND DISCUSSION

The result of KSO analysis as PUFA source showed that saponification number was 120.19 with iodine number was 54. It means that saponification of 1 g KSO required 120.19 mg KOH, wheareas the amounts of iodine which can be bound by 100 g KSO is 54 g. Iodine number depended on amounts of double bound or unsaturation rate of fatty acids in KSO (Tranggono *et al.*, 1989). The Result of KSO analysis completely showed in Table 1. Those indicated that 74.24% of total fatty acid content in KSO was PUFA. Linoleic Acid proportion was 62.32% from total UFA in KSO. Those values were slightly higher than those reported by Sarosa (1990) which showed that UFA proportion was 71.95%, whereas linoleic acid proportion was 54.26% from total FA content in KSO.

The influence of kapok seed supplementation on *in vitro* ruminal cellulolytic enzyme activity

Cellulolytic enzyme activity from local sheep rumen fluid was reflected by reduced sugar production rate with CMC as substrate. The reducing sugar was intermediary product of cellulose degradation, before producing the final products, such as: volatile fatty acids (VFA) and fermentation gas. It can be seen in Table 2 that the reduced sugar production data per minute, which indicated the reduced sugar production rate from CMC, catalyzed by ruminal cellulolytic enzyme. The average reduced sugar production

Table 1. Relative Proportion of Fattty Acids in Kapok Seed Oil (KSO)

Fatty acid component	Proportion (%)
Palmitic acid (C 16 : 0)	22.88
Stearic acid (C 18 : 0)	2.09
Oleic acid (C 18 : 1)	24.01
Linoleic acid (C 18 : 2)	44.86
Linolenic acid (C 18 : 3)	3.11
Another fatty acids	3.15

rate in treatment groups of T0, T1, T2, and T3, were : 2.58; 2.93; 2.08, and 1.58 mg/g CMC per minute, respectively.

KSO supplementations was significantly influenced ($P<0.05$) the reduced sugar production rate. Reduced sugar production rate decreased ($P<0.05$) caused by 10% KSO supplementation, those so much the bigger along with increasing of supplementation level (up to 15%). The rate of reduced sugar production from CMC without KSO supplementation was not significantly different from that supplemented by 5% of KSO. According to Jenkins (1992), lipid can inhibit ruminal cellulose degradation throughout several mechanism, such as lipid coating, direct antimicrobial effect, microbial population modification in related to cellulose degradation and decreasing of Ca which available to microbial function. In this experiment, the incubation temperature was 50°C and pH was 5.5, whereas the optimum temperature in rumen was 38°C, and pH was 7 (Baldwin and Allison, 1983). Those incubation condition inactivate microbes, therefore the catalytic process depended on the amount of enzyme which had existed when the incubation started.

Inhibition of CMC degradation was taken place because the lipid coated to substrate particle, therefore it reduced the direct contact between cellulolytic enzyme and substrate. It was effective in 10% KSO supplementation level, therefore the reduced sugar production rate decreased significantly. Imming *et al*. (1991) stated that the existence of free fatty acids in the mixture which consisted of ruminal cellulase enzyme and CMC, decreased the attachment between enzyme and substrate, therefore the cellulase enzyme activity decreased. The decreasing of cellulose enzyme activity was higher with the increasing of KSO supplementation level (up to 15%), which was reflected by the reduced sugar production rate in T3 treatment group. The KSO supplementation at 5% level did not influence the rate of reduced sugar production compared to without KSO supplementation. Those finding as reconciled to Byers and Schelling opinion (1988), who reported that feeding lipid up to 5% dry matter (DM) did not inhibit fiber biodegradation. Dijkstra *et al*. (2000) reported that degree of fiber degradation inhibition by lipid was also influenced by fiber content in feed. In their experiment, the increasing lipid supplementation from 4 to 8% in low fiber ration, decreased the fiber digestibility 5.3%, whereas in high fiberation only 1.6%. The fiber proportion in substrate used in this experiment was 100% (CMC), so that it did not result in altering the fiber degradation significantly, which is reflected by non significant reduced sugar production rate. Reduced sugar production rate per minute in T0 treatment group tended to be lower than T1 treatment group. Perhaps, the reverse event had been taken place, that reduced sugar production in T0 tended to be higher than T1, but those compound was readily hydrolyzed by saccharolytic enzyme. The faster reduced sugar production resulted in higher substrate concentration (in this case was reduced sugar). The increasing of substrate concentration

Table 2. The Average of Reducing Sugar Production and Ruminal Fermentation Ruminal Fermentation Gas Production from CMC

Variable	T0	T1	T2	T3
Reduced sugar production (mg/g CMC/minute)	2.58^{a}	2.93^{a}	2.08^{b}	1.58^{c}
Fermentation gas production (ml/g CMC)	15.97^{a}	13.26^{b}	10.54^{c}	7.57^{d}

a,b,c,d : The different superscript in same row indicates significantly difference ($P< 0.05$). T0 : KSO supplementation 0%; T1 : KSO supplementation 5%; T2 : KSO supplementation 10%; T3 : KSO supplementation 15%

increased the catalytic reaction velocity, therefore the detected reduced sugar in T0 treatment group tended to be lower than those in T1. Those fenomenon is in agreement with Michaelis–Menten law, which stated that increasing of substrate concentration with fix amount of enzyme concentration, will increase the enzymatic reaction velocity (Murray *et al*., 1997)

The influence of kapok seed oil supplementation on rumen microbial fermentation activity

Gas production data as a result of fermentation of CMC substrate supplemented by KSO, are shown in Table 2. The KSO supplementation decreased gas production per gram fermented CMC ($P<0.05$). The decreasing of fermented gas production was bigger along with the increasing of KSO supplementation level (up to 15%). Table 2 showed that gas production at 5% KSO supplementation level (T1) was lower than those without supplementation (T0) although reduced sugar production per minute did not significantly different. Those can take place, because UFA content in KSO depressed the methanogenic bacteria, so that methane gas production decreased (Baldwin and Allison, 1983; Byers and Schelling, 1988; Johnson *et al*., 2002; Fievez *et al*., 2003). A part of hydrogen gas which was not used for methane gas synthesis, was used for biohydrogenation of UFA, whereas another was used for propionic acid synthesis (Van Soest, 1994). Synthesis of 1 mole of propionic acid required 1 mole of hydrogen (Preston and Leng, 1987). The availability of hydrogen increased becaused methanogenesis inhibition, so that result in the increasing of propionic acid synthesis and decreasing of acetic acid production. According to Hungate (1966), to form acitic acid also produced CO_2 gas. Thus, the decreasing of acetic acid production also decreased CO_2 gas production. The decreasing of methane and CO_2 gas production, in turn decreased fermented gas production, that was reflected by gas production of T1 treatment group was lower than T0. The increasing of KSO supplementation level started at 10%, resulted in decreasing of gas production significantly ($P<0.05$). Those fenomenon can be occurred, because fiber degradation decreased up to 50% and fat supplementation level were lower than 10% (Jenkins, 1992).

The decreasing of those structural carbohydrate degradation was accompanied by the decreasing of methane, hydrogen, volatile fatty acids production. Baldwin and Allison (1983) stated that high fiber proportion in ration increased the acetic acid production in ruminal degradation. The production of 1 mole acetic acid was accompanied by 1 mole CO_2 gas and 2 atoms hydrogen (H) production. The atom H and CO_2 were methane gas precursor. Thus the decreasing of cellulose degradation by rumen microbes decreased CO_2 and methane gas production, which was reflected by the decreasing of total fermentation gas production. The increasing of KSO supplementation level (up to 15%) resulted in the decreasing of gas production ($P<0.05$). The depressing of methanogenic and cellulolytic bacteria which was accompanied by the decreasing of acetic acid/ propionic acid ratio, limited CO_2 as well as methane gas production, therefore it lowered ruminal fermentation gas production.

CONCLUSION AND SUGGESTION

Kapok seed oil supplementation up to 5% DM of cellulose substrate (CMC) did not influence the ruminal cellulolytic enzyme activity. The KSO supplementation level 10%-15% decreased the ruminal cellulolytic enzyme activity. In suggestion, KSO supplementation more than 5% DM need to be protected to avoid the decreasing of fibrous feed utility.

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