*Full Length Research Paper*

# The influence of protected kapok seed oil supplementation on *in vitro* ruminal fermentability and linoleic acid status with Etawah crossbred goat rumen fluid and elephant grass as feed

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This investigation was conducted to study the effect of protected kapok (*Ceiba pentandra*) seed oil supplementation upon the *in vitro* relative proportion of linoleic acid and ruminal fermentation parameters using rumen fluid of Etawah crossbred goat and elephant grass (*Pennisetum purpureum*) as feed. Research materials used were elephant grass (EG), kapok seed oil (KSO), lactating Etawah crossbred goat (ECG) rumen fluid, potassiumhydroxide (KOH) and calciumchloride($CaCl_2$). There were two treatment factors, namely KSO supplementation as factor I (S) and protection as factor II (P). The first factor consists of four levels, namely 5% (S1), 10% (S2), 15% (S3) and without KSO supplementation (S0), while the second factor consists of 5 levels, namely: 0% (P0), 25%(P1), 50% (P2), 75% (P3) and100% (P4), thus there were 15 combinations of treatment and one control (S0P0). Measured variables consisting of: *in vitro* neutral detergent fiber digestibility (IVNDFD), the molar proportion of acetic acid, propionic acid, butyric acid, acetic acid: propionic acid (A/P) ratio, the relative proportion of linoleic acid, stearic acid and ruminal lipid iodine number (IN). The results showed that 5% of unprotected KSO supplementation increased the molar proportion of propionic acid from 21.30% in the EG without supplementation becomes to 23.67% (P <0.05) without any negative effect on IVNDFD. Supplementation of protected KSO at 10 and 75% protection level increased (P <0.05) the molar proportion of propionic acid significantly (35.49%) with A / P ratio: 1.68 without significant IVNDFD variation. Increasing levels of KSO supplementation (up to 15%) increased the relative proportion of ruminal linoleic acid, followed by increasing in iodine number. The combination between KSO supplementation and protection increased the ruminal linoleic acid relative proportion and lipid iodine number, were 47.14% and 51.09, respectively. The KSO supplementation at the level of 10% with 75% protection level can resulted in the same proportion of linoleic acid to 15% KSO supplementation with 75% protection level (45.39 and 46.50 and 97%, respectively) with higher IVNDFD (P<0.05), namely 50.97 vs. 45.44%).

**Key words:** Kapok seed oil, protection, linoleic acid, ruminal fermentability, elephant grass, Etawah crossbred goat, *in vitro*, linoleic acid.

## INTRODUCTION

Consumer demand for improved quality of livestock products recently is higher not only in terms of nutritional value, but also for healthy products (Nuernberg et al., 2006). The increasing of goat products, both meat and

goat milk consumption still often face constraint such as cholesterol phobia issue, because of the high saturation degree of fatty acid and cholesterol levels in the livestock products. Feed manipulation was required to overcome this, among other with the supplementation of linoleic acid -rich oil in this case was kapok seed oil (KSO), because of lower lipid levels in goats conventional feed (forage), which was only about 3% despite a high proportion in linoleic acid (Jalc et al., 2007). Oil supplementation may also increase the energy density of the ration (Cieślak et al., 2010).

Linoleic acid makes up about 23% of fatty acids in phosphatidylcholine, a major phospholipid constituent of high density lipoprotein (HDL) (Bauchart, 1993). That lipoprotein plays an important role in cholesterol controlling by cholesterol transporting from extrahepatic tissues and other lipoproteins to the liver (reverse cholesterol transport), for various processes. The role of linoleic acid in the control of cholesterol also occured through the increasing of low density lipoprotein (LDL) receptors number and the increasing of LDL catabolic rate (Murray et al., 1997).

Linoleic acid as part of phosphatidylcholine also plays an important role in the maintenance of membrane integrity, both the cell membrane and mitochondria membrane as well as nuclei membranes, to maintain the membrane fluidity. These conditions allow the maintenance the normal activity of membrane bounded enzymes, receptors affinity and membrane permeability. It makes possible for the increasing of the nutrients transport into the cells and activation of intracellular enzymes which is reflected in the stimulation of growth, maintenance of reproduction performance and other aspects of the livestock production that in turn increases the livestock productivity (Sardesai, 1992). Supplementation of the linoleic acid-rich oils also allow deposition of essential polyunsaturated fatty acids occure in animal products so that can make the consumers healthy (Jayanegara, 2013).

Level of linoleic acid in ruminant products is low, due to low level of that essential fatty acid inconventional feed, it is also due to microbial biohydrogenation in the rumen (Varadyova et al., 2013). Thus, protection needs to be done to protect linoleic acid from ruminal biohydrogenation, thus ensuring the sufficient supply of these nutrients for the post-rumen absorption (Jayanegara, 2013). Protection is also required to minimize the negative effect of unsaturated fatty acids upon the fiber degradation by cellulolytic microbes. Partial protection is applied as an attempt to capitalize on the positive influence of polyunsaturated fatty acids (PUFA) to raise the ruminal fermentation efficiency up to a certain level that does not cause the decreasing of

fibrous feed utility, significantly (Renno et al., 2014). Linoleic acid can affect the ruminal fermentation, which lowers the availability of substrates for methanogenesis so that the methane formation is reduced, which means reduce the waste of energy that to go to waste through the formation of methane gas. The decreasing of methanogenesis has impact on the increasing of molar proportion of propionic acid and / or the decreasing of acetate / propionate ratio which also means the increasing of energy efficiency (Cieślak et al., 2009). Metabolism of propionic acid will produce higher adenosine triphosphate (ATP) while the heat increment (HI) per mole lower than acetic acid metabolism (Banerjee, 1978).

In the view of above concepts, this study was conducted to assess the effect of supplementation of linoleic acid source (that is, KSO) upon ruminal fermentation. The study also yields the technology of protected KSO supplementation and information about its potency to increase the supply of linoleic acid for absorption in the post-ruminal digestive tract, which is reflected in the status of ruminal lipids, especially the relative proportions of ruminal linoleic acid, without reducing the utility of fibrous feed as the main feed ingredient for ruminants.

## MATERIALS AND METHODS

The materials used in this study was the elephant grass in air dry state as a single feed, KSO as supplements and rumen fluid from lactating Etawah crossbred goat which fed a standard diet with elephant grass as a basal feed. Reagents used among other reagents to protect KSO, such as KOH and $CaCl_2$.

The results of the analysis of fatty acid composition of KSO listed in Table 1, the saponification number was 119.06 whereas the iodine number was 53.93. Nutrient composition of elephant grass showed in Table 2. Goat rumen fluid taken from four fistulated lactating goats on 3 h after the morning feeding.

### Research method

There were 2 factors in this study, namely KSO supplementation as factor I and KSO protection as a factor II. The factor I consists of three levels, namely 5 (S1); 10 (S2) and 15 (S3)%, while the factor II consists of 5 levels of protection, namely 0 (P0); 25 (P1); 50 (P2); 75 (P3) and 100 (P4)%. Both of these factors resulted 15 combinations of treatment and 1 control, namely without supplementation and without protection (S0P0).

### Protection procedure of KSO

Protection of KSO was conducted through saponification with KOH, which then transformed into calcium salt by using $CaCl_2$. The amount of KOH used was suitable to the protection level and

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**Table 1.** Fatty acid composition in kapok seed oil (KSO).

| Fatty acid composition | Proportion (%)* |
|---|---|
| Palmitic acid (C16:0 ) | 23.62 |
| Stearic acid( C18 :0) | 2.38 |
| Oleic acid (C18 :1) | 24.59 |
| Linoleic acid (C18 :2) | 43.68 |
| Linolenic acid (C18 : 3) | 2.92 |

*Proportion from total fatty acids in KSO.

**Table 2.** Nutrient composition of experimental feed (%) (dry matter basis).

| Feed | CP[a] | CF[a] | NFE[b] | EE[a] | Ash[a] | TDN[c] |
|---|---|---|---|---|---|---|
| Elephant grass | 9.52 | 31.13 | 41.82 | 2.27 | 15.26 | 55.43 |

Explanation : CP : crude protein ; CF : crude fiber ; NFE : nitrogen free extract ; EE : ether extract ; TDN : total digestible nutrient : [a] Determined, [b] DM – Ash- CP – CF – EE, [c]calculated based on TDN formulation for goat.

calculated based on the saponification number of KSO that were determined according to the method of Cabatit (1979). A certain amount of KSO (based on the supplementation levels) put into the beaker glass, then heated in a water bath until the temperature reached 90°C. A number calculated KOH were weighed, diluted with distilled water and then added to KSO which was being heated while stirring for 10 min to form a suspension of potassium soap. To transform potassium soap into calcium salt, a number of $CaCl_2$ were calculated using stoichiometry, weighed and diluted with distilled water. The $CaCl_2$ solution was added to the suspension of potassium soap while heated in a water bath at the temperature of 90°C and stirred until the precipitation of calcium salts then formed. After centrifugation at 2500 rpm for 10 min, the supernatant was removed, the precipitate was mixed with a portion of KSO which was not protected (suitable to the supplementation level), ready to be used as a supplement.

### Variables were measured and chemical analysis of samples

The measured variables included fiber digestibility, in this case the *in vitro* neutral detergent fiber digestibility (IVNDFD), molar proportions of volatile fatty acids (VFAs), the relative proportion of linoleic acid and stearic acid, and ruminal lipid iodin number (IN). Elephant grass IVNDFD was determined according to the method of Tilley and Terry one stage (1963). Amount of 0.5 g sample were incubated in 50 ml of a mixture of rumen fluid and McDougall's buffer solution (1: 4), for 48 h. Fermentation was stopped after 48 h using 2 ml of 50 g / kg $HgCl_2$. To the initial sample, residue and blank also boiled (refluxed) in neutral detergent solution to determine NDF level (Goering and Van Soest, 1970), for calculating IVNDFD.

Amount of 9 ml of supernatant from each fermentation tube was taken, preserved by the addition of 1 ml of 50% (vol. / vol.) $H_2SO_4$, then stored at -20°C, prior to analysis. Towards the analysis, then sample was thawed and centrifugated (15000 g for 15 min). Concentration of volatile fatty acids (acetic acid, propionic acid and butyric acid) were determined by gas chromatography (Galyean, 1980).

After incubation the content of fermentation tubes were dried by freeze-drier at a temperature of -50°C . Lipids were extracted from 0.5 g of culture that has been dried by a freeze-drier using mixture

of chloroform: methanol 2:1, the samples were purified using a 20% HCl (Bligh and Dyer, 1959). Concentrations of long chain fatty acids were determined by gas chromatography (Galyean, 1980). Iodine number (IN) of the extracted lipid was determined by the method of Plumer (1971).

### Statistical analysis

The collected data included IVNDFD, partial VFAs molar proportions, A / P ratio, the relative proportion of linoleic and stearic acids and iodine number variables were processed statistically by analysis of variance (ANOVA) with factorial treatment patterns, in a completely randomized design (Steel et al., 1997 ). Data processing was done by using co-stat program.

## RESULTS AND DISCUSSION

### *In vitro* neutral detergent fiber digestibility (IVNDFD)

The IVNDFD and molar proportions of partial volatile fatty acids in this study reflected the fibrous feed utility, in this case the elephant grass (EG), and the effect of protected KSO supplementation upon these variables (Narimani-Rad et al., 2012). Data of those variables were summarized in Table 3. Elephant grass IVNDFD without supplementation (S0P0) was 51.63%, whereas the treatment group of KSO supplementation with 5% level and 25; 50; 75 and 100% protection level (S1P0, S1P1, S1P2, S1P3, and S1P4) were 50.87; 50.93; 51.03; 51.09 and 51.12%, respectively.

Between the elephant grass with no supplementation and supplemented by 5% KSO with and without protection, there were no significant difference in IVNDFD. According to Byers and Schelling (1988) and Patra (2013), levels of fat in the diet up to 5%, did not inhibit the microbial fermentation in the rumen. Elephant

**Table 3.** The *in vitro* NDF digestibility (IVNDFD), VFAs molar proportion (%) and acetic acid/propionic acid ratio.

| Treatment | IVNDFD | Acetic acid | Propionic acid | Butyric acid | Acetic acid/Propionic acid ratio |
|---|---|---|---|---|---|
| $S_0P_0$ | 51.6±1.3[a] | 72.3±1.0[a] | 21.3±0.5[i] | 6.4±1.0[cd] | 3.4±0.1[a] |
| $S_1P_0$ | 50.9±0.3[a] | 64.2±1.3[d] | 23.7±0.7[h] | 12.1±1.4[a] | 2.7±0.1[d] |
| $S_1P_1$ | 50.9±0.4[a] | 67.5±1.5[c] | 22.5±0.5[hi] | 8.0±1.9[ab] | 3.0±0.1[c] |
| $S_1P_2$ | 51.0±0.6[a] | 68.4±1.3[bc] | 23.0±0.8[h] | 6.9±2.2[b] | 3.0±0.1[c] |
| $S_1P_3$ | 51.1±0.6[a] | 71.3±1.9[a] | 22.4±0.6[hi] | 6.3±1.4[cd] | 3.2±0.2[b] |
| $S_1P_4$ | 51.1±0.5[a] | 72.0±0.9[a] | 21.3±1.8[i] | 6.7±2.6[c] | 3.4±0.3[a] |
| $S_2P_0$ | 40.9±0.3[cd] | 55.8±1.1[g] | 42.1±0.6[b] | 2.0±0.9[fh] | 1.3±0.1[hi] |
| $S_2P_1$ | 45.6±0.6[b] | 56.0±1.2[g] | 40,7±1.1[c] | 3.2±2.1[efgh] | 1.4±0.1[h] |
| $S_2P_2$ | 45.9±0.3[b] | 58.7±0.5[ef] | 37.3±1.0[d] | 3.2±1.4[effh] | 1.6±0.1[fg] |
| $S_2P_3$ | 51.0±0.4[a] | 59.9±2.0[e] | 35.5±0.9[e] | 4.6±2.8[cde] | 1.7±0.1[f] |
| $S_2P_4$ | 51.0±0.3[a] | 69.5±1.3[b] | 26.6±0.6[g] | 4.0±1.3[defgh] | 2.6±0.1[d] |
| $S_3P_0$ | 40.3±0.7[d] | 52.7±0.9[h] | 45.2±1.2[a] | 2.1±1.3[fgh] | 1.2±0.1[j] |
| $S_3P_1$ | 41.4±0.9[c] | 52.9±1.4[h] | 42.6±0.7[b] | 4.5±1.6[cdef] | 1.2±0.1[ij] |
| $S_3P_2$ | 41.6±0.9[c] | 56.3±0.5[g] | 42.2±0.8[b] | 1.6±0.7[h] | 1.3±0.1[hi] |
| $S_3P_3$ | 45.4±0.9[b] | 58.1±0.8[f] | 37.5±0.7[d] | 4.4±0.6[cdefg] | 1.5±0.1[g] |
| $S_3P_4$ | 46.0±1.3[b] | 63.9±1.0[d] | 29.9±1.7[f] | 6.2±2.4[cd] | 2.1±0.1[e] |

The values were arithmetic means (n=5); IVNDFD : *in vitro* neutral detergent fiber digestibility; VFAs: volatile fatty acids. S0,S1,S2, S3 : KSO supplementation levels : 0; 5; 10; 15%, respectively. P0, P1, P2, P3, P4 : protection levels : 0; 25; 50; 75 and 100% respectively. Different superscript within column denote significantly different means (P <0.05).

grass fat content was 2.27%, with KSO supplementation, levels of fat in the ration became to more than 5%. The high proportion of fibrous feed in the ration could reduce the negative effect of fat on the rumen microbial metabolism (Delgado et al., 2013). Feedstuff that was used as a research material in this study was fibrous feed as a single feed, so that 5% KSO supplementation did not influence the IVNDFD significantly.

The IVNDFD began to decrease at the level of 10% of KSO supplementation without protection (S2P0), namely 40.93% and the decreasing tend to be larger at the level of 15% of KSO supplementation (S3P0), namely 40.28%. The decreasing of IVNDFD primarily could occurred due to the influence of unsaturated fatty acids (UFA) contained in KSO. Unsaturated fatty acids could inhibit fibrolytic bacteria causing reduced fiber digestibility (McGinn et al., 2004; Messana et al., 2013). Anaysis of KSO in this study showed the proportion of unsaturated fatty acids in KSO (from total fatty acid in KSO) was 71.19%, which consist of 43.68% linoleic acid, 24.59% oleic acid and 2.92% linolenic acid (Table 1).

The IVNDFD of elephant grass supplemented by 10% KSO with protection level of 25, 50, 75 and 100%, were 45.60; 45.92; 50.97 and 51.05%, respectively while elephant grass supplemented by 15% KSO were 41.37; 41.64; 45.44 and 45.96%, respectively. Protection of KSO could reduce the negative effect of these supplements on ruminal fermentation, which were reflected in the increasing of IVNDFD, both at 10% as well as 15% supplementation levels. Combination

between 75 as well as 100% protection level and 10% KSO supplementation level resulted in not significantly different IVNDFD from elephant grass without supplementation (50.97 and 51.05 vs 51.63%). Binding of the carboxyl group with the calcium in the protection process reduced the unsaturated fatty acids toxicity on rumen microbe resulting reduced ruminal metabolism inhibition (Bhatt et al., 2013)

## Molar proportions of volatile fatty acids

Analysis of variance showed the effect of KSO supplementation and protection as well as its interaction upon the molar proportions of partial volatile fatty acids . The major volatile fatty acids as ruminal fermentation product were acetic acid, propionic acid and butyric acid.

## Acetic acid

Most of the acetic acid produced from ruminal fermentation of fibrous feed, by fibrolytic bacteria, among other Ruminococcus, Butyrivibrio and Bacteroides (Hungate, 1966). The rumen microbes which were depressed by unsaturated fatty acid supplementation were primarily fibrolytic bacteria (Patra and Yu, 2013), thus will resulted in a decreasing of ruminal acetic acid production (Al-Dobaib and Kamel, 2012; Sun et al., 2013). It was seen from the molar proportion of acetic acid in the fermentation of elephant grass supplemented

with 5, 10 and 15% KSO without protection (S1P0, S2P0 and S3P), namely 64.22; 55.84 and 52.68% which lower (P <0.05) than the molar proportion of acetic acid in the treatment group without KSO supplementation (S0P0), namely 72.26%.

Protection of KSO reduced the cytotoxic effect of unsaturated fatty acids upon fibrolytic microbes, so that the molar proportion of ruminal acetic acid from the fermentation of elephant grass supplemented with protected KSO were higher than the treated group with unprotected KSO supplementation. The molar proportion of acetic acid were more and more high along with the increasing of protection level. Protection level of 75 and 100% at 5% KSO supplementation level (S1P3 and S1P4) resulted in molar proportion of acetic acid which were equivalent to the treatment group without KSO supplementation (S0P0), namely 71.34 and 72.03% vs. 72.26%. This phenomenon showed that protection of KSO began the 75% level could eliminate the inhibition upon fibrolytic microbes.

## Propionic acid

Unsaturated fatty acids also have depressing effect on the methanogenic microbes, which have an impact on the decreasing of methane production (Patra, 2013; Thanh and Suksombat, 2013). Decreasing of methane production resulted in the increasing of external hydrogen pressure that could potentially inhibit the thermodynamics of reoxidation reaction of the reduced coenzyme ($NADH_2 \rightarrow NAD^+ + H_2$), so that the microbes are encouraged to reduce pyruvic acid to propionic acid, in order to maintain hydrogen balance (Baldwin and Allison, 1983). That mechanism was reflected in the increasing (P <0.05) of molar proportion of propionic acid due to KSO supplementation. The molar proportion of propionic acid in the treatment group without supplementation (S0P0) was lower (P <0.05) than the treatment group of KSO supplementation at the level of 5% (S1P0), 10% (S2P0) and 15% (S3P0), which were 21.30 vs. 23.67; 42.13 and 45.24%.

Protection of KSO could reduce the pressure on methanogenic microbes, so that the molar proportion of propionic acid in protected KSO treatment group was lower than KSO supplementation without protection treatment group (P<0.05). In line with the increasing of protection level, the decreasing in the molar proportion of propionic acid became greater. Ruminal propionic acid molar proportion in 5% KSO supplementation level with 100% protection level treatment group (S1P4) did not significantly different from without supplementation treatment group (S0P0), namely 21.30 vs 21.27%.

## Butyric acid

Ruminal butyric acid production essentially one of reaction mechanism to allow reoxidation of reduced coenzyme (in this case $NADH_2$ become to $NAD^+$) in for ensure the continuity of fermentation in anaerobic systems (Baldwin and Allison, 1983). Pyruvic acid as the central intermediate compound in ruminal fermentation will be converted into various end products, among other butyric acid (Hungate, 1966). Reaction process of pyruvic acid to butyric acid involves the formation NAD from $NADH_2$, namely in reduction of acetoacetyl-CoA to betahydroxybutyril-CoA and reduction of crotonyl-CoA to butyril-CoA (Baldwin and Allison, 1983). The reaction increases for reduced coenzyme reoxidation, along with the decreasing of methane production as a result of pressure on methanogenic microbes by unsaturated fatty acids in the KSO, because hydrogen production in the butyric acid formation is lower than in the acetic acid formation.

The change of butyric acid production was appeared significantly at 5% KSO supplementation level. The molar proportion of butyric acid increased sharply (P <0.05) from 6.43% in the without supplementation treatment group to 12.10% in 5% KSO supplementation without protection treatment group (S1P0). The molar proportion of butyric acid decreased gradually along with the increasing of protection levels, because the decreasing of methanogenesis pressure. The molar proportion pattern of butyric acid at the higher levels of KSO supplementation (10 and 15%), was not specific. This phenomenon might be occurred because in the high level KSO supplementation, the main hydrogen controlling mechanism was through the increasing of propionic acid production.

## Acetic acid / propionic acid ratio

The molar proportion ratio of acetic acid to the butyric acid (A / P) can be used as one of the indication in the energy efficiency of ruminal metabolism. Decreasing of A / P value means the increasing of energy efficiency of ruminal metabolism, which in line with the increasing of the molar proportion of propionic acid and decreasing of methane production (Rahbar et al., 2014). In general A / P data in Table 3 illustrated that KSO supplementation improved the energy efficiency of ruminal metabolism, as reflected by the decreasing of A / P value. The decreasing of A / P were occurred in the KSO supplemented treatment group, either unprotected or protected. Protection level of 100% resulted in the higher A / P value than the lower protection level for each level of KSO supplementation. The value of A/P in 5% KSO supplementation with 100% protection level treatment group (S1P4) was not significantly different from without supplementation treatment group (S0P0), namely 3.39 vs. 3.41.

## Status of ruminal linoleic acid

The relative proportion of ruminal linoleic acid, stearic

**Table 4.** Relative proportion of linoleic and stearic acids (%) and IN.

| Treatment combination | Linoleic acid | Stearic acid | IN |
|---|---|---|---|
| S0P0 | nd | nd | nd |
| S1P0 | 18.5±1.6[i] | 8.2±1.1[c] | 11.2±0,5[ij] |
| SIP1 | 22.5±1.1[g] | 7.9±1.1[cd] | 13.5±1.0[i] |
| S1P2 | 31.5±1.2[f] | 7.4±0.6[cd] | 20.4±1.2[h] |
| S1P3 | 34.9±1.1[e] | 6,0±1.2[ef] | 26.2±0.5[g] |
| S1P4 | 38.0±1,1[d] | 3.4±0.6[h] | 28.5±1.0[f] |
| | | | |
| S2P0 | 20.0±1.3[h] | 15.7±1.2[a] | 13.4±1.6[i] |
| S2P1 | 38.9±0.8[d] | 6.3±0.6[ef] | 29.0±0.7[f] |
| S2P2 | 43.6±0.7[c] | 5.4±0.8[fg] | 37.8±1.5[d] |
| S2P3 | 45.4±1.1[b] | 4.6±0.5[g] | 42.8±1.1[c] |
| S2P4 | 46.1±0.8[ab] | 3.0±0.6[h] | 48.6±1.0[b] |
| | | | |
| S3P0 | 33.5±1.6[e] | 10.0±0.9[b] | 35.5±1.6[e] |
| S3P1 | 43.5±0.5[c] | 6.9±0.9[de] | 47.2±1.6[b] |
| S3P2 | 43.4±0.9[c] | 5.7±1.3[fg] | 48.2±2.2[b] |
| S3P3 | 46.6±1.1[ab] | 5.5±0.5[fg] | 49.0±1.8[b] |
| S3P4 | 47.1±1.1[a] | 3.2±0.4[h] | 51.1±2.0[a] |

The values were arithmetic means (n=5);nd : non detected; IN : iodine number. S0,S1,S2, S3 : KSO supplementation levels : 0; 5; 10; 15%, respectively. P0, P1, P2, P3, P4 : protection levels : 0; 25; 50; 75 and 100% respectively. Different superscript within column denote significantly different means (P <0.05).

acid and iodine number (IN) were showed in Table 4. In the without supplementation treatment group (S0P0), linoleic acid and stearic acid were undetected. In general, forage lipid content is low, and main fatty acid in forages was linolenic acid (Byers and Schelling, 1988; Mir et al., 2006). Lipids were metabolized and used rapidly by rumen microbes, so that its metabolic products were not detected (Varadyova et al., 2007). The lowest relative proportion of ruminal linoleic acid in 5% KSO supplementation treatment groups was found in S1P0, namely 18.53% whereas in S1P4 was highest, namely 38.02%. The portion of the hydrogenated linoleic acid in S1 treatment groups was in S1P0, because it was not protected. It can be seen from the relative proportions of stearic acid as biohydrogenation product which higher than other treatment combinations in S1 groups, namely 8.21% (S1P0). Protection decreased the relative proportions of stearic acid that the value of it was getting the lower, it was in line with the decreasing the level of protection at S1P4 namely 3.41%. The magnitude of biohydrogenation in S1P0 treatment combination was also visibled from the lower IN value (11.21), than that in protected S1 combination. Protection at the level of 100% gave a higher degree of unsaturation compared to unprotection and protection at a lower level (the value of IN was 28.46).

Phenomenon as described above was also found in the 10% KSO supplementation treatment groups (S2). Treatment combination of 10% KSO supplementation without protection (S2P0) showed the lowest linoleic acid relative proportion and IN value (20.04 and 13.42%), with the highest relative proportion of stearic acid (15.71%), while the opposite value was seen in the treatment combination between 10% KSO supplementation and 100% protection (relative proportions of linoleic acid was 46.05% and IN value was 48.63) whereas the relative proportion of stearic acid was 2.99%. It showed that the treatment combination of KSO supplementation with protection could increase the proportion of available linoleic acid for the post-ruminal absorption, for productive purpose.

Treatment combination of 15% KSO supplementation without protection (S3P0) supplied the linoleic acid more than S2P0, but its biohydrogenation products, namely stearic acid, had lower relative proportion (P <0.05) than S2P0 (10.04 vs. 15.71%). That were presumably becaused the increasing of uncomplete biohydrogenation process and its product were accumulated as intermediate compounds, namely cis 9, trans 11 linoleic acid and trans-11 vaccenic acid (Khamal and Dhiman, 2004; Panatuk et al., 2013; Castano et al., 2014 ).That assumption was supported by the higher IN of S3P0 (P <0.05) than S2P0 (35.46 vs. 13.42). That intermediate compounds were unsaturated fatty acids derivatives, thus contributed to the magnitude of ruminal lipid unsaturation degree which was reflected on the magnitude of the iodine number (IN). Uncomplete biohydrogenation was occurred becaused inhibition in second step of

biohydrogenation, the change of trans vaccenic acid into stearic acid by the increasing of free linoleic acid as a result of   trilinolein lipolysis by ruminal *Anaerovibrio lipolytica* that increased due to the increasing of KSO supplementation level (15%) without protection (Jenkins et al., 2008).

## Conclusion

Kapok seed oil supplementation on elephant grass as fibrous feed lowers the ratio of acetic acid / propionic acid. Protected kapok seed oil supplementation up to 10% supplementation level  and 75%  protection level increased the molar proportion of ruminal propionic acid without affected the    *in vitro* fiber digestibility with lactating Etawah crossbred goat rumen fluid .

The Increasing of KSO supplementation level (to 15%) increased the relative proportion of ruminal linoleic acid, followed by the   increasing of ruminal fatty acid unsaturation degree (IN). Combination of KSO supplementation with protection increased the ruminal linoleic acid proportion and lipid iodine number. The inhibition of biohydrogenation by free linoleic acid significantly occurred at the level of 15% unprotected KSO supplementation, with the decreasing of ruminal stearic acid proportion. The KSO supplementation at the level of 10% with 75%  protection level could resulted in the same  linoleic acid proportion with 15% of KSO and 75% of protection level with a higher *in vitro* NDF digestibility.

## Conflict of Interests

The authors have not declared any conflict of interests.

## ACKNOWLEDGEMENTS

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